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Review Article

STEM CELL: A REVIEW

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ABSTRACT

Stem cell research holds great promise for improving human health by control of degenerative diseases and restoration of damage organs by various injuries. Though this technique is very complicated and success rate is low, the clinical trials that followed have shown satisfactory results. Many therapies based on stem cells are designed today to treat successfully diseases like Parkinson's disorder, Diabetes, Alzheimer's disease, Cardiac disorders, Obesity, etc. This review attempts to give brief overview on stem cells and various stem cell based researches going on in different regions of the world.

Key words: Stem cell, Stem cell therapy, Embryonic stem cells, Adult stem cells

INTRODUCTION

With the discovery of DNA, in the 1990s biological researchers took a big leap in the field of molecular biology. Even though most prestigious project of biology completed today i.e. 'Human Genome Project' the field of therapy still has numerous unsolved mysteries ranging from treatment of common cold to HIV. The issue of stem cell research burst on the scientific scene of embryonic stem and embryonic germ cells, which offer great promise for new ways of treating diseases. Perhaps this is because stem cells are the master organizers of all living multicellular organisms, giving rise to every tissue in the body [1].

Stem cells are "unspecified cells or undifferentiated cell". These cells have the ability to give rise to many different cell types like skin, liver, kidney, heart, nerve cells etc [2, 3]. Scientists work with two kinds of stem cells from animals and humans. These are, (1) Embryonic stem cells (ESC's), are pluripotent derived from the inner cell mass (ICM) of the blastocyst, an early-stage embryo. Human embryos reach the blastocyst stage 4–5 days post fertilization, at which time they consist of 50–150 cells [4] (Fig. 1), and (2) Adult stem cells (ASC's) or somatic stem cell found throughout the body after embryonic development. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found. The ASC's can be found in bone marrow, blood stream, cornea and retina of the eyes, liver, skin etc.

All the these stem cells have unique capabilities to self-renew, grow indefinitely, and differentiate or develop into multiple types of cells and tissues but they all are found in very small populations. Therefore, it is very difficult to identify and examine them under a microscope as they look like any other cell in the tissue where they are found. Scientists use "stem cell markers" to identify these rare types of cells found in many different cells and tissues (Fig. 2).

What are stem cell markers? Every cell in the body carries specific proteins on their surface called receptors, which are differing in their structure and have the capability of selectively binding to signaling molecules. Normally, cells use these receptors and the molecules that bind to them as a way of communicating with other cells and to carry out their proper functions in the body. These same cell surface receptors are the stem cell markers. Researchers use the signaling molecules that selectively adhere to the receptors on the surface of the cell to identify stem cells. Many years ago, a technique was developed to attach to the signaling molecule to another molecule (or the tag) that has the ability to fluoresce or emit light energy when activated by an energy source such as an ultraviolet light or laser beam [5].

Two techniques are use to identify specific populations of stem cells. (1) Fluorescence-activated cell sorting (FACS) [6-8], a suspension of tagged cells (i.e., bound to the cell surface markers are fluorescent tags) is sent under pressure through a narrow nozzle, so cells must pass through one at a time. Upon exiting the nozzle, one by one cells are pass, through a light source, usually a laser, and then through an electric field. The fluorescent cells become negatively charged, while non-fluorescent cells become positively charged. The charge difference allows stem cells to be separated from other cells (Fig.3), and (2) Visual assessment, a thin slice of tissue is prepared and the stem cell markers are tagged by the signaling molecule that has the fluorescent tag attached. The fluorescent tags are then activated either by special light energy or a chemical reaction. The stem cells will emit a fluorescent light that can easily be seen under the microscope.

