

# Stem cells and future medical applications



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While somatic cells can only replicate themselves to have the same functions, for instance, replacement of skin and red blood cells, stem cells can differentiate into any type of functional cell. They have the potential to innovatively rehabilitate terminal illnesses, such as cancer, diabetes, cardiovascular diseases, and brain damage; however, there are still multiple limitations to their usage in the medical field. This paper aims to serve as an introduction for people seeking to know more about stem cells in general; it provides a collection of comprehensible principal information about stem cells, such as their types and preservation methods. Moreover, it focuses on the future of stem cell adaptations for health and treatments for diseases and conditions. The research also covers the importance of ethical concerns, since they are extracted from living human beings. Hence, it would allow a wider range of audiences to acknowledge the advancement in stem cell science and technology. Stem cells are considered a novel area of science, so their current uses are rather obscure but will definitely play a significant role in the future for existing and unprecedented diseases.

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Watch a video introduction by the authors at <https://youtu.be/KEkJ1zQvFEo>

## 1. Definition of stem cells

Stem cells are unspecialized cells that have two key features in its definition. First, they are self-renewing, meaning that they can self-replicate, and second, they can also give rise to more mature or differentiated cells (Ramalho-Santos and Willenbring 2007). For years, this remarkable capacity has helped scientists in

understanding the process of our cell development in greater detail and may open new pathways for further development of new medical treatment of major public health problems like juvenile (Type 1) diabetes, cancers, and Parkinson's disease.

There are three primary types of stem cells: embryonic stem cells, adult stem cells, and induced pluripotent stem cells.

Human Embryonic stem cells (hESCs) are the cells which are found within the first two weeks of the fetus's development and are the original cells which all specialised cells in the fetus originate from. (Lee and Lee 2011)

Embryonic stem cells appear primarily in two cell varieties, each with different abilities of cell differentiation: one is found after zygote development and another is found during the blastocyst stage. First, totipotent stem cells are formed shortly after the fertilization of an egg cell and sperm. These are the most versatile embryonic stem cells and have the potential to become any kind of cell. Four or five days after forming, they develop into 'pluripotent stem cells' which are less versatile, but can still give rise to other cell types as illustrated in Figure 1 (Hildreth 2020).

Apart from the previously mentioned embryonic stem cells, another type of stem cell is the adult stem cell, which is limited to forming into only certain numbers of cell types. These stem cells are multipotent, meaning that they can only become a few different cell types relative to the organs where they are found; for example, the skin, bone marrow, and blood vessels (National Institutes of Health 2001)

With the advent of multiple stem cell breakthroughs, many researchers and scientists have been studying and testing the most effective and morally acceptable ways to extract stem cells. These attempts have been carried out for the purpose of harvesting a steady supply of stem cells which can be used for regenerative medicine and future trials. In the past, the process of stem cell extraction called Somatic Cell Nuclear Transfer (SCNT) (Murnaghan 2020) from which the inner cell mass region from human embryonic stem cells was used. But this procedure received a major backlash; the public claimed that this procedure involves the killing of embryos, therefore, it was thought to be unethical and would ultimately be rejected. Hence, the new alternative called ANT or Altered Nuclear Transfer, a procedure which involved reprogramming of somatic cells or Adult Stem cells to obtain embryonic stem cells without the destruction of human embryos, was introduced (Murnaghan 2020). These types of cells are called the pluripotent stem cells and are not as controversial (National Institutes of Health 2001).

Another type of stem cell regarding its pluripotent characteristic is called induced pluripotent stem cells (iPSCs). The discovery of this kind of stem cell was a major breakthrough to stem cell technology since it allowed adult cells to be modified to pluripotent stem cells. This new technology of iPSCs introduced in 2006 by Shinya Yamanaka, who was awarded the 2012 Nobel prize, suggested the transcription of four specific genes

namely c-Myc, Oct3/4, Sox2 and Klf4 into somatic cells, giving rise to pluripotent stem cells. In fact, the Yamanaka factor, unlike the ANT, does not require the nuclear transfer process; instead, the procedure involves the introduction of the genes of the four Yamanaka factors into somatic cells through retroviral transduction. These Yamanaka factors regulate the expression of pluripotency and the induction of pluripotent stem cells. Finally, with a method of directly converting somatic cells to pluripotent stem cells, scientists are now able to circumvent both the high-risk nature, practical issues associated with the nuclear transfer method, and the ethical issues of killing embryos, in their pursuit to retrieve a supply of stem cells to be used for medical purposes (Takahashi and Yamanaka 2006). The new technique of producing iPSCs opens up new ways of treating damaged cells, tissues, organs, whether damaged by disease or significant medical events such as stroke and neurological problems, by transplanting laboratory-grown cells to replace the lost or damaged cells. An obvious example that is now being used more widely in the stem cell therapies is the hematopoietic stem cell transplantation. The current program in Thailand requires stem cells that originate in bone marrow and has the main purpose of curing diseases in the hematopoietic system, for example, thalassemia, hematological malignancies, malignant lymphoma, and multiple myeloma (Issaragrisil 2008).

Additionally, Mesenchymal stem cell-based drugs (MSCs) are another popular variety of stem cells found in the human body which are present in nearly all tissues. They allow an immune modulation to effectively suppress both acute and chronic inflammations and further work against cancer (National Cancer Institute 2020).

