Review Article

Review on: Stem Cell Therapy and Regenerative Medicine

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ABSTRACT

Stem cells are the cells that are capable of forming the entire human body. They can divide over and over again to produce new cells; they can change into the other types of cell that make up the body. Adult stem cells have been found in tissues such as the brain, bone marrow, blood, blood vessels, skeletal muscles, skin, and the liver. Embryonic stem cells are derived from a four- or five-day-old human embryo that is in the blastocyst phase of development. Stem cells are either extracted from adult tissue or from a dividing zygote in a culture dish. Stem-cell therapy is the use of stem cells to treat or prevent a disease or condition. Advancements in gene editing and tissue engineering technology have endorsed the ex vivo remodeling of stem cells grown into 3D organoids and tissue structures for personalized applications. This review outlines the what is stem cell, Types, Properties, sources of stem cells, Application in regenerative medicine, clinical research and clinical trials.

Keywords: Stem cells, regenerative medicine, cell cultures, blastocyst.

INTRODUCTION

A stem cell is a cell with the unique ability to develop into specialized cell types in the body. In the future they may be used to replace cells and tissues that have been damaged or lost due to disease.[2]

Stem cells are distinguished from other cell types by two important characteristics. First, they are unspecialized cells capable of renewing themselves through cell division, sometimes after long periods of inactivity. Second, under certain physiologic or experimental conditions, they can be induced to become tissue- or organ-specific cells with special functions. In some organs, such as the gut and bone marrow, stem cells regularly divide to repair and replace worn out or damaged tissues. In other organs, however, such as the pancreas and the heart, stem cells only divide under special conditions.

Laboratory studies of stem cells enable scientists to learn about the cells' essential properties and what makes them different from specialized cell types. Scientists are already using stem cells in the laboratory to screen new drugs and to develop model systems to study normal growth and identify the causes of birth defects.

Research on stem cells continues to advance knowledge about how an organism develops from a single cell and how healthy cells replace damaged cells in adult organisms. Stem cell research is one of the most fascinating areas of contemporary biology, but, as with many expanding fields of scientific inquiry, research on stem cells raises scientific questions as rapidly as it generates new discoveries.

What is a Stem Cell? [1,9]

Stem cells are the cells that are capable of forming the entire human body. They're amazing since the very beginning of life, a fertilized egg cell divides repeatedly to form a group of stem cells that will go on to eventually make all the organs and tissues in your body. Stem cells are also present in adults, where they play an important role in repairing and replacing damaged cells and tissues.

The main feature of a stem cell is that it can divide to give rise to both more stem cells and also specialized cells like nerve cells or muscle cells. No other type of cell can do this! In this way, stem cells replenish themselves at the same time that they provide new specialized cells to create or repair organs and tissues. Stem cells are unique cells: They have the ability to become many different types of cells, and they can replicate rapidly. Stem cells play a huge part in the body's healing process, and the introduction of new stem cells has always showed great promise in the treatment of many conditions. It wasn't until we found out where and how to isolate these cells that we started using them for transplants. Although a person's own stem cells are always 100 percent compatible, there are risks in using someone else's stem cells, especially if the donor and recipient are not immediately related. The discovery of certain markers allows us to see how compatible a donor's and host's cells will be. The relatively recent discovery of stem cells in the umbilical cord's blood has proven advantageous over acquiring stem cells from other sources. Researchers are currently conducting clinical trials with stem cells, adding to the growing list of 80 diseases which they can treat.

Properties of Stem Cells [3,6,10]

- Our body is made up of many different types of stem cells.
- Most cells are specialized to perform particular functions, such as red blood cells? That carry oxygen around our bodies in the blood, but they are unable to divide.
- Stem cells provide new cells for the body as it grows and replace specialized cells that are damaged or lost. They have two unique properties that enable them to do this:
- They can divide over and over again to produce new cells.
- As they divide, they can change into the other types of cell that make up the body. [2]

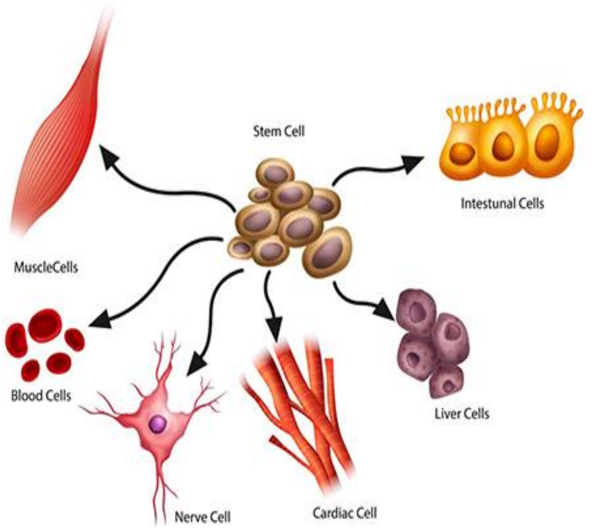

Figure 1: A stem cell has the potential to become one of many different types of cells.