Recently, researchers have applied a genetic engineering approach that uses fluorescence, but isn't dependent on cell surface markers. The importance of this new technique is that it allows the tracking of stem cells as they differentiate or become specialized. Scientists have inserted into a stem cell a "reporter gene" called green fluorescent protein [9]. The gene is only activated or reports when cells are undifferentiated and is turned off once they become specialized. Once activated, the gene directs the stem cells to produce a protein that fluoresces in a brilliant green color (Fig.4). Researchers are now coupling this reporting method with the FACS and microscopic methods described earlier to sort cells, identify them in tissue and now track them as they differentiate or become specialized.

KEY STEM CELL RESEARCH EVENTS

- **1908:** The term stem cell was proposed for scientific use by the Russian histologist Alexander Maksimov at congress of hematologic society in Berlin.
- **1960:** Scientific evidence presented by Joseph Altman and Gopal Das on adult neurogenesis, ongoing stem cell activity in the brain.
- **1963:** McCulloch and Till illustrate the presence of self-renewing cells in mouse bone marrow.
- **1968:** Bone marrow transplant between two siblings successfully treats severe combined immunodeficiency (SCID).
- **1978:** Haematopoietic stem cells are discovered in human cord blood.
- **1981:** Mouse ESC's are derived from the ICM reported by G. R. Martin and his team.
- **1992:** Neural stem cells are cultured *in vitro* as neurospheres.
- **1998:** James Thomson derives the first human embryonic stem cell line [16].

- **2001:** Scientists at Advanced Cell Technology clone first early (four to six-cell stage) human embryos for the purpose of generating embryonic stem cells [17].
- **2003:** Dr. Songtao Shi discovers new source of ACS's in children's primary teeth [18].
- **2004-2005**: Hwang Woo-Suk claims to have created several human ESC's lines from unfertilised human oocytes.
- **2006:** Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors [19].
- **2006:** Scientists in England create the first ever artificial liver cells using umbilical cord blood stem cells.
- **2007:** Scientists at Wake Forest University led by Dr. Anthony Atala and Harvard University report discovery of a new type of stem cell in amniotic fluid [20].
- **2007:** Research reported by three different groups shows that normal skin cells can be reprogrammed to an embryonic state in mice [21].
- **2007:** Shoukhrat Mitalipov reports the first successful creation of a primate stem cell line through somatic cell nuclear transfer [22].
- **2007:** Human induced pluripotent stem cells: Two similar papers released by their respective journals prior to formal publication: in Cell by Kazutoshi Takahashi and Shinya Yamanaka, "Induction of pluripotent stem cells from adult human fibroblasts by defined factors [23] and in Science by Junying Yu, et al., from the research group of James Thomson, "Induced pluripotent stem cell lines derived from human somatic cells [24] pluripotent stem cells generated from mature human fibroblasts.
- **January 2008:** Human embryonic stem cell lines were generated without destruction of the embryo [25].
- **January 2008:** Development of human cloned blastocysts following somatic cell nuclear transfer with adult fibroblasts.
- **February 2008:** Generation of Pluripotent Stem Cells from Adult Mouse Liver and Stomach: these induced pluripotent stem cells (iPS) seem to be more similar to embryonic stem cells than the previous developed iPS cells and not tumorigenic, moreover genes that are required for iPS cells do not need to be inserted into specific sites, which encourage the development of non-viral reprogramming techniques [26].
- **March 2008**: The first published study of successful cartilage regeneration in the human knee using autologous adult mesenchymal stem cells is published by Clinicians from Regenerative Sciences [27].
- **October 2008:** Sabine Conrad generate pluripotent stem cells from spermatogonial cells of adult human testis by culturing the cells *in vitro* under leukemia inhibitory factor (LIF) supplementation [28].
- **30 October 2008**: Embryonic-like stem cells from a single human hair [29].
- **05 March 2009**: Australian scientists find a way to improve chemotherapy of mouse muscle stem cells.
- **28 May 2009**: Kim et al. announced that they had devised a way to manipulate skin cells to create patient specific "induced pluripotent stem cells" (iPS), claiming it to be the ultimate stem cell solution [30].
- **09 March 2009:** US President Obama lifted federal funding limits on human embryonic instituted by former President Bush.
- **11 October 2010:** First trial of embryonic stem cells in humans [31].

