MESENCHYMAL STEM CELLS: HELP OR HYPE?

by

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ABSTRACT

In recent years, stem cells have been credited as the "greatest discovery of modern science" with claims that stem cells will be a "cure-all" for a multitude of diseases including cerebral palsy, Alzheimer's disease, autoimmune disorders, and many others. As of January 2020, the U.S. Food & Drug Administration (FDA) has only approved seventeen stem cell therapies all of which are used only in treatment of blood malignancies.[1] There has been much debate whether or not stem cells have potential to live up to these claims, or is it all hype? This paper will explore the history and development of stem cell research, the potential uses of stem cells in disease treatment, and the future for stem cell therapies.

I. HISTORY OF RESEARCH

In 1981, Drs. James Till and Ernest McCulloch described the first stem cells and successfully derived embryonic stem cells from mouse embryos.[2] Till and McCulloch found that the stem cells they had harvested from mouse bone marrow were able to differentiate into many types of cells, leading to the well-known term of pluripotent stem cells (PSCs). This research later led to the discovery of ways to derive human embryonic stem cells for growth in the laboratory setting in late 1998 by Dr. James Thompson. In 2006, researchers were able to "reprogram" specialized adult cells to become a "stem-like" cell genetically.[3] These new types of stem cells are known as induced pluripotent stem cells (iPSCs). Over the last 20 years, researchers have been able to distinguish many kinds of stem cells within three primary categories: adult stem cells (ASCs), embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs).

Adult stem cells are undifferentiated cells which can be harvested from many differentiated tissues the human body. ASCs are typically specific to the organ or tissue from which they are derived.[4] For example, ASCs from the liver will regenerate liver tissue, and ASCs from the muscle will regenerate muscle tissue. With that being said, these cells are incredibly specialized and are limited to regenerating into their original specialized tissue. ASCs can be found in locations such as the bone marrow, brain, muscle, fat tissues, gastrointestinal tract, umbilical cord, and placenta. There are currently five types of adult stem cells that have been identified: hematopoietic stem cells (blood stem cells), mesenchymal stem cells, epithelial stem cells, and skin stem cells.[5]

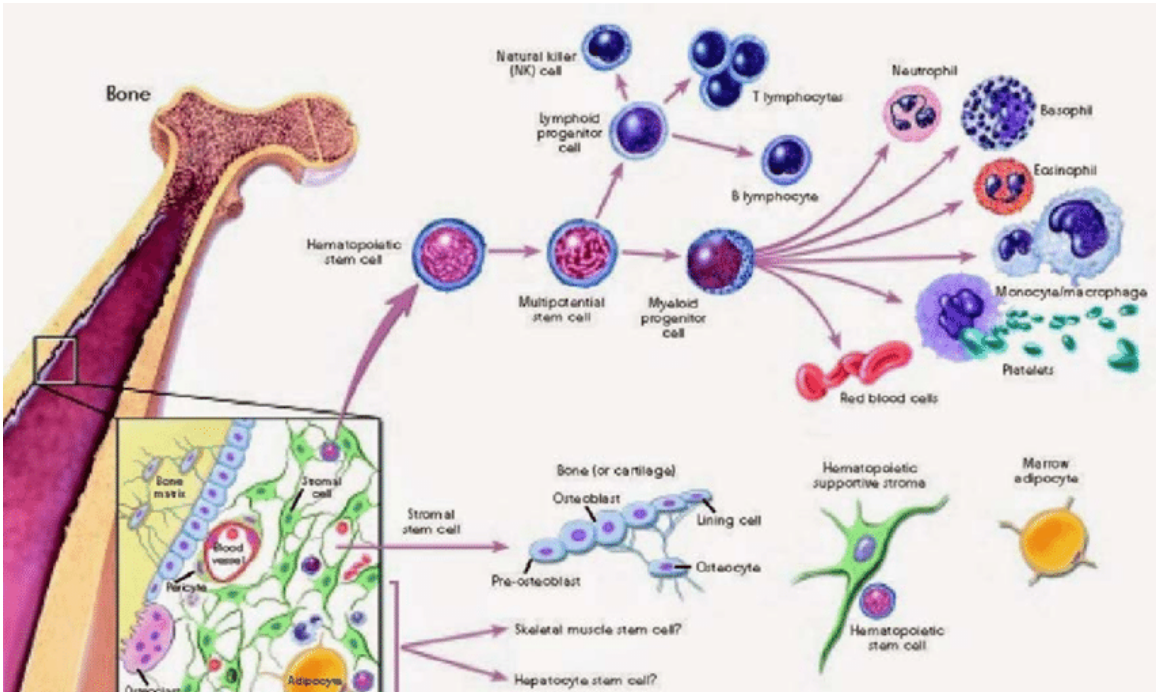

Illustration 1. Hematopoietic and Stromal Cell Differentiation (© 2001 Terese Winslow (assisted by Lydia Kibiuk))