TYPES OF STEM CELLS [2,3,8]

Adult stem cells

Adult or somatic stem cells exist throughout the body after embryonic development and are found inside of different types of tissue. These stem cells have been found in tissues such as the brain, bone marrow, blood, blood vessels, skeletal muscles, skin, and the liver. They remain in a quiescent or non-dividing state for years until activated by disease or tissue injury.

Adult stem cells can divide or self-renew indefinitely, enabling them to generate a range of cell types from the originating organ or even regenerate the entire original organ. It is generally thought that adult stem cells are limited in their ability to differentiate based on their tissue of origin, but there is some evidence to suggest that they can differentiate to become other cell types.

Embryonic stem cells

Embryonic stem cells are derived from a four- or five-day-old human embryo that is in the blastocyst phase of development. The embryos are usually extras that have been created in IVF (in vitro fertilization) clinics where several eggs are fertilized in a test tube, but only one is implanted into a woman.

Sexual reproduction begins when a male's sperm fertilizes a female's ovum (egg) to form a single cell called a zygote. The single zygote cell then begins a series of divisions, forming 2, 4, 8, 16 cells, etc. After four to six days - before implantation in the uterus - this mass of cells is called a blastocyst. The blastocyst consists of an inner cell mass (embryoblast) and an outer cell mass (trophoblast). The outer cell mass becomes part of the placenta, and the inner cell mass is the group of cells that will differentiate to become all the structures of an adult organism. This latter mass is the source of embryonic stem cells - totipotent cells (cells with total potential to develop into any cell in the body).

In a normal pregnancy, the blastocyst stage continues until implantation of the embryo in the uterus, at which point the embryo is referred to as a fetus. This usually occurs by the end of the 10th week of gestation after all major organs of the body have been created.

However, when extracting embryonic stem cells, the blastocyst stage signals when to isolate stem cells by placing the "inner cell mass" of the blastocyst into a culture dish containing a nutrient-rich broth. Lacking the necessary stimulation to differentiate, they begin to divide and replicate while maintaining their ability to become any cell type in the human body. Eventually, these undifferentiated cells can be stimulated to create specialized cells.

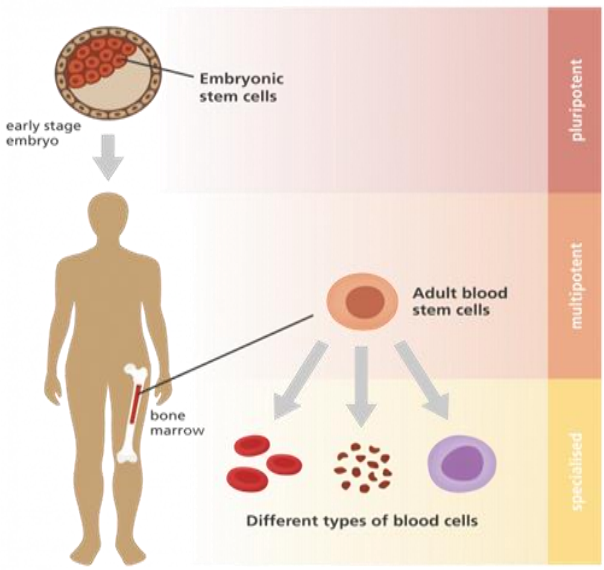

Figure 2: Types of stem cells

Stem Cell Cultures

Human embryonic stem cell colony

Stem cells are either extracted from adult tissue or from a dividing zygote in a culture dish. Once extracted, scientists place the cells in a controlled culture that prohibits them from further specializing or differentiating but usually allows them to divide and replicate. The process of growing large numbers of embryonic stem cells has been easier than growing large numbers of adult stem cells, but progress is being made for both cell types.

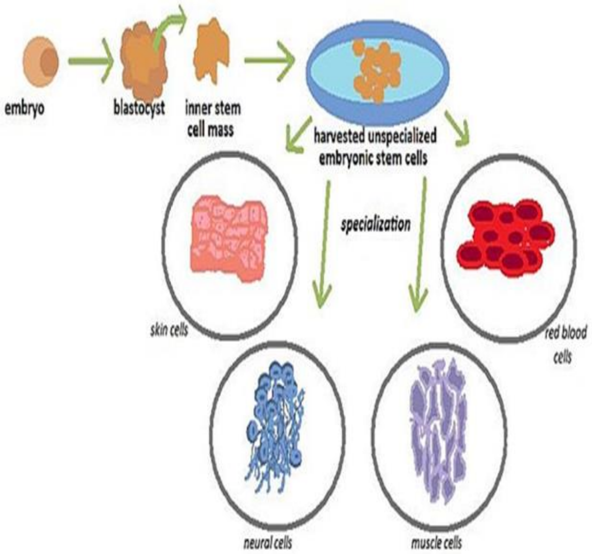

Figure 3: Stem cell culture

APPLICATIONS OF STEM CELL [2,10]