Although there are several clinical trials on stem cell therapies and its medical properties, they are yet to be proven fully effective and safe for patients. Therefore, further specific experimental treatments are required for the prospective generation of medicine and pharmacy (Pham 2016).

## 2. Types of stem cells

Even though all stem cells can replicate and differentiate into other specialized cells, each type of stem cell varies in versatility and amounts. Stem cells are categorized into 3 main types: embryonic, adult, and induced pluripotent stem cells.

1. Embryonic Stem Cells: Embryonic stem cells are found in embryos that are 3-5 days old. Most of these stem cells come from fertilized eggs in vitro fertilization clinics which are then donated for research (They do not come from fertilized eggs

in a woman's body). Cells from preimplantation-stage embryos are then transferred into a petri dish to create embryonic stem cells. When these cells crowd the dish, they will be placed into numerous new petri dishes and repeated for several cycles which can produce millions of embryonic stem cells . Scientists use characterization, or the testing of cells, to measure their fundamental properties during embryonic stem cell creation. Embryonic stem cells are the most versatile and are commonly used to regenerate damaged tissues and organs.

2. **Adult Stem Cells:** Adult stem cells are found among differentiated tissues in the stem cell niche and organs are categorized into different types based on the origin of the tissue. These stem cells maintain and repair tissues in humans and may remain undivided for extended periods until an activation is present. Once these cells are extracted from the body, their ability to divide happens to be more narrow. Thus, the generation of extensive numbers of stem cells becomes more challenging . Adult stem cells typically generate mature cells that belong to a particular tissue and have specific functions. Nevertheless, some adult stem cells can differentiate into other cell types during transdifferentiation. Adult stem cells are less versatile but are present in small numbers in most of their types.
3. **Induced Pluripotent Stem Cells:** Induced pluripotent stem cells are somatic (adult) stem cells from skin cells, blood cells, or other adult stem cells that were genetically reconditioned into an embryonic stem cell by imposing them to convey genes and components crucial to retain the features of embryonic stem cells . These stem cells are commonly generated for therapeutic purposes, notably regenerative medicine, and are able to convert into any other cell type.

### 3. Current uses of each type of stem cell

With the capability to differentiate, stem cells are considered to be pluripotent and implemented to cure diseases where human cells are impaired. Moreover, stem cells also play a special role in medical examinations by allowing them to test in various cell conditions before applications into patients. However, current medical technology has not developed to the point that we can utilize stem cells to their fullest potential. If scientists can develop a way to use stem cells efficiently, many lives may be saved from these incurable diseases.

Stem cells that are used in regenerative medicine can be collected from two main sources: the patient and the others. They are called autologous and allogeneic,

respectively. Both are used in different situations and one is not better than the other. However, autologous stem cells have fewer complications when transplanting since they are harvested from the patient's body.

#### 3.1 Tissue regeneration

Tissue engineering or regenerative medicine refers to attempts to create a functional tissue or organ. Tissues and organs of patients may be damaged by chronic diseases or traumatic injuries which may cause difficulties later in life. These tissues will need to be repaired in order to comfort and let the patients have a fully functional body; human bodies can heal themselves naturally but there are some limitations. If there are too many damages, the tissue might not be able to regenerate itself to the normal state or it may partially reform. This is why stem cells can be significant since they can replace these damaged tissues.

Stem cells are grouped according to their abilities of differentiation which are multipotent stem cells and pluripotent stem cells. Embryonic stem cells are more limited and controversial than adult stem cells. Adult stem cells can be extracted from multiple parts of grown humans. For example, mesenchymal stem cells (MSCs) can be extracted from bone marrow and they are used in MSCs treatments to help bone and cartilage damages. There is also evidence that MSCs help form new blood vessels in damaged tissues. Even though stem cells might be able to reconstruct damaged tissues, the process still depends on signals from the surrounding microenvironment (Wan, Wang and Wang 2015), which provides the homeostasis for cell growth and maintenance. .

Stem cells will diverge into tissue and organs when surrounded by a suitable environment. It requires a mixture called the extracellular matrix or ECM which consists of glycosaminoglycans, inorganic hydroxyapatite crystals, and specific protein. For stem cells to develop, it also requires growth factors and cellular behavior guidelines which come in the form of ligands. These ligands also act as a skeletal system for the physical strength of the cell.

To simplify, the process of tissue regeneration begins with creating a framework containing essential proteins and growth factors. Then, stem cells are introduced which will continue to develop into tissues.

Tissue replacement which is created by stem cells is still complicated and produces uncertain results; researchers are still working on a way to hold these new generated tissues in place and guide them to develop into desired organs.

### 3.2 Cardiovascular disease treatment

Cardiovascular diseases, such as heart muscle failure, cause the most death worldwide. Researchers are trying to find a way to replace defective tissues. Unlike using the medicine for symptoms-focus treatment, regenerative medicine will provide a long term benefit with a stable replacement. When a heart is damaged, there are mainly three types of vital heart tissues that might need replacement including cardiomyocytes, cardiac pacemaker cells, and endothelial cells.