Three to five days after fertilization, embryonic stem cells are found within the inner cell mass, which generates specialized tissue in the developing embryo (embryo is a blastocyte at this stage).[6] Embryonic stem cells are pluripotent and can differentiate into any cell type during the early developmental periods. One important consideration is that these stem cells are harvested from eggs that have been fertilized in vitro; they are not derived from fertilized eggs within the female body at this time.

Induced pluripotent stem cells are created in the lab. They are considered a "happy medium" of embryonic stem cells and adult stem cells in that they are adult stem cells that have been genetically altered to behave like an embryonic stem cell.[7] Scientists have claimed that by creating iPSCs, they have been able to "de-differentiate" specialized cells that otherwise would only be able to produce a single tissue type. iPSCs are the

most unstudied stem cells at this time; it is unknown if iPSCs and embryonic stem cells differ in a significant way during tissue regeneration.

Mesenchymal stem cells are adult stem cells which are non-hematopoietic, multipotent stem cells that have the ability to differentiate into mesodermal lineage such as chondrocytes (cartilage), adipocytes (fat), osteocytes (bone).[8] It was initially believed that MSCs could differentiate into other types of cells, including cardiac cells, liver cells, endothelial cells, and nerve cells. This has since been disproven in clinical trials.[9] MSCs are easily accessible and easily cultured in vitro for research. They also have exceptional genomic stability and few ethical issues making them a popular choice of study in regenerative research.[10]

Prior to November 2017, the FDA had no policy or guidelines guiding the research into regenerative medicine and stem cell research. Many companies were creating therapies not reviewed by the FDA with no reliable evidence. Therapies were usually costly and sometimes dangerous. Public concern turned the attention of the FDA towards these questionable therapies and resulted in significant changes in FDA oversight on stem cell therapy and research.

> FDA Commissioner Scott Gottlieb, M.D. stated, "One of the most promising new fields of science and medicine is the area of cell therapies and their use in regenerative medicine. These new technologies, most of which are in early stages of development, hold significant promise for transformative and potentially curative treatments for some of humanity's most troubling and intractable maladies. Recent advances in our basic knowledge of the pathways involved in tissue damage and regeneration have combined with remarkable progress in adult

stem cell biology to put us at a genuine inflection point in the history of medicine."[11]

The FDA has since released four guidance documents that outline its regulations on regenerative medicine. These documents aim to: clarify the distinctions between products that require full drug approval and those that do not as well as introduce the new review process for therapies and reduce specific regulatory requirements on developers.[12] The regenerative medicine advanced therapy (RMAT) designation was created in 2016 under the 21st Century Cures Act, which allows regenerative therapy developers to conduct smaller and shorter trials to expedite the process of approval. Before 2017, the FDA had notoriously failed to implement the RMAT designation but pledged to fully implement and improve the program to include certain gene therapies and products.

After the FDA released the revised guidelines, there was an exponential drop in the amount of available elective procedures utilizing stem cells and related therapies. In turn, there has also been a reduced amount of adverse events reported with stem cell therapies. As of January 2020, the FDA has only approved seventeen stem cell therapies for use in the clinical setting but has approved over 50 new trails in the last two years alone, which are ongoing. The ongoing trials have "showed promising results" and "are anticipated to be approved for use in clinical medicine within the next three years."[13]

II. Significance

Many ask, why do we need stem cells? Why should we spend so much time and money on these studies? As mentioned above, stem cells are crucial for living organism development and functioning, starting at the embryonic stage. Stem cells are able to regenerate themselves, giving the potential for disease treatments. Despite the vast amount of research that has been performed and is ongoing, there are still many questions which must be explored before stem cells are able to cure diseases. Research on stem cells over the last 30 years has been advancing knowledge of not only how the body develops from a single cell, but also how the body continues to replace damaged cells throughout an individual's life. By studying how the body replaces damaged cells with new ones, researchers are able to test how to eliminate diseased cells and replace them with healthy cells.