Model System

Cell culture are used as model system to study basic cell biology and biochemistry, to study the interaction between cell and disease causing agents like bacteria, virus, to study the effect of drugs, to study the process of aging and also it is used to study triggers for ageing[4]

Cancer Research

The basic difference between normal cell and cancer cell can be studied using animal cell culture technique, as both cells can be cultured in laboratory. Normal cells can be converted into cancer cells by using radiation, chemicals and viruses. Thus, the mechanism and cause of cancer can be studied. Cell culture can be used to determine the effective drugs for selectively destroy only cancer cells. [4]

Virology

Animal cell cultures are used to replicate the viruses instead of animals for the production of vaccine. Cell culture can also be used to detect and isolate viruses, and also to study growth and development cycle of viruses. It is also used to study the mode of infection. [4]

Toxicity Testing

Animal cell culture is used to study the effects of new drugs, cosmetics and chemicals on survival and growth of a number of types of cells. Especially liver and kidney cells. Cultured animal cells are also used to determine the maximum permissible dosage of new drugs. [4]

Vaccine Production

Cultured animal cells are used in the production of viruses and these viruses are used to produce vaccines. For example, vaccines for deadly diseases like polio, rabies, chicken pox, measles and hepatitis B are produced using animal cell culture. [4]

Genetically Engineered Protein

Animal cell cultures are used to produce commercially important genetically engineered proteins such as monoclonal antibodies, insulin, hormones, and much more. [4]

Replacement Tissue or Organ

Animal cell culture can be used as replacement tissue or organs. For example, artificial skin can be produced using this technique to treat patients with burns and ulcers. However, research is going on artificial organ culture such as liver, kidney and pancreas. Organ culture techniques and research are being conducted on both embryonic and adult stem cell culture. These cells have the capacity to differentiate into many different types of cells and organs. It is believed that by learning to control the development and differentiation of these cells may be used to treat variety of medical conditions. [4]

Genetic Counseling

Fetal cell culture extracted from pregnant women can be used to study or examine the abnormalities of chromosomes, genes using karyotyping, and these findings can be used in early detection of fetal disorders. [4]

Genetic Engineering

Cultured animal cells can be used to introduce new genetic material like DNA or RNA into the cell. These can be used to study the expression of new genes and its effect on the health of the cell. Insect cells are used to produce commercially important proteins by infecting them with genetically altered baculoviruses. [4]

Gene Therapy

Cultured animal cells can be genetically altered and can be used in gene therapy technique. First cells are removed from the patient lacking a functional gene or missing a functional gene. These genes are replaced by functional genes and altered cells are culture and grown in laboratory condition. Then these altered cells are introduced into the patient. Another method is by using viral vector, functional gene is inserted into the genome of viral vector and then they are allowed to infect the patient, in the hope that the missing gene will be expressed with the help of the viral vector. [4]

Drug Screening and Development

Animal cell cultures are used to study the cytotoxicity of new drug. This is also used to find out the effective and safe dosage of new drugs. Now these tests are being conducted in 384 and 1536 well plates. Cell-based assay plays an important role in pharmaceutical industry. [4]

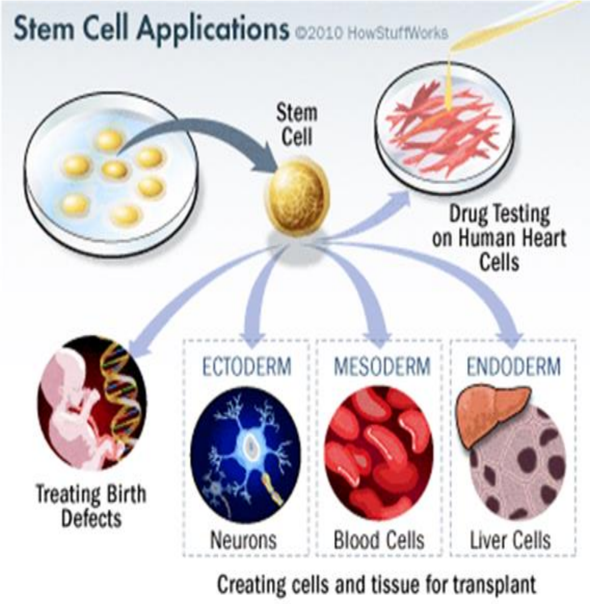

Figure 4: Stem cell application

Stem-Cell Therapy [1,5]

Stem-cell therapy is the use of stem cells to treat or prevent a disease or condition.

Bone marrow transplant is the most widely used stem-cell therapy, but some therapies derived from umbilical cord blood are also in use. Research is underway to develop various sources for stem cells, and to apply stem-cell treatments for neurodegenerative diseases and conditions such as diabetes, heart disease, and other conditions.