STEM CELL THERAPY

Many medical researchers believe that stem cell treatments have the potency to change the face of human disease and alleviate suffering. The ability of stem cells to self-renew and give rise to subsequent generations that can differentiate offers a large potential to culture tissues that can replace diseased and damaged tissues in the body, without the risk of rejection. Medical researchers anticipate one day being able to use technologies derived from adult and embryonic stem cell research to diverse range of diseases.

Stem cell and diabetes

Diabetes is characterized by abnormally high levels glucose in the bloodstream. This excess glucose is responsible for most of the complications of diabetes, which include blindness, kidney failure, heart disease, stroke etc. Type 1 diabetes or juvenile-onset diabetes, typically affects children and young adults. It develops when the body's immune system seen islet cells as foreign and attacks and then destroys them. As a result, the islet cells of the pancreas, which normally produce insulin, are destroyed. In the absence of insulin, glucose cannot enter the cell and glucose accumulates in the blood. Type 2 diabetes or adult-onset diabetes, tends to affect older and occurs when the body cannot use insulin effectively. This is called insulin resistance diabetes and the result is the same as with type 1 diabetes.

A team of scientist in Brazil has published the results of a study on 23 patients of type 1 diabetes (aged 13-31 years) treated with a new diabetes treatment using the patient's own stem cells. The doctors collected blood from the patients and isolated stem cells called CD34, which can differentiate into white blood cells. Around the same time, chemotherapy is used to partly destroy the patient's own bone marrow cells. Afterward, each patient received his own stem cells back by injection. The scientists traced blood levels of C-peptide, produced by beta cells, in order to confirm that whatever remaining beta cells in patient were now able to grow again and repopulate the pancreas and produce insulin. The results show that 12 of the patients now life free of insulin. Their pancreas, no longer being attacked, can now make its own insulin. 8 people need less insulin than before and only 3 patients show no benefit at all. These stem cell transplants apparently work by 'resetting' the immune system so that the body stops attacking the pancreas. The researchers themselves say that this treatment can only be used when the condition is caught early enough (within six weeks of diagnosis), before the pancreas has been irreversibly damaged [32]. The university of Miami Diabetes Research Institute conducted the successful pilot study with 25 patients of type 2 diabetes. Once researchers removed the stem cells from the patient's bone marrow, it was purified, concentrated and then injected into arteries near the pancreas. Researchers then put the patients into hyperbaric oxygen chambers and subjected them to 10 hours of pure oxygen at 2.4 times the atmospheric pressure at ground level. Researchers believe the high-pressure oxygen pulled extra stem cells from the patient's bone marrow, adding to the stem cells injected near the pancreas and the immature stem cells developed into pancreatic cells, regenerating the pancreas's ability to produce natural insulin. After treatment, symptoms significantly lessened, with increased insulin production, lower blood-sugar levels and a reduced need for those dreaded insulin injections [33].

Table 1: Markers used to identify ASCs in various tissues and organs

Cell type	Markers	Significance	Reference
Embryonic stem cell and embryonic carcinoma	Oct-4, germ cell nuclear factor	Essential for the establishment and maintenance of PSC'S	Bongso et al [15]
Ectodermal, neutral and pancreatic progenitors	Nestin, vimetin	Found during the formation of neuro ectoderm	Herzenberg & de rosa [7]
Endoderm	α-fetoprotein, GATA-4	Endoderm differentiation	Shamblott et al +
Mesoderm	Bone morphogenetic protein-4 brachyury	Formation and differentiation of mesoderm	Itskovitz-Eldor et al [3]

Table 2: Markers (ESC's) used to identify pluripotent stem cell.