1. **Cardiomyocytes:** They are the primary group with the responsibility of heart contraction. If they are damaged, the hearts may not be able to pump naturally. Cardiomyocytes are terminally differentiated cells which means they cannot replicate to repair themselves when damaged. Dysfunction cardiomyocytes may lead to heart failure or heart attack. Currently, the only treatment for dysfunctional cardiomyocytes is a heart transplant.
2. **Cardiac pacemaker cells:** These cells generate impulses that control blood pumping which directly contribute to the heart rate; the sinoatrial (SA) node is a biological pacemaker.
3. **Endothelial cells:** They help to line blood vessels and transfer oxygen to the cardiocytes.

Researchers are mainly using adult stem cells and pluripotent cells to reconstruct the affected heart tissue. They transplant stem cells into the patients with a defective heart in effort to create a working one.

#### 3.2.1 Adult stem cell treatment

There are some experiments conducted dealing with adult stem cells which are shown to improve function of the hearts. However, those transplants died shortly after. Researchers expect to use adult stem cells to enhance cardiac function by releasing signals without replacing pre-existing muscle. Clinical trials show that transplants on the defected hearts by using adult stem cells extracted from the bone marrow are achievable and effectively safe. On a larger scale with placebo-controlled and randomized trials display less improvements of the cardiac function. An agreement has been concluded where researchers believe that adult stem cells have the most potential to be beneficial to cardiac functions.

#### 3.2.2 Pluripotent stem cell treatment

Beating human heart muscle cells can be formed by using pluripotent cell-derived cardiomyocytes. When

a heart attack occurs, newly-formed cardiomyocytes can generate vital signals and substitute the lost ones. They can have substantial benefits. These results are shown in animal-based transplants ranging from mice to monkeys dealing with heart diseases. Currently, there are not many clinical trials using pluripotent stem cells on humans, but researchers are trying to build patches created by human heart muscle cells which are created from pluripotent stem cells. Early results indicate that with further developments, these patches can be beneficial for failing hearts patients.

### 3.3 Brain disease treatment

The brain is amazingly complex. It contains more than eighty billion nerve cells which consist of a trillion connections between these neurons. As a result, when the brain is damaged, it can negatively have an impact on various functions. One of the main developments in neuroscience research is the study of neural stem cells; neural stem cells can be defined as multipotent cells that have the ability to renew themselves. There have been several approaches that stem cells can already be used in patients. Despite the excitement, the therapies have only been seen in the part of neurons obtained from embryonic stem cells and induced pluripotent stem cells (iPSCs) (Gage and Temple 2013). When the central neuron is damaged, there are some possible ways for future medical care in the following examples of brain disorders.

#### 3.3.1 Stroke

Stroke is a medical condition when a blood vessel that carries nutrients and oxygen is blocked or ruptures, resulting in poor blood flow to the brain which can cause disability or death (American Stroke Association 2020). Consequently, neural stem cell transplant examination is required in order to reconstitute possible brain damages. The current research proves that the stem cells injection is feasible in decreasing initial neurologic deterioration, apoptosis, and brain edema formation (Ul Hassan, Hassan and Rasool 2009).

#### 3.3.2 Vision restoration

Vision restoration is the damage to the brain and retinal which possibly leads to the loss of vision which is considered to be irreversible (Sabel, Henrich-Noack, Fedorov, et al. 2011). Recently, the medical staff have achieved new treatments from the use of stem cells. In effort to repair vision, these cells are transplanted to regenerate retinal pigment epithelial (RPE). By addressing the various types of blindness conditions, AMD or age-related macular degeneration affects a huge number of a population around the world.

### 3.3.3 Parkinson's disease

Parkinson's disease is a long-term neurological disorder that mainly comprehends movement such as shaking, slowness and difficulty with walking. Most researchers are using aborted human embryonic tissue which indicates that both clinical and long-term benefit does occur with transplants.

### 3.3.4 Alzheimer's disease

Alzheimer's disease is known as an irreversible disease that could lead to dementia which is considered a long-term brain disorder. The patient who is diagnosed with Alzheimer's disease is associated with the decline of cognitive function such as arousal, attention, learning, and emotion. Currently, the stem cells treatment for Alzheimer's disease is not available yet. However, the latest research provides the strategies of new stem cell therapies apart from using drug treatment (Ul Hassan, Hassan and Rasool 2009).

## 3.4 Autoimmune disease treatment

Normally, the immune system is responsible for defending against detrimental diseases. However, people with autoimmune diseases have the immune system that reciprocates its function by attacking self-antigens. Since this disease could either occur in a particular organ or across different tissues in the human body, the symptoms could be quite severe without proper treatment. Although there are many possibilities for autoimmune disease treatments, some of the methods are not effective enough to cure this life-long disease (Riordan 2017). To exemplify, the use of steroids and other immunosuppressive drugs often have inconsistent effects and involve toxicities which will negatively affect the users in the long term period. Therefore, regenerative medicine is an interesting alternative to assist the body system in recovering the destroyed cells and recognizing self-antigens while preserving the ability to defend the unprecedented cells that could be harmful to the body system. Accordingly, there are several adult stem cells that are required in this treatment (e.g. hematopoietic stem cells and mesenchymal stem cells) (Choi 2009).