Stem-cell therapy has become controversial following developments such as the ability of scientists to isolate and culture embryonic stem cells, to create stem cells using somatic cell nuclear transfer and their use of techniques to create induced pluripotent stem cells. This controversy is often related to abortion politics and to human cloning. Additionally, efforts to market treatments based on transplant of stored umbilical cord blood have been controversial

What is Stem Cell Therapy

Everybody is born different, some are born perfectly healthy and remain healthy for the rest of their lives, some are born with certain neuromuscular disorders, while some may develop degenerative disorders. Stem Cell Therapy (SCT) is the treatment of various disorders, non-serious to life threatening, by using stem cells. These stem cells can be procured from a lot of different sources and used to potentially treat more than 80 disorders, including neuromuscular and degenerative disorders.

Hematopoietic disorders (e.g. leukemia, thalassemia, aplastic anemia, MDS, sickle cell anemia, storage disorders etc.) affect the bone marrow and manifest with various systemic complications. Stem cells from a donor (either from cord blood or bone marrow) are known to reconstitute the defective bone marrow and permanently overcome the disorder.

Degenerative disorders arise from degeneration or wear and tear of bone, cartilage, muscle, fat or any other tissue, cell or organ. This could occur due to a variety of reasons, but it's normally the process known as aging, or 'getting old' that is the biggest cause. The disorders have a slow and insidious onset but once contracted, can be long-standing, pain-staking and lifelong. These disorders can affect any organ of the body. The common degenerative disorders are diabetes, osteoarthritis, stroke, chronic renal failure, congestive cardiac failure, myocardial infarction, Alzheimer's disease, Parkinson's disease etc.

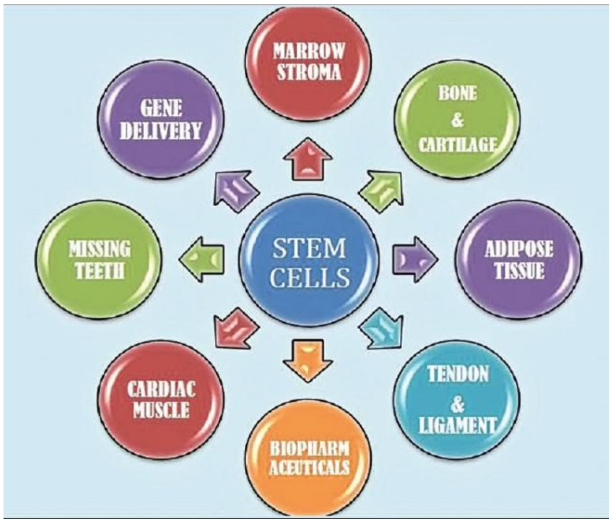

Figure 5: Stem cell therapy

Sources of Stem Cells[4,9]

1.Adult stem cells

- Cord blood, umbilical cord blood
- Bone marrow
- Blood, peripheral blood stem cells
- Menstrual blood
- Skin
- Teeth
- Placental tissue

All of these sources of adult stem cells share some characteristics. Others are unique and options are being researched daily.

2. Embryonic stem cells

- Human embryos
- Fetal tissue

❖ What are the Best Sources of Stem Cells?

The Placenta (Chorion + Decidua), Amniotic Sac and Amniotic Fluid are the best sources of stem cells, in terms of both - quality and quantity.

- Placenta - The outermost membrane around the embryo.
- Amniotic Sac - The bag of fluid inside the uterus where the baby is born.
- Amniotic Fluid - This is the fluid inside the amniotic sac that the developing baby inhales and Completely Safe and Ethical

Unlike embryonic stem cells, these are completely safe and there is no chance of tumour formation. They have the least chance of rejection when used in therapy. They are also non-embryonic, hence completely ethical.

Easy to Obtain

Stem cells from these three sources can be obtained easily during the birth of your child - whether it is a normal delivery or a caesarean section. The procedure is completely non-invasive and can be easily procured along with the cord and cord blood, without any added inconvenience.

It's very efficient and there is no need to have a separate procedure to procure them. For the Entire Family

These cells can be used for treatment by the baby's siblings, parents or even grandparents (subject to HLA matching).

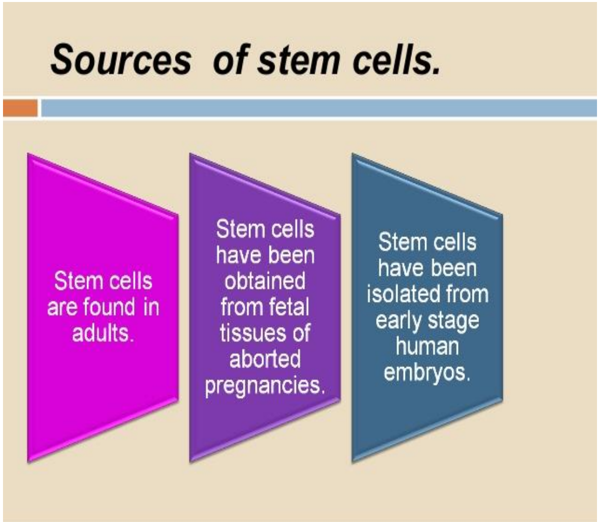

Figure 6: Sources of Stem cell

Medical Uses of Stem Cell Therapy [2,6,7]

Hematopoietic stem cell transplantation

For over 30 years, bone marrow has been used to treat cancer patients with conditions such as leukemia and lymphoma; this is the only form of stem-cell therapy that is widely practiced. During chemotherapy, most growing cells are killed by the cytotoxic agents. These agents, however, cannot discriminate between the leukemia or neoplastic cells, and the hematopoietic stem cells within the bone marrow. It is this side effect of conventional chemotherapy strategies that the stem-cell transplant attempts to reverse; a donor's healthy bone marrow reintroduces functional stem cells to replace the cells lost in the host's body during treatment. The transplanted cells also generate an immune response that helps to kill off the cancer cells; this process can go too far, however, leading to graft vs host disease, the most serious side effect of this treatment.