Tissue/organ	Cell type	Marker	Significance	Reference
Blood vessel	Smooth muscles	Cell specific myosin heavy chain, cadherin	Indentifies smooth muscle cells in blood vessel walls	Jackson et al [10]
Bone	Osteoblasts	Hydroxyapatite	Marker in bone formation	Herzenberg & De Rosa [7]
Liver	Hepatocytes	Albumen	Indicates functioning of fully differentiated hepatocyte	Alison & ponlson [11]
Skin	Epidermal cells	Keratin/pigment	For various skin damages due to burns or accidents	Yamane et al [12]
Nervous system	Neurons	Neutral tubulin	Identifies differentiated	Woodbury et al [13]
Blood	WBC	CD4 and CD 146	Importance in hematopoiesis	Shamblott et al [14]

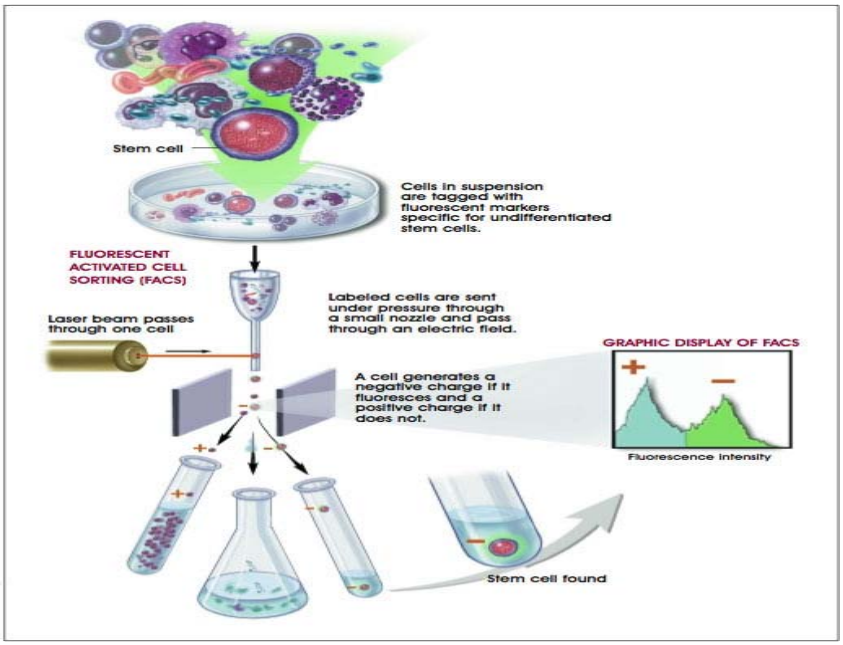

Fig. 3: Various steps involved in identification of stem cell

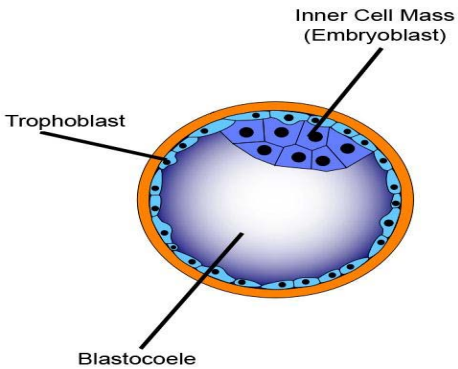

Fig. 1: The blastocyst (a hollow sphere made of approximately 150 cells and contains three distinct areas: the trophoblast, which is the surrounding outer layer that contains the trophoblast stem cells and later becomes the placenta, the blastocoel, which is a fluid-filled cavity within the blastocyst, and the inner cell mass, also known as the embryoblast, which can become the embryo proper, or fetus, and is where human embryonic stem cells are isolated from).