Major research projects at this time include cancer treatment, skin graft generation, neuronal damage treatment, gene therapy, and many autoimmune treatments. While most of these projects are in the early stages, there have been promising results and advances in treating many of the above issues. In 2017, Dr. Donald Kohn at UCLA was able to successfully treat nine babies with a genetic mutation called adenosine deaminase-deficient severe combined immunodeficiency (ADA-SCID or bubble baby disease) which can be fatal within the first year of life. Dr. Kohn extracted fetal hematopoietic stem cells and inserted the gene responsible for making adenosine deaminase enzyme and then transplanted the corrected stem cells which led to improved immune system functioning and the individuals no longer require isolation.[14] Scientists at UC Irving devised a stem cell-based technique to identify and destroy cancer cells, which had

metastasized to other locations in the body, preventing progression of the disease.[15] Finally, one of the most well-known stem cell studies in the last decade was conducted by Asterias Biotherapeutics on quadriplegic spinal cord injury victims by stem cell therapy resulting in some improved movement of paralyzed limbs.[16] The study by Asterias Biotherapeutics received major media attention specifically for their first patient undergoing the study, Kris Boeson, a teenager who was left paralyzed from the neck down after a car accident. Kris was treated with 10 million embryonic stem cells, which had been differentiated into oligodendrocyte progenitors. Within 90 days of treatment, Kris was regaining the ability to lift his arms and is now able to write his name and perform daily tasks. [17]The above studies demonstrate some of the major successes in stem cell research in the last decade. While there have been tremendous successes in trials such as those above, there have also been failures and many ethical concerns with stem cell research.

Embryonic stem cells, such as those used in the study on spinal cord injury, are derived from an embryo. Many individuals question whether or not it is ethical to destroy a human embryo to pursue novel therapies in the quest of curing disease. This ethical and moral dilemma is primarily founded by individual opinions meaning there will never be a correct answer to the question. In Italy, there is a prohibition of all human embryonic stem cell research. In the United States, producing hESC's for the purpose of destroying an embryo is no longer allowed as of 2001.[18] Ethical concerns for using hESC's have led many researchers to transition their studies to using mesenchymal stromal cells, which function as adult multipotent stem cells.

A major safety concern for stem cell research is the rapid and uncontrollable growth of undifferentiated stem cells. In a study with undifferentiated stem cell implantation, 33-100% of mice had later developed a tumor or teratoma. The only way to ensure tumors or teratomas do not develop as of this time is to differentiate the stem cell prior to implantation, which still has shown possibilities of uncontrolled cell division in studies.[19] A search in the PubMed database for studies with adverse events after stem cell therapy revealed 885 results with keywords “Death,” “infection,” “tumor,” “neoplasm,” and “complication.” A total of 35 cases revealed acute or chronic complications or death as a result of these trials. It is important to note that majority of these adverse events occurred before the FDA regulation implementation in 2017.

III. In Vitro Expansion and Differentiation

As mentioned above, there are three categories of stem cells: adult stem cells (ASCs), embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs). Adult stem cells are the most studied stem cells in modern science due to the fact they mature and differentiate into many different cell types with specialized structures and functions. ASCs have been shown to differentiate into hematopoietic stem cells, mesenchymal stem cells, neural stem cells, epithelial stem cells, and skin stem cells. Mesenchymal stem cells are one type of adult stem cell that is extremely easy to isolate and culture.

The primary culture systems utilized in mesenchymal stem cell studies are CFU-F assays, the analysis of bone marrow stroma, and the cultivation of MSC lines. [20] MSCs are plastic adherent and generate colonies when plated at low densities while retaining their multipotentiality. Mesenchymal stem cells are able to generate colonies after they are plated at low densities on large plates. Single cells have been shown to generate multiple colonies due to their ability to detach during expansion and then reseed the plate forming a new colony. [21]

Mesenchymal stem cells are easily isolated from tissues such as bone marrow and adipose tissue. From adipose tissue, a stromal vascular fraction pellet (SVF) is isolated by mechanical and enzymatic digestion. The pellet is then plated and kept in culture. After about 21 days, the SVF derives into a homogenous mesenchymal cell population. [22] After bone marrow extraction from a long-bone, bone marrow is filtered and incubated. Nonadherent cells are removed after 72 hours of incubation by enzyme washing. After

the culture reaches 70% confluence, the cells are subcultured into different MSC lines and are characterized as osteogenic, adipogenic, and chondrogenic. [23]