Another stem-cell therapy called Prochymal, was conditionally approved in Canada in 2012 for the management of acute graft-vs-host disease in children who are unresponsive to steroids. It is an allogenic stem therapy based on mesenchymal stem cells (MSCs) derived from the bone marrow of adult donors. MSCs are purified from the marrow, cultured and packaged, with up to 10,000 doses derived from a single donor. The doses are stored frozen until needed.

The FDA has approved five hematopoietic stem-cell products derived from umbilical cord blood, for the treatment of blood and immunological diseases.In 2014, the European Medicines Agency recommended approval of limbal stem cells for people with severe limbal stem cell deficiency due to burns in the eye.

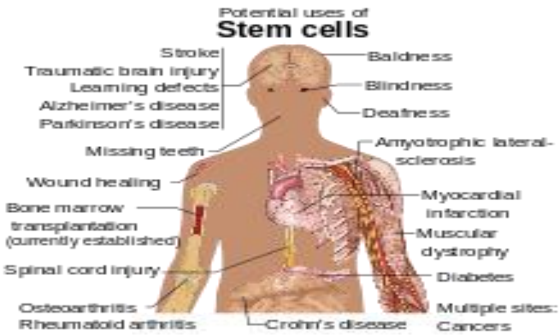

Figure 7: Diseases and conditions where stem cell treatment is promising or emerging.

Stem cells are being studied for a number of reasons. The molecules and exosomes released from stem cells are also being studied in an effort to make medications. The paracrine soluble factors produced by stem cells, known as the stem cell secretome, has been found to be the predominant mechanism by which stem cell-based therapies mediate their effects in degenerative, auto-immune and inflammatorydiseases.

Applications

Neurodegeneration

Research has been conducted on the effects of stem cells on animal models of brain degeneration, such as in Parkinson's, Amyotrophic lateral sclerosis, and Alzheimer's disease. There have been preliminary studies related to multiple sclerosis.

Healthy adult brains contain neural stem cells which divide to maintain general stem-cell numbers, or become progenitor cells. In healthy adult laboratory animals, progenitor cells migrate within the brain and function primarily to maintain neuron populations for olfaction (the sense of smell). Pharmacological activation of endogenous neural stem cells has been reported to induce neuroprotection and behavioral recovery in adult rat models of neurological disorder.

Brain and spinal cord injury

Stroke and traumatic brain injury lead to cell death, characterized by a loss of neurons and oligodendrocytes within the brain. Clinical and animal studies have been conducted into the use of stem cells in cases of spinal cord injury.

Heart

Stems cells are being studied in those with severe heart disease.

The work by Bodo-Eckehard Strauer has been discredited by the identification of hundreds of factual contradictions. Among several clinical trials that have reported that adult stem-cell therapy is safe and effective, powerful effects have been reported from only a few laboratories, infarcts as well as heart failure not arising from myocardial infarction. While initial animal studies demonstrated therapeutic effects, later clinical trials achieved only modest, though statistically significant, improvements.

Stem-cell therapy for treatment of myocardial infarction usually makes use of autologous bone-marrow stem cells (a specific type or all), however other types of adult stem cells may be used, such as adipose-derived stem cells. Adult stem cell therapy for treating heart disease was commercially available in at least five continents as of 2007.

Possible mechanisms of recovery include:

- Generation of heart muscle cells
- Stimulation of growth of new blood vessels to repopulate damaged heart tissue
- Secretion of growth factors
- Assistance via some other mechanism

It may be possible to have adult bone-marrow cells differentiate into heart muscle cells.

The first successful integration of human embryonic stem cell derived cardiomyocytes in guinea pigs (mouse hearts beat too fast) was reported in August 2012. The contraction strength was measured four weeks after the guinea pigs underwent simulated heart attacks and cell treatment. The cells contracted synchronously with the existing cells, but it is unknown if the positive results were produced mainly from paracrine as opposed to direct electromechanical effects from the human cells. Future work will focus on how to get the cells to engraft more strongly around the scar tissue. Whether treatments from embryonic or adult bone marrow stem cells will prove more effective remains to be seen.