### 3.4.1 Hematopoietic stem cells

Hematopoietic stem cells have been implemented as a treatment to cure the autoimmune disease since 1990 (Choi 2009). The intention of the transplantation is to destroy the defective cells and generate a proper immune system. To exemplify, the hematopoietic stem cell was injected into the body so that the body bone marrow could release the stem cell through the bloodstream. Then, after the dysfunction cells have been through

a cytotoxic process (the process where the cells are destroyed), the patient will gradually recover through the self-healing process. There were numerous trials being placed among several types of patients with autoimmune disease and the results were not completely positive; there were several deaths and some patients were persistent from stem cell transplantations. Nevertheless, the overall outcome was quite pleasurable as the success rate still greatly outweighs the mortalities in most types of autoimmune disease patients (Choi 2009)

### 3.4.2 Mesenchymal stem cells

Alternatively, with protective and anti-inflammatory abilities, mesenchymal stem cells are another source of adult stem cells that can be implemented in cellular-based therapy. However, the concept of transplanting mesenchymal stem cell and hematopoietic stem cell is quite different; hematopoietic stem cell transplantation requires the patient to be immunosuppressed in order to acquire transplantation while mesenchymal stem cell has the potential to inhibit unsophisticated memory and activated necessary cells for example T cells, B cell, NK cells, and dendritic cells in order to correct mismatched lymphocytes. Moreover, mesenchymal stem cell transplantation also has additional benefits for patients with systemic lupus erythematosus(SLE): bone marrow reconstruction and multiorgan dysfunction correction (Choi 2009).

### 3.4.3 Adipose tissue-derived mesenchymal stem cells

With some limitations of mesenchymal stem cells (e.g. abundance and availabilities), the scientists are developing adipose tissue-derived mesenchymal stem cells (AT-MS) purposely for it to substitute the mesenchymal stem cell in the transplanting process as it is easier to obtain. Even though AT-MS is now applicable mainly for regenerative treatment and wound healing, the AT-MS itself also contains immunosuppressive qualities which scientists hypothesize that it could act as a substitution for MSC so that the transplantation treatment for autoimmune diseases could be done with fewer difficulties (Choi 2009).

### 3.4.4 Autoimmune diseases

1. Multiple Sclerosis: Multiple sclerosis is concerned as a demyelinating disease in which the spinal cord and nerve system in the brain have been damaged by the activation of T cells. As a result, it may produce a variety of neurological symptoms that can worsen over time. Currently, the disease cannot be cured yet but the new advancement in technology and therapeutic like autologous hematopoietic stem cell transplantation have allowed us to slow the multiple sclerosis progression.



2. Autoimmune Cytopenias: The autoimmune disorder that could lead to thrombocytopenia and bleeding as a consequence of the decline in platelet level. According to the experiment, the treatment strategies remained to be diverse for the scientist to discover the best source for stem cell transplantation.
3. Crohn's Disease: Currently, there is no clear explanation of what causes Crohn's disease but it is thought to be a consequence of the abnormal activation of the helper T-cell in the gastrointestinal tract. Thus the patient who is diagnosed with Crohn's disease usually experienced stomach pain and diarrhea. Although there is no surgical way to cure the disease, the new medication can now be associated. The latest research shows that stem cell therapy can effectively decrease the negative immune response.

### 3.5 Cancer treatment

Generally, the human body is made up of trillions of cells that will grow and replicate to construct new cells which perform different functions in the body. Cancer is a disease caused by certain DNA changes to when the cells replicate themselves. The individual has a different set of genetic mutations as a consequence of certain environmental exposures. Although the body's immune system would normally get rid of abnormal or damaged cells, cancer has the ability to elude the protection and change the surrounding microenvironment. In addition, there are more than a hundred types of cancer whereby they are usually named it from the area of occurrence (eg. breast cancer, brain cancer, ovarian cancer). The scientists have found that stem cells can contribute to the procedure of cancer and autoimmune diseases treatment (National Cancer Institute 2015).

#### 3.5.1 Cancer

1. Breast Cancer: In the last few decades, more than a million women have suffered from breast cancer. The new advancement in the medical field has developed various options to cure the disease, for example, chemotherapy, radiotherapy, surgery, endocrine therapy. However, allogeneic hematopoietic stem cell transplant has also been introduced as an option to a more effective procedure to reduce the relapse of cells (Hawsawi, Al-Zahrani, Mavromatis, et al. 2018).
2. Lung Cancer: The most common type of lung cancer is small-cell lung carcinoma or SCLC. The frequently used treatment options for this are chemotherapy and radiotherapy. Recently, the combination of chemotherapy and autologous hematopoietic stem cell transplant is commonly used in order to

reduce the chance of relapse (Hawsawi, Al-Zahrani, Mavromatis, et al. 2018).

3. Leukemia: Leukemia and lymphoma are resulting from the uncontrollable rise of white blood cells. The procedure of chemotherapy and radiotherapy has been replaced by the use of bone marrow or hematopoietic cell transplantation (Hawsawi, Al-Zahrani, Mavromatis, et al. 2018).

Presently, the theory of cancer stem cells is controversial as its affirmation is based on the experiments of a particular cell to illustrate that it does feed tumors within the body. Thus the cells would be able to cure the patients. However, if it leaves cancer stem cells behind after the therapy then it could relapse and cause tumors to grow again. There are many questions for scientists to be explored for future cancer therapies (Hawsawi, Al-Zahrani, Mavromatis, et al. 2018).