Phenotypic characterization of MSCs is based on cell surface markers. It has been reported that the characterization of MSCs is often inaccurate due to the fact that many of the epitopes are shared by both hematopoietic stem cells and mesenchymal stem cells. There have also been studies that demonstrate that MSCs in different species display different cell surface markers. Overall, scientists have come to a consensus that undifferentiated cultured MSCs "typically express CD29, SH2 (CD105), SH3 and SH4 (CD73), CD44, CD90, and CD166 while fail to express common hematopoietic and endothelial markers, such as CD11b, CD14, CD31, CD34, and CD45".[24]

MSCs are often a target of genetic manipulation by replication-deficient viral vectors in trails aiming to enhance cell function or to deliver proteins. MCSs are also able to produce and secrete a variety of cytokines and chemokines which are used in tissue repair. One of the major questions yet to be answered is in regard to the physiological conditions induce and cease cell division and differentiation. Studies have been conducted using both chemical and biological growth factors to induce differentiation. However, many scientists believe "there are more factors at play which have yet to be discovered."[25]

IV. Therapeutic Efficacy in Burn Trauma

Burn trauma is an extremely common injury globally and can cause debilitating injury or even death. Burns are classified based on several criteria. First, the type of burn is classified as thermal, chemical, electrical, or radiation. Second, the extent of the burn is evaluated and expressed as a percentage of the total body surface area. Next, the depth of the burn is classified as superficial (first degree), partial thickness (second degree), or full-thickness (third-degree). Finally, other criteria take into account additional risk factors such as age, medical history, and location of the burns. The majority of burns are caused by thermal injury and are more prevalent in people of low or middle-income people, military personal, and people in low-income countries.

Stem cell therapies for burn trauma have been said to have the potential to revolutionize therapeutic approaches for the US military in the treatment of burn trauma in soldiers both on and off the battlefield.[26] Current preclinical research efforts of the US military include development of a dispersive biofilm graft to promote bone healing while preventing infection, regenerate muscle and tissues, and develop a readily available full-thickness skin graft. [27]

Stem cells have been used to treat burns in several studies and have been shown to induce faster and better wound healing than alternative treatment options. Studies have been conducted with diverse applications of stem cells, including topical application, local injection, intravenous or systemic injection, and dermal application.[28] Studies have shown that introduction of MSCs have had a proliferative effect on keratinocytes promoting more efficient re-epithelialization. The stem cells also increased the vascular

density of the recovering tissue allowing greater blood flow and thus greater healing to the affected tissues.

In 2004, a study conducted by Rasulov et al. in Russia used bone marrow-derived MSCs on a female patient with extensive skin burns to greater than 30% of her body surface area. [29] The stem cells were applied topically to a portion of the burns, and the other burns were left without treatment as a control. The burns treated with topical MSCs healed faster, have better granulation of wound tissue, and exhibited active neoangiogenesis when compared to the area which was used as a control. The patient was able to return home after just 64 days in the burn unit, which is almost four times faster than the average burn victim being treated in the United States. This study revolutionized the research into the use of MSCs in burn trauma. Shortly after publication, the United States military and many other countries began their own trials and studies using similar protocols.

Another study exhibited a significantly elevated amount of MSCs in the bloodstream after acute large surface area burns. This study concluded that the percentage of MSCs in circulation was correlated with the size and severity of the burns indicating that MSCs play an essential biological role in skin repair. [30] The majority of burn trauma studies are conducted using bone marrow, or adipose-derived MSCs rather than embryonic or umbilical cord-derived MSCs due to the ethical and legal concerns previously discussed. More recently, the focus has shifted from bone marrow-derived MSCs to adipose-derived MSCs due to the greater yield of MSCs when culturing adipose tissue. Studies have also successfully cultured MSCs from burned adipose tissue, which

is removed during wound debridement, thus eliminating the need for a procedure to harvest bone marrow from the patient.