In 2013 the pioneering reports of powerful beneficial effects of autologous bone marrow stem cells on ventricular function were found to contain "hundreds" of discrepancies.Critics report that of 48 reports there seemed to be just five underlying trials, and that in many cases whether they were randomized or merely observational accepter-versus-rejecter, was contradictory between reports of the same trial. One pair of reports of identical baseline characteristics and final results, was presented in two publications as, respectively, a 578 patient randomized trial and as a 391 patient observational study. Other reports required (impossible) negative standard deviations in subsets of patients, or contained fractional patients, negative NYHA classes. Overall there were many more patients published as having receiving stem cells in trials, than the number of stem cells processed in the hospital's laboratory during that time. A university investigation, closed in 2012 without reporting, was reopened in July 2013.

One of the most promising benefits of stem cell therapy is the potential for cardiac tissue regeneration to reverse the tissue loss underlying the development of heart failure after cardiac injury.

Blood-cell formation

The specificity of the human immune-cell repertoire is what allows the human body to defend itself from rapidly adapting antigens. However, the immune system is vulnerable to degradation upon the pathogenesis of disease, and because of the critical role that it plays in overall defense, its degradation is often fatal to the organism as a whole. Diseases of hematopoietic cells are diagnosed and classified via a subspecialty of pathology known as hematopathology. The specificity of the immune cells is what allows recognition of foreign antigens, causing further challenges in the treatment of immune disease. Identical matches between donor and recipient must be made for successful transplantation treatments, but matches are uncommon, even between first-degree relatives. Research using both hematopoietic adult stem cells and embryonic stem cells has provided insight into the possible mechanisms and methods of treatment for many of these ailments.

Fully mature human red blood cells may be generated *ex vivo* by hematopoietic stem cells (HSCs), which are precursors of red blood cells. In this process, HSCs are grown together with stromal cells, creating an environment that mimics the conditions of bone marrow, the natural site of red-blood-cell growth. Erythropoietin, a growth factor, is added, coaxing the stem cells to complete terminal differentiation into red blood cells. Further research into this technique should have potential benefits to gene therapy, blood transfusion, and topical medicine.

Regrowing teeth

In 2004, scientists at King's College London discovered a way to cultivate a complete tooth in mice and were able to grow bioengineered teeth stand-alone in the laboratory. Researchers are confident that the tooth regeneration technology can be used to grow live teeth in human patients.

In theory, stem cells taken from the patient could be coaxed in the lab turning into a tooth bud which, when implanted in the gums, will give rise to a new tooth, and would be expected to be grown in a time over three weeks. It will fuse with the jawbone and release chemicals that encourage nerves and blood vessels to connect with it. The process is similar to what happens when humans grow their original adult teeth. Many challenges remain, however, before stem cells could be a choice for the replacement of missing teeth in the future.

Cochlear hair cell regrowth

Heller has reported success in re-growing cochlea hair cells with the use of embryonic stem cells.

Blindness and vision impairment

Since 2003, researchers have successfully transplanted corneal stem cells into damaged eyes to restore vision. "Sheets of retinal cells used by the team are harvested from aborted fetuses, which some people find objectionable." When these sheets are transplanted over the damaged cornea, the stem cells stimulate renewed repair, eventually restore vision. The latest such development was in June 2005, when researchers at the Queen Victoria Hospital of Sussex, England were able to restore the sight of forty patients using the same technique. The group, led by SherazDaya, was able to successfully use adult stem cells obtained from the patient, a relative, or even a cadaver. Further rounds of trials are ongoing.

Pancreatic beta cells

Diabetes patients lose the function of insulin-producing beta cells within the pancreas. In recent experiments, scientists have been able to coax embryonic stem cell to turn into beta cells in the lab. In theory if the beta cell is transplanted successfully, they will be able to replace malfunctioning ones in a diabetic patient.

Orthopaedics

Clinical case reports in the treatment orthopaedic conditions have been reported. To date, the focus in the literature for musculoskeletal care appears to be on mesenchymal stem cells. Centeno et al. have published MRI evidence of increased cartilage and meniscus volume in individual human subjects. The results of trials that include a large number of subjects, are yet to be published. However, a published safety study conducted in a group of 227 patients over a 3-4-year period shows adequate safety and minimal complications associated with mesenchymal cell transplantation.

Wakitani has also published a small case series of nine defects in five knees involving surgical transplantation of mesenchymal stem cells with coverage of the treated chondral defects.

Wound healing

Stem cells can also be used to stimulate the growth of human tissues. In an adult, wounded tissue is most often replaced by scar tissue, which is characterized in the skin by disorganized collagen structure, loss of hair follicles and irregular vascular structure. In the case of wounded fetal tissue, however, wounded tissue is replaced with normal tissue through the activity of stem cells. A possible method for tissue regeneration in adults is to place adult stem cell "seeds" inside a tissue bed "soil" in a wound bed and allow the stem cells to stimulate differentiation in the tissue bed cells. This method elicits a regenerative response more similar to fetal wound-healing than adult scar tissue formation. Researchers are still investigating different aspects of the "soil" tissue that are conducive to regeneration.

Infertility

Culture of human embryonic stem cells in mitotically inactivated porcine ovarian fibroblasts (POF) causes differentiation into germ cells (precursor cells of oocytes and spermatozoa), as evidenced by gene expression analysis.