### 3.6 Diabetes

Diabetes is a common chronic disease that can be categorized into 2 main types: type 1 diabetes and type 2 diabetes. Currently, type 1 diabetes is the only type with qualified treatment implementing regenerative tissues; although the particular research seems to be beneficial to people with diabetes, patients with type 1 diabetes are not the majority cases compared to the widespread amount of people with type 2 diabetes; type 1 diabetes is considered to be an autoimmune disease and normally associates with juveniles while type 2 diabetes cases are common in adults and are mostly caused by improper habits such as consuming diets that lead to obesity (Centers for Disease Control and Prevention 2020).

Patients that are experiencing type 1 diabetes will lose the pancreas' capability to produce sufficient amounts of insulin; insulin is responsible for enabling sugar molecules in the bloodstream to enter the process of cellular respiration. Therefore, stem cell therapy is one of the treatments recommended as it has the ability to reverse and cure diabetes in the long-term period (Centers for Disease Control and Prevention 2020).

There are several approaches toward the patients in tissue regenerative medications: growing cells, direct differentiation, reprogramming, and self-regulation (Harvard Stem Cell Institute 2020).

1. Growing Cells: In this approaching process, the researchers aim to utilize embryonic stem cells as a source to produce pancreatic endocrine cells, then expand the quantity of cells by a special bioreactor. Unfortunately the differentiation of pancreatic endocrine cells into beta cells, which is a crucial part

in insulin production and secretion, has not yet been confirmed that it is possible in humans. However, if this approach has succeeded, it could verify that the patients would get the precise amount and type of cell.

2. **Direct Differentiation:** This approach is quite similar to the first one, nevertheless, it requires different types of pancreatic cells: pancreatic acinar (digestive) cells. This method is confirmed to be successful in the treatment process by differentiating the pancreatic acinar cells into beta cells and stimulating the process of insulin production. Therefore, apart from implementing this approach towards diabetes treatment, the scientists are currently adapting the particular theory to different cells in the body (e.g liver cells).
3. **Reprogramming:** This approach implements the reprogramming techniques with extracted cells from patients; it aims to produce induced pluripotent stem cells (iPS) and convert them to beta cells. However, even though numerous trials have been experienced in the laboratory, the conversion from iPS to beta cells is not yet applicable to humans.
4. **Self-regulation:** Differently, instead of evolving other types of cells into beta cells, this approach aims to stimulate the intensification of beta cells by themselves; it could be done through the process called self-replication. Correspondingly, after the scientists have examined the process closely, the beta cells do replicate themselves in the pancreas but with an extremely slow rate; some researchers are discovering medications that would enhance the replication rate.

Following the approaches of stem cell transplantation, immunologists and bioengineers are concerned about the possible destruction of transplanted cells from the body immune system. Hence, they invented semi-permeable membranes for the cells using cellular engineering deliberately to strengthen them; the membranes act as gateways to authorize specific molecules to proceed the cell (i.e. glucose and insulin) and inhibit the entrance of immune cells (Harvard Stem Cell Institute 2020)

## 4. Methods of checking stem cells

### 4.1 Checking embryonic stem cells

The research of stem cells in labs is vital for the intuition gained on how diseases and certain medical conditions develop (Mayo Foundation for Medical Education and Research (MFMER) 2019). By observing how stem cells derive, transform, and mature into certain types of

tissues produced in a human body allows researchers to understand functions of stem cells and reasons why many of the adverse infections or diseases occur (Stanford Medicine 2020). Before using these stem cells for tests or therapy, researchers have to learn how to grow them in a lab and check to ascertain that they exhibit the characteristics and functionality of the desired replaceable cells. Methods of checking stem cells are important factors in making sure that the stem cells not only divide correspondingly to the patients' needs but also do not become malignant or show signs of possibilities to cause auto-immune diseases.

Scientists need to come up with a process to test and check embryonic stem cells for the exhibition of their fundamental functional features. Although the process is not yet fully standardized, some tests are used to identify the necessary properties. Specific techniques and procedures are carried out to identify cell surface markers and transcription factors, of which are generated by undifferentiated stem cells. Other checking methods include growing, subculturing, examining these cells under a microscope, and testing for their pluripotency, which is defined as the ability of a cell to generate all of the cell types of an organism .

1. **Growing and Subculturing:** Checking embryonic stem cells can be done by growing and subculturing them for many months. This is done to eradicate early problems associated with the cells' inability to self-replicate and maintain a prolonged development. Once these issues are decimated by observing these cells under a microscope, long-term growth of stem cells and their self-renewal capability will be achieved if they remain healthy and undifferentiated during the culture. Furthermore, these embryonic stem cells can be checked for eligibility if they are experimented to have the capacity to regrow and be subcultured after going through freezing, thawing, and re-plating.
2. **Observing:** Identifying the functionality and completeness of embryonic stem cells involves observing these stem cells under a microscope. Because during the subculturing process the chromosomes in stem cells might be damaged and mutated, a microscope can help magnify them for a thorough and ensuring assessment. If any dubious damage or a change in the number of chromosomes can be seen, those embryonic stem cells are likely incapable of being used. Though, this process does not ascertain the genetic mutations that might have occurred among stem cells.
3. **Surface Markers:** Checking these pluripotent stem cells involves techniques to identify particular cell surface markers, which are normally constructed by embryonic stem cells. Antigens which are capable of

isolating and identifying embryonic stem cells, which can be discovered on human embryonal carcinoma cells, include TRA-1-60 and TRA-1-81 (Andrews, Banting, Damjanov, et al. 1984).