The US military has begun implementing many burn trauma protocols in their burn clinics using MSCs. While there are no FDA approved trials for the general public at this time, military researchers and physicians are confident there will be treatments available in the coming years. [31] Most recently, a study being conducted by the US Department of Defense and the University of Miami was approved by the FDA for phase 1 of a 15 civilian patient trial with 2nd-degree skin burns to less than 20% of the total body surface area. This study is researching the efficacy of MSCs in 2nd-degree burns.[32]

V. Future of Stem Cell Research

Stem cell research has become a popular topic both in the science community as well as the general public. Despite great advances in recent years, there are still many questions which must be answered before stem cell therapies are widely available. Given current advances, it is reasonable to believe that stem cell therapies may someday be a cure to many diseases. First and foremost, researchers must be able to further understand the mechanisms by which stem cells differentiate and repair themselves. Scientists then must develop more reliable and efficient efficacy and safety tests, which are now required by the FDA for approval.

The FDA has currently approved nineteen stem cell therapies using hematopoietic stem cells for the treatment of malignancy. In 2018, more than 25,000 hematopoietic stem cell transplantations were performed in the United States for the treatment of lymphoma, leukemia, myelodysplastic and myeloproliferative syndromes, and hemoglobinopathies. [33] Dr. Antonio Liras states, "while 25,000 transplants were performed, there still over one hundred thousand patients in the united states suffering from the same illnesses who did not receive stem cell treatments." [34] Before stem cells can be known as the "magic solution," there must be an exponential increase in the availability of these treatments for patients across the world.

Before stem cell treatments are made widely available to the general public, the FDA believes there is a necessity for more investigation into the disadvantages and possible adverse events in the use of each specific type of stem cells. [35] Scientists admit that many of these advantages and disadvantages are still under investigation. For example, embryonic stem cells have shown to be very easy to isolate and be very

productive in culture. They have also exhibited a great amount of rejection by the recipient as well, as cases of uncontrollable differentiation leading to teratomas or tumors. Adult stem cells have a greater differentiation potential than embryonic stem cells. They are also less likely to induce immune rejection responses, but they are much harder to isolate and differentiate in culture. One study has also suggested that adult stem cells may carry inherited genetic abnormalities and have shown telomere shortening. [36] Many of the ongoing FDA approved clinical trials aim to discover the advantages and disadvantages of each respective stem cell category with hopes to improve clinical outcomes.

Production and storage of the majority of stem cell therapies are extremely high. Currently, a single stem cell treatment can cost up to $40,000. This is primarily due to the fact that each therapy is created on a low, sometimes even individual scale. There are many requirements that contribute to a single treatment, including surgical procedures, maintenance of aseptic conditions, specific specialized training of medical staff, specialized facilities, the need for small quantity batches of stem cells, and further research. All of the above cost thousands of dollars, and many question whether these costs will be compatible with the available government funding, medical insurance companies, health institutions, and private funding.[37] Many health insurance companies have also stated that they will not financially support the experimental use of stem cell therapies until the risks and complications are further investigated.

Stem cell research has often run into ethical concerns, specifically regarding the use of embryonic stem cells. In the US, restrictions are limited only to federally funded projects. Over recent years, there have been a number of privately funded projects

utilizing embryonic stem cells. The majority of the ethical concerns are related to the "donor consent" clause in the FDA's guidelines on cell therapies as well as the destruction of a human embryo. [38] Ultimately, it is at the scientists' discretion if they utilize human embryonic stem cells. Many independent governing bodies such as the Kennedy Institute of Ethics at Georgetown University and the Presidential Commission on Bioethics have set standards and legal guidelines regarding stem cell research and the use of live cells for therapeutic purposes. [39] Many private institutions have also vowed to create and enforce stricter regulations and guidelines for future products.

VII. Conclusions

In specific cases of burn trauma, stem cell therapies have begun to show great potential for widescale use in treatment of burns and skin regeneration. With further research, stem cells "may become the new standard of care for burn victims" and "could change patients' lives post incident with faster healing times and less disabling effects".[40] Current research trials both military and privately funded offer exciting advances into the field of stem cell research.

Given the excellent potential stem cells have exhibited for future treatments and possibly even cures of diseases, many institutions and researchers have joined the race to discover the full potential of stem cells. Research focuses are primarily geared towards answering some of the most important research questions such as: [41]

> *What physiological conditions allow stem cells to maintain themselves in an undifferentiated state? What conditions induce and cease cell division? What environmental and genetic signals affect differentiation? What physiological properties guide the functional integration of newly generated tissues into existing organs?*

Once these questions have been fully investigated and answered, scientists believe "the flood gates will open with treatment possibilities for a wide variety of diseases and illnesses".[42]

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