Human embryonic stem cells have been stimulated to form Spermatozoon-like cells, yet still slightly damaged or malformed. It could potentially treat azoospermia.

In 2012, oogonial stem cells were isolated from adult mouse and human ovaries and demonstrated to be capable of forming mature oocytes. These cells have the potential to treat infertility.

HIV/AIDS

Destruction of the immune system by the HIV is driven by the loss of CD4+ T cells in the peripheral blood and lymphoid tissues. Viral entry into CD4+ cells is mediated by the interaction with a cellular chemokine receptor, the most common of which are CCR5 and CXCR4. Because subsequent viral replication requires cellular gene expression processes, activated CD4+ cells are the primary targets of productive HIV infection. Recently scientists have been investigating an alternative approach to treating HIV-1/AIDS, based on the creation of a disease-resistant immune system through transplantation of autologous, gene-modified (HIV-1-resistant) hematopoietic stem and progenitor cells (GM-HSPC).

Stem Cell Uses in Veterinary Medicine[3,6]

Research has been conducted on horses, dogs, and cats can benefit the development of stem cell treatments in veterinary medicine and can target a wide range of injuries and diseases such as myocardial infarction, stroke, tendon and ligament damage, osteoarthritis, oste ochondrosis and muscular dystrophy both in large animals, as well as humans. While investigation of cell-based therapeutics generally reflects human medical needs, the high degree of frequency and severity of certain injuries in racehorses has put veterinary medicine at the forefront of this novel regenerative approach. Companion animals can serve as clinically relevant models that closely mimic human disease.

Sources of stem cells

Terinary applications of stem cell therapy as a means of tissue regeneration have been largely shaped by research that began with the use of adult-derived mesenchymal stem cells to treat animals with injuries or defects affecting bone, cartilage, ligaments and/or tendons. There are two main categories of stem cells used for treatments: allogeneic stem cells derived from a genetically different donor within the same species and autologous mesenchymal stem cells, derived from the patient prior to use in various treatments. A third category, xenogenic stem cells, or stem cells derived from different species, are used primarily for research purposes, especially for human treatments.

Hard-tissue repair

Bone has a unique and well documented natural healing process that normally is sufficient to repair fractures and other common injuries. Misaligned breaks due to severe trauma, as well as things like tumor resections of bone cancer, are prone to improper healing if left to the natural process alone. Scaffolds composed of natural and artificial components are seeded with mesenchymal stem cells and placed in the defect. Within four weeks of placing the scaffold, newly formed bone begins to integrate with the old bone and within 32 weeks, full union is achieved. Further studies are necessary to fully characterize the use of cell-based therapeutics for treatment of bone fractures.

Stem cells have been used to treat degenerative bone diseases. The normally recommended treatment for dogs that have Legg–Calve–Perthes disease is to remove the head of the femur after the degeneration has progressed. Recently, mesenchymal stem cells have been injected directly into the head of the femur, with success not only in bone regeneration, but also in pain reduction.

Because of the general positive healing capabilities of stem cells, they have gained interest for the treatment of cutaneous wounds. This is important interest for those with reduced healing capabilities, like diabetics and those undergoing chemotherapy. In one trial, stem cells were isolated from the Wharton's jelly of the umbilical cord. These cells were injected directly into the wounds. Within a week, full re-epithelialization of the wounds had occurred, compared to minor re-epithelialization in the control wounds. This showed the capabilities of mesenchymal stem cells in the repair of epidermal tissues.

Soft-palate defects in horses are caused by a failure of the embryo to fully close at the midline during embryogenesis. These are often not found until after they have become worse because of the difficulty in visualizing the entire soft palate. This lack of visualization is thought to also contribute to the low success rate in surgical intervention to repair the defect. As a result, the horse often has to be euthanized. Recently, the use of mesenchymal stem cells has been added to the conventional treatments. After the surgeon has sutured the palate closed, autologous mesenchymal cells are injected into the soft palate. The stem cells were found to be integrated into the healing tissue especially along the border with the old tissue. There was also a large reduction in the number of inflammatory cells present, which is thought to aid in the healing process.

Ligament and tendon repair

Autologous stem cell-based treatments for ligament injury, tendon injury, osteoarthritis, osteoch ondrosis, and sub-chondral bone cysts have been commercially available to practicing veterinarians to treat horses since 2003 in the United States and since 2006 in the United Kingdom. Autologous stem cell based treatments for tendon injury, ligament injury, and osteoarthritis in dogs have been available to veterinarians in the United States since 2005. Over 3000 privately owned horses and dogs have been treated with autologous adipose-derived stem cells. The efficacy of these treatments has been shown in double-blind clinical trials for dogs with osteoarthritis of the hip and elbow and horses with tendon damage.