4. **Transcription Factor:** This method uses specific techniques to inspect on the presence of transcription factors whether they are present on the undifferentiated cells. To exemplify, octamer-binding transcription factor 4, or OCT4, and NANOG are the crucial transcription factors located on pluripotent stem cells. These transcription factors are important due to its significance in turning the genes on and off at the right moment. In this case, both NANOG and OCT4 are responsible for self-renewability of stem cells by maintaining them in an undifferentiated state. In addition, NANOG has been seen to be expressed during the development of malignant cancer cells. Embryonic stem cells and cancer stem cells, a subpopulation of cancer cells within the tumor, therefore, are considered to possess similar phenotypes. The correspondence between the two suggests that NANOG influences the process of carcinogenesis and is possibly a marker for malignant tumors (Gawlik-Rzemieniewska 2016). In contrast, OCT4 is the central composition important for stem cells' pluripotency that drastically decreases in presence during differentiation. This transcription factor is considerably crucial for the re-establishment of pluripotency in embryonic stem cells (Shi and Jin 2010).

#### 4.2 Checking adult stem cells

Contrarily, compared to the embryonic stem cells, scientists implement steps of procedures in order to disseminate the eligibility of adult stem cells; they can replicate via cell culture process. Additionally, the procedures can be used to prove the accurate evolution capability of adult stem cells. Also, to make sure of the candidate stem cells' functionality, the purified population of them is transplanted into a human or animal to see whether they repopulate or not. If they repopulate to produce identical cell types, then they are qualified .

1. In the first method, the cells in a living tissue are labeled with molecular markers. Later, scientists would be able to identify what specialized cells they generate by separating those without markers from those with markers.
2. On the other hand, the second method requires the removal of the cells from a living animal. After that, they are labelled, put in a culture, and transplanted back into another animal, such as a mouse. Whether the cells repopulate into the correct identical cell type is the determining factor in this method of evaluating adult stem cells.

#### 4.3 Preservation of stem cells

Preserving stem cells means the collection and cryopreservation of stem cells which is from Umbilical cord blood and also Umbilical cord tissue for future application (Cells4Life).

This is necessary for tissue regeneration and its medical applications; it allows the cell banks to develop among protein-coding genes found on the cell surfaces which help the immune system in recognizing unfamiliar substances. In humans, the system is called the human leukocyte antigen system (HLA) (Hanna and Hubel 2009).

In order to predict or operate, the collection of stem cells from numerous resources comes with difficulties, but the preservation process permits the cells storage until later use. Furthermore, the fulfillment of safety and quality trials along with cell movement between sections (i.e. section of compilation and processing) are authorized before utilization.

##### 4.3.1 Reasons to preserve stem cells

Since stem cells are similar to other kinds of cells, they can be easily succumbed to the external environment. Cells need a proper environment and also sufficient energy. So these are the two main reasons why cells can not survive. However, a scientist stated that if stem cells are frozen and preserved properly, they can stay alive for years.

##### 4.3.2 Where umbilical cord blood stem cells are stored (WebMD 2020)

Since stem cells provide a load of benefits, people usually reserve their stem cells for their future use. These are some places where people store their stem cells.

1. Public cord banks - free of charge and donation is available for people who are in need.
2. Private cord banks - store the blood for use only in family and charge storage fee annually
3. Direct-donation banks - are an association of public and private cord banks. They store blood for public utilize and likewise store for family use.

##### 4.3.3 How long stem cells can be reserved (Cells4Life 2014)

The maximum preservation period of umbilical cord stem cells is still a moving target. As cord blood banking has been in progress for 25 years and there is no scientific



information which indicates that stem cells can be accumulated for more than that. Nevertheless, from the latest data, scientists illustrated that these preserved stem cells have no expiration date.

#### 4.3.4 Process of preserving stem cells (Cordlife Sciences 2020)

1. Step 1: Collect the stem cells by cutting a sector of the baby's umbilical cord cell and storing them in a sterile container.
2. Step 2: The collected stem cells are sent to the laboratory for processing the identity of stem cells to make sure that every unit belongs to the exact client.
3. Step 3: Next, all the other components were removed from the stem cells, leaving only cord lining. Then utilize a wash buffer solution to clean the cord lining and divide it into small pieces.
4. Step 4: After that, add the cryoprotectant solution into the decontaminated parts; it will be transferred into cryovials as a viability protection throughout the cryopreservation process.
5. Step 5: Then, at one time when the cryoprotectant solution was added. The cord lining sectors have to be frozen in the controlled-rate freezer. At the temperature lower than 1-2 celsius to preserve the cell viability.
6. Step 6: Lastly, after the controlled-rate temperature procedure, the cryovials are changed into a MVE anti-contamination. Consequently, long-term store the product at -190 degrees Celsius in a particular storage system: a vapour-phase liquid nitrogen.