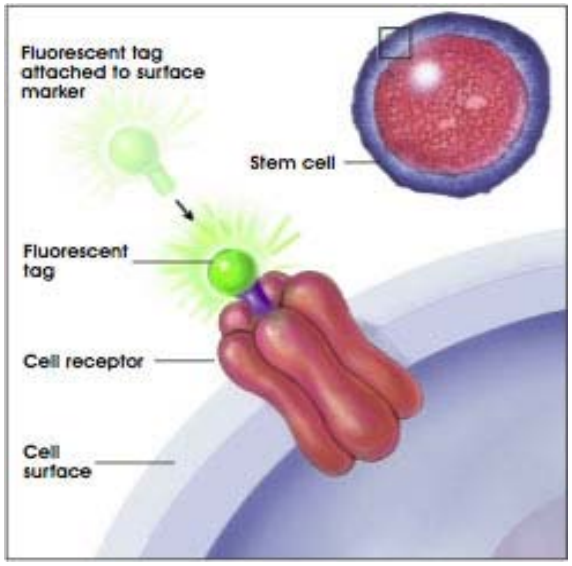

Fig. 2: Identifying Cell Surface Markers Using Fluorescent Tags

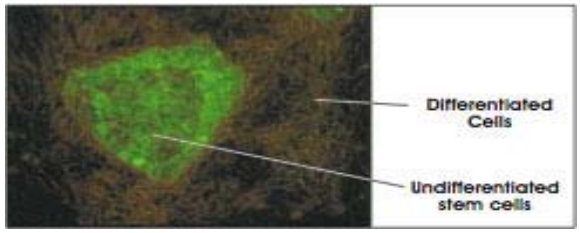

Fig. 4: Microscopic Image of Fluorescent-Labeled Stem Cell

Stem cell and spinal cord injury

This is a major cause of paralysis and the associated trauma destroys numerous cell types, including the neurons that carry messages between the brain and the rest of the body. In many spinal injuries, the cord is not actually severed and at least some of the signal carrying nerve cells remain intact. However, the surviving nerve cells may no longer carry messages because oligodendrocytes, which comprise the insulating sheath of the spinal cord, are lost. Currently, no effective drug treatment is available to reverse this disabling condition. So researcher looking forward at stem cell to treat the same and it was found that both embryonic stem cells and adult stem cells are effective to reverse spinal injury.

A successful method is developed by using of human embryonic stem cell (hESC) to treat paralyzed rats. Stem cells are injected into the spinal cords of rats that were paralyzed by spinal cord injury. The stem cells then developed into neurons called oligodendrites that produce a substance called myelin. Myelin is stripped away in certain spinal cord injuries, but the new oligodendrites replaced the missed myelin.

As a result, nerve impulses could once again travel through the damaged area, and the rats regained the ability to walk [34]. In January 2009, the U.S. Food & Drug Administration gave Geron Corporation based in Menlo Park, California, permission to treat the individuals having thoracic spinal cord injuries, which occur below the neck.

Another similar type of research carried out by using adult stem cells to treat the paralyzed rats. Here stem cells were obtained from the lining of the rat's owns spinal cord, called ependymal stem cell. When these cells were transplanted into animals with spinal cord injury, they regenerated ten times faster in the transplant subject than similar cells derived from healthy control animals. One week after the injection it was found that rats regained a significant amount of motor function [35]. Unlike human embryonic stem cell treatment for paralysis, adult stem cell treatment is not yet available for clinical trials in humans.

Stem cell and Acute Lung Injury (ALI)

In ALI, the layer of cells that forms the lining of the blood vessels surrounding the lung's air sacs is damaged, allowing fluid to leak in and fill the sacs. Repair of these breaks in the endothelium, or lining, is complicated by the fact that endothelial cells are long-lived.

There is no effective drug treatment. At the University of Illinois at Chicago College of Medicine, Kishore Wary and his colleagues were able to identify progenitor stem cells, named fetal liver kinase-1 (Flk-1) and CD34 for the proteins on their surfaces, in the bone marrow of mice that could prevent and treat experimentally-induced ALI. These stem cells stud their surface with molecules called integrins that allow the cells to stick to their targets and affect the repair. When mice that had been injected with compounds that causes ALI were injected with the purified and cultured Flk-1 and CD34 stem cells, the progenitor cells were able to repair the lung injury, prevent fluid build-up, and led to improved survival. The researchers hope to explore the possibility of using stem cell therapy in human acute lung injury [36].

Stem cell and re-growth of tooth

A Japanese team from the Tokyo University of Science, reported that stem cell had successfully regrown a tooth from cells extracted from mouse embryos. Teeth in mice, much like those in humans, form during embryonic development from two major cell types: the mesenchyme- originated ondontoblasts that are responsible for the production of dentin and the epithelium- derived ameloblasts the form the enamel.

Takashi's team isolated both kinds of cells from multiple mouse embryos and then transferred them to a collagen gel culture, in which the cells interacted to form a tooth bud and then transplanted the bud into the liver of an adult mouse, where the increased blood supply aided further tooth formation. Finally, Takashi inserted the tooth into an empty cavity within the mouse's mouth, in which it grew to full size [37].

Another research was performed at Odontis, a UK based dental company started by Professor Paul Sharpe. Here the researchers are working on developing human teeth from stem cells. This biological replacement tooth has been trademarked as BioTooth. By 2007 Dr. Paul Sharpe and his team claimed to have achieved the first promising results in experiment with a mouse, where Sharpe's team took early tooth buds from growing embryos and switched on a gene known to be active in growing molars. They implanted the buds in the front of the jaws of mice, where incisors would normally grow. The rodents emerged with molars in front and back [38].

Stem cell and heart

Despite many breakthroughs in cardiovascular medicines, heart attack and congestive heart failure remain most prominent health challenges. Recent researcher's evidence showed that adult stem cells and embryonic stem cells may able to developed into cardiomyocytes, vascular endothelial cells and the smooth muscle cell which are essential for the normal functioning of heart.

Orlic and his colleagues [39] describe an experimental application of mouse hematopoietic stem cells for the regeneration of the tissues in the heart. They induced heart attack in mice by tying off the left main coronary artery. The researcher then isolated a selected group of adult primitive bone marrow cells with a high capacity to develop into cells of multiple types and injected into the damaged wall of the ventricle, these cells led to the formation of new cardiomyocytes, vascular endothelium, and smooth muscle cells, thus generating de novo myocardium, including coronary arteries, arterioles, and capillaries.

The newly formed myocardium occupied 68 percent of the damaged portion of the ventricle nine days after the bone marrow cells were transplanted, in effect replacing the dead myocardium with living, functioning tissue. The researchers found that mice that received the transplanted cells survived in greater numbers than mice with heart attacks that did not receive the mouse stem cells.

A second study, Jackson and his colleagues [40] used adult stem cells from mouse bone marrow to regenerate the cardiac tissue damaged in the mouse heart due to attack. In this model, investigators purified a "side population" of hematopoietic stem cells from a genetically altered mouse strain. These cells were then transplanted into the marrow of lethally irradiated mice approximately 10 weeks before the recipient mice were subjected to heart attack via the tying off of a different major heart blood vessel, the left anterior descending (LAD) coronary artery.

At two to four weeks after the induced cardiac injury, the survival rate was 26 percent. Stem cell applications for heart repair have not only seen success in rodent models, but also in human clinical trials. In Germany, Gustav Steinhoff at the University of Rostock purified stem cells from bone marrow removed from the hips of six heart attack patients. The following day the stem cells were injected, during a bypass operation, into the boundary between the living and dead heart tissue of each patient. "For all six patients, the heart's strength and blood supply improved, suggesting the stem cells had differentiated into heart muscle and blood vessel cells [41].