## 5. Potential applications and limitations

Stem cells have made a considerable impact in the field of science, representing the future of regenerative medicine. The study has shown that stem cells contribute to the treatment of many deadly diseases intertwined with the degenerative or malfunctioning cells. Hence, many biotechnology organizations and researchers around the globe have conducted studies and experiments on stem cells to acquire a better understanding of the subject by exploring its applications and limitations.

The potential of stem cells is theoretically limitless; they are the building blocks of life, and an entire human body is developed from the rudimentary stem cells. However, intensive research is required before the promise of stem cells can be approved for therapeutic uses in the clinic. Therefore, clinical studies and treatment attempts

have been gathered in order to predict the possible applications of stem cells.

### 5.1 Drug testing (Cynober 2018)

Cells that differentiate from the stem cell lines allow the drug testing process to be safer and more convenient. For example, cancer cells are also used to test the effectiveness of anti-tumor drugs. Pluripotent stem cells, possessing the ability to develop into various types of cells, would allow for the greater range of drug testing. Nevertheless, the condition for the drug testing process to be successful is relatively strict and controlled. Therefore, researchers need to be able to control the product of each cell differentiation precisely in order for this method to be possible.

### 5.2 Cell-based therapies

#### 5.2.1 Heart disease

There are no natural means of regenerating cardiac tissue. Therefore, the only option for people with severe myocardial infarction is heart transplantation. Scientists have attempted to use mesenchymal stem cells in treatments, but long-term effects are not so positive. However, embryonic stem cells from cardiac cells have been founded to be able to rebuild cardiac cells after infarction in rodents, even though experiments on primates show some complications (Carfagno 2019).

#### 5.2.2 Diabetes

Type I diabetes is a condition when the immune system erroneously attacks the cells producing insulin, hormone regulating the amount of glucose in the blood. Insulin-producing cells are not capable of healing themselves, however, according to recent experiments on rodents, they have been reported to be formed by the use of embryonic stem cells both inside and outside of the test-tube. Transplantation on the human subject, however, is yet to be approved of being useful (International Society for Stem Cell Research (ISSCR) 2020).

#### 5.2.3 Neural tissue

Like beta-cells, neural tissue does not actively regenerate. Once the brain gets damaged, the natural healing process cannot be depended on. However, studies in mice have reported that neuron deriving from pluripotent stem cells could be an effective treatment against Parkinson's disease (Panchision 2016). Further potential applications of other types of stem cells are being studied by researchers.

Stem cells provide many honored achievements in the medical field. Nonetheless, the technology still possesses

certain limitations. According to the current medical knowledge and academic testing, the instances of stem cell limitations include the potential immune rejection, low number of muscle cells, and limited capacity of CSCs.

1. **The Potential Immune Rejection:** The patient receiving treatment from the embryonic stem cells may have transplant rejection resulting from the receiver's immune system; the genome of the donor and the recipient are different. Hence, during the process of stem cell implants which utilize the stem cells of an embryo then deliver the cells to the patient, the immune system might reject; it can lead to the possible failure of the process (Sun, Zhang and Sun 2014).
2. **Low Number of Muscle Cells:** The research suggests that embryonic stem cells can only generate small amounts of muscle cells, which is a boundary for scientists to break (Sun, Zhang and Sun 2014).
3. **The Limited Capacity of CSCs:** Earlier, scientists opined that the heart muscles could not be renewed until the cardiac stem cells (CSCs) were introduced. CSCs can produce muscle cells and vessels. Still, the capacity of the cardiac stem cells is too limited; in other words, it is insufficient for therapeutic use (Sun, Zhang and Sun 2014).
4. **Wound Healing:** The intensive studies demonstrate the possibility of using induced pluripotent stem cells to heal the wound. However, there are not enough research and safety protocols for the process. The process of generating induced pluripotent stem cells with non-technology is still not unproductive and ambiguous (Gorecka, Kostiuk, Fereydooni, et al. 2019).

## 6. Ethical concerns

In the past decades since stem cells were discovered, many scientists started to pay more attention to stem cell research. In the year 1999, certain ethical issues in stem cell research were brought to a consideration in a meeting; there are many different perspectives on collecting stem cells in terms of ethical concerns. This creates an ongoing debate about the potential negative effects of science in the society. The common concerns are mainly about human cloning, the commodification of human organs, the hybridization of human and animal species, and the aim for regenerative endurance.

### 6.1 Human cloning

Cloning is a method of creating genetically identical copies of a cell, a tissue, or an organism with desired traits. There are several types of cloning; take animal

cloning as an instance. In 1997, the researchers published the delivery of Dolly in Scotland, a sheep that was cloned by nuclear transplant from a differentiated cell. They performed the vital dedifferentiation of a mammary cell donor nuclei by culturing cells in a fertile environment. The cultured mammary cell fused with enucleated sheep eggs from egg cell donors. Consequently, the resulting diploid cells arranged to create initial embryos that were embedded in the uterus of the surrogate parent. As the development was successfully completed and normal, Dolly was born. Later analysis showed that Dolly's chromosomal DNA was certainly identical to the nucleus donor.

Although the use of animal and plant clonings is currently acceptable, it is a very different case for humans due to the fact that humans are different from other organisms in terms of anatomy and rights; an example of differences in anatomy is in the brain. Collaborative research from Weizmann Institute of Science in Rehovot, Israel, and Itzhak Fried of the University of California, Los Angeles, shows a difference in neurons in the brain of humans and other primates. The researchers proved that human neurons have greater efficiency in processing information compared to other primates; this result might help us clarify the unique intelligence in humans (Abbott 2019).

Animal cloning is a process which frequently comes with failures; the scientists are well-aware that human cloning could possibly generate failures as well. It took several years along with numerous attempts to successfully clone a monkey, one of the closest human relatives in the animal kingdom, in 2018. From trials and errors, many primates had to sacrifice their lives for the experiment (Kolata 2001). Consequently, if human cloning is to be done, many human beings would have died from the experiment as well; in terms of individual rights, concerns among the society could possibly be about human health and safety, dehumanization, and the definition of the ancestor. Thus, some people might not accept cloned humans as human beings. Therefore, it may lead to the system of slavery and discrimination. Moreover, the definition of parent would also be affected by the evolution of cloning and confusion might occur whether or not clones should be considered in the family tree.

### 6.2 Commodification of human organs

During the developments in pharmacological, surgical, and public health have been unprecedented advances in health care. While many medical unsolved problems such as genetic abnormalities and damage and dysfunctional tissues or organs were partly solved, they still have not successfully fulfilled human's desire. Recently, a new regenerative medicine has generated significant interests

among people; it aims to repair or replace dysfunctional, diseased, or dead body parts. This discovery is applicable to mammalian stem cells by using chemicals in order to induce those cells in various tissues. Therefore, it is theoretically also possible to apply to human cells as replacements for body parts.

Although those stem cells are acceptable, commercialization could not be allowed. To illustrate, the patent on stem cells and the sale of stem cells products such as tissue or organs should not be authorized. In Europe, politicians and the media disagreed with the European Patent Office's (EPO) decision for authorizing the patent to the University of Edinburgh for isolating genetic engineering on stem cells; European patent guidelines prohibit the patent of genetically engineered human stem cells. Especially when the university's patent used the word "animal", it led to many arguments from the protestors saying that the process would have been implemented in humans as well. (Hangman, 2000) This issue helped to urge public opposition to the commercialization of stem cells in Europe. Currently, many biotech companies and traditional pharmaceutical companies have enrolled and invested a lot of money in the stem cell industry. In some countries, it is illegal to sell or patent embryos. And in many countries, selling human organs is also prohibited. The rationale behind this is the uniqueness in our bodies; there is the distinctiveness in reflection, expression, action, reception, and relation. Therefore, bodies are priceless and should not be commercialized as if they are properties.

### 6.3 Hybridization of human and animal species

Studies show that stem cells can be used for hybridization or crossing over between species, including the hybridization between humans and animals; hybridization is a process of crossbreeding between organisms from different species (Wittler 2020). Recently in 2019, a Japanese stem-cell scientist named, Hiromitsu Nakauchi, has created a government funded experiment where embryos from rats and mice were generated from human cells and inserted into selected animals; the main goal is to create animals with human celled organs which can replace the limited human organs. A committee of experts in the science ministry approved these human-animal hybrid embryos to be officially the first in the world (Cyranoski 2019).

Some bioethicists are concerned about the scattering of cells beyond the intention due to ethical issues. Perhaps, the developing animal's brain and its cognition might be affected by the roaming cells. However, the scientists claim that the experiment design process has already considered the possible concerns. Although there are risks of conducting this innovative experiment, it would be a helpful advancement in the medical industry.

### 6.4 Regenerative endurance

The utilization of stem cells and related developmental medication is recently problematic due to their exaggerated commercials, bringing moral threats to the hospitals; stem cells that dissimulate anti-aging remedies imply a case of unlawful therapeutics that are undeviatingly conferred to victims. In non-human research, these treatments hold a pointed consequence in aging by building a life extent and repairing torn devices such as brain injury in Alzheimer. While those bits of knowledge experiments resemble encouragement, the scientists are still remote from owning quality proof. At the same time, unverified and harmful therapies on anti-aging and rejuvenating are already widespread by clinics at the exorbitant cost to the patient. Consequently, this innovative treatment is approachable for both health conditions treatment and beauty purposes.

However, even though a few hospitals do have stem cell treatment, they tend to prioritize safety and feasibility rather than only meeting the patients' desires. Accordingly, many patients are indeed ignorant and affluent. Thus, immoral clinics and clinicians take this opportunity by fleeing from regulation; they export their treatments across the country. Hence, medical tourism is increasing. To illustrate, Dr. Robert Trossel has been fired from the United Kingdom membership by the General Medical Council because he delivered untruthful diagnoses that require regenerative stem cell therapy; for example, the requirement for sclerosis. Then, he charged the patients up to 12,000 pounds which was declared by the General Medical Council to be aimless and illogical. In South Korea, Dr. Hwang woo-Suk who had succeeded in creating human embryonic stem cells via cloning was charged with fraud and bioethics law violations by using eggs from his students and from the black market. After finding his embezzlement, he was sentenced to a 2 years suspended prison at Seoul.

In conclusion, there are both benefits and downsides to the use of stem cells. This is what created concerns and debates about stem cells and their applications. Some common ethical concerns are about cloned humans, turning human organs into commodities, human-animal hybrids, and the use of self-regeneration. In the future, these concerns among the people might be lessened as the process is more transparent and people are becoming more understanding.

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