Stem cell and Parkinson's disease

Parkinson's disease occurs as a result of a gradual loss of a specific type of nerve cell, located in an area of the brain called the substantia nigra. These nerve cells produce a natural chemical called

dopamine. The lack of dopamine makes patients with Parkinson's disease have difficulty in moving freely, holding a posture, talking and writing. Stem cell based therapies for Parkinson's disease are not yet a routine clinical procedure. Scientists are agreed that more information is needed about the causes of Parkinson's disease and the biology of stem cells before safe, effective and long-lasting therapies can be developed. Because a single, well-identified type of cell is affected in Parkinson's disease, stem cells offer great potential for treatment. The basis for such treatment would be to replace the cells that have died with other identical dopaminergic nerve cells, obtained from stem cells.

Dr. Omar Gonzales, uses stem cells therapy for treatment of Parkinson's disease and claims that the patients who received the stem cell therapy experienced drastic improvement. After the treatment, the patients are reported to experience improvement in balance and coordination for 80 percent, decrease of stiffness and rigidity for 70 percent, improved mental clarity for 50 percent and mood improvement for 80 percent, having less tremor for 60 percent and reduced dependency on other people for 90 percent. In addition, Dr. Gonzales says, his patients even experience reversal of the symptoms of Parkinson's disease after the treatment. Stem cells used in Dr. Gonzales' therapy are prepared and kept at the Center's laboratory [42].

Stem cell and Alzheimer's disease

Alzheimer's disease (AD) is a slowly progressive disease of the brain that is characterized by impairment of memory and eventually by disturbances in reasoning, planning, language, and perception. Many scientists believe that Alzheimer's disease results from an increase in the production or accumulation of a specific protein (beta-amyloid protein) in the brain that leads to nerve cell death. A human growth factor i.e. the granulocyte-colony stimulating factor (GCSF) stimulates blood stem cells to proliferate in the bone marrow reverses memory impairment in mice by reducing the level of brain clogging protein beta amyloid deposited in excess in the brains of the Alzheimer's mice. Based on this, researchers at University of South Florida injected filgrastim (Neupogen®), one of three commercially available GCSF compounds under the skin which increased the production of new neurons and promoted nerve cell connections within the brain and both of these actions led to improved memory and learning behavior in the Alzheimer's mice. Based on the promising findings in mice, the Alzheimer's Drug Discovery Foundation is funding a pilot clinical trial at USF's Byrd Alzheimer's Center. The randomized, controlled trial, led by Dr. Sanchez-Ramos [43] and Dr. Ashok Raj, will test the safety and effectiveness of filgrastim in 12 patients with mild to moderate Alzheimer's disease. GCSF reduced the burden of beta amyloid deposited in the brains of the Alzheimer's mice by several means, the researchers found. One was by recruiting reinforcements to clear beta amyloid accumulating abnormally in the brain. The growth factor prodded bone-marrow derived microglia outside the brain to join forces with the brain's already-activated microglia in eliminating the Alzheimer's protein from the brain. Microglias are brain cells that act as the central nervous system's main form of immune defense. Like molecular "Pac-men," they rush to the defense of damaged or inflamed areas to gobble up toxic substances.

CONCLUSION:

Remarkable progress has been achieved in studying stem cells. This review has summarized the role of stem cells in basic biological processes *in vivo*. The most exciting uses of embryonic stem cells as well as adult stem cell gives promise to cure many devastating diseases like Parkinson's, heart diseases, paralysis, diabetes and many more. Of the stem cells discussed, ESC's have the most capacity to differentiate into a variety of cells and their proliferation capacity is also unsurpassed by any other cell type. But In the future, somatic stem cells from the patient will be extracted and manipulated and then reintroduced into the same patient to cure debilitating diseases. This would preclude the use of embryonic stem cells for cell therapy, eliminate the ethical objections against stem cell research, and also resolve immunological rejection problems. However, at present the cell proliferation and differentiation potential of embryonic stem cells remains far more likely to produce a cure than do the somatic cells.

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