Race horses are especially prone to injuries of the tendon and ligaments. Conventional therapies are very unsuccessful in returning the horse to full functioning potential. Natural healing, guided by the conventional treatments, leads to the formation of fibrous scar tissue that reduces flexibility and full joint movement. Traditional treatments prevented a large number of horses from returning to full activity and also have a high incidence of re-injury due to the stiff nature of the scarred tendon. Introduction of both bone marrow and adipose derived stem cells, along with natural mechanical stimulus promoted the regeneration of tendon tissue. The natural movement promoted the alignment of the new fibers and tendocytes with the natural alignment found in uninjured tendons. Stem cell treatment not only allowed more horses to return to full duty and also greatly reduced the re-injury rate over a three-year period.

The use of embryonic stem cells has also been applied to tendon repair. The embryonic stem cells were shown to have a better survival rate in the tendon as well as better migrating capabilities to reach all areas of damaged tendon. The overall repair quality was also higher, with better tendon architecture and collagen formed. There was also no tumor formation seen during the three-month experimental period. Long-term studies need to be carried out to examine the long-term efficacy and risks associated with the use of embryonic stem cells. Similar results have been found in small animals.

Joint repair

Osteoarthritis is the main cause of joint pain both in animals and humans. Horses and dogs are most frequently affected arthritis. Natural cartilage regeneration is very limited and no current drug therapies are curative, but rather look to reduce the symptoms associated with the degeneration. Different types of mesenchymal stem cells and other additives are still being researched to find the best type of cell and method for long-term treatment.

Adipose-derived mesenchymal cells are currently the most often used because of the non-invasive harvesting. There has been a lot of success recently injecting mesenchymal stem cells directly into the joint. This is a recently developed, non-invasive technique developed for easier clinical use. Dogs receiving this treatment showed greater flexibility in their joints and less pain.

Muscle repairs

Stem cells have successfully been used to ameliorate healing in the heart after myocardial infarction in dogs. Adipose and bone marrow derived stem cells were removed and induced to a cardiac cell fate before being injected into the heart. The heart was found to have improved contractility and a reduction in the damaged area four weeks after the stem cells were applied.

A different trial is underway for a patch made of a porous substance onto which the stem cells are "seeded" in order to induce tissue regeneration in heart defects. Tissue was regenerated and the patch was well incorporated into the heart tissue. This is thought to be due, in part, to improved angiogenesis and reduction of inflammation. Although cardiomyocytes were produced from the mesenchymal stem cells, they did not appear to be contractile. Other treatments that induced a cardiac fate in the cells before transplanting had greater success at creating contractile heart tissue.

Nervous system repairs

Spinal cord injuries are one of the most common traumas brought into veterinary hospitals. Spinal injuries occur in two ways after the trauma: the primary mechanical damage, and in secondary processes, like inflammation and scar formation, in the days following the trauma. These cells involved in the secondary damage response secrete factors that promote scar formation and inhibit cellular regeneration. Mesenchymal stem cells that are induced to a neural cell fate are loaded onto a porous scaffold and are then implanted at the site of injury. The cells and scaffold secrete factors that counteract those secreted by scar forming cells and promote neural regeneration. Eight weeks later, dogs treated with stem cells showed immense improvement over those treated with conventional therapies. Dogs treated with stem cells were able to occasionally support their own weight, which has not been seen in dogs undergoing conventional therapies. Treatments are also in clinical trials to repair and regenerate peripheral nerves. Peripheral nerves are more likely to be damaged, but the effects of the damage are not as widespread as seen in injuries to the spinal cord. Treatments are currently in clinical trials to repair severed nerves, with early success. Stem cells induced to a neural fate injected into a severed nerve. Within four weeks, regeneration of previously damaged stem cells and completely formed nerve bundles were observed.

Stem cells are also in clinical phases for treatment in ophthalmology. Hematopoietic stem cells have been used to treat corneal ulcers of different origin of several horses. These ulcers were resistant to conventional treatments available, but quickly responded positively to the stem cell treatment. Stem cells were also able to restore sight in one eye of a horse with retinal detachment, allowing the horse to return to daily activities.

Stem Cells Applications in Regenerative Medicine[2,5]

Regenerative medicine, the most recent and emerging branch of medical science, deals with functional restoration of tissues or organs for the patient suffering from severe injuries or chronic disease. The spectacular progress in the field of stem cell research has laid the foundation for cell-based therapies of disease which cannot be cured by conventional medicines. The indefinite self-renewal and potential to differentiate into other types of cells represent stem cells as frontiers of regenerative medicine. The transdifferentiating potential of stem cells varies with source and according to that regenerative applications also change. Advancements in gene editing and tissue engineering technology have endorsed the ex vivo remodeling of stem cells grown into 3D organoids and tissue structures for personalized applications. Regenerative medicine, the most recent and emerging branch of medical science, deals with functional restoration of tissues or organs for the patient suffering from severe injuries or chronic disease.[5]

Stem Cell Research [9,10]

- **Research is looking to better understand the properties of stem cells so that we can**
- Understand how our bodies grow and develop
- Find ways of using stem cells to replace cells or tissues[7] That have been damaged or lost.
- We can use stem cells to study how cells become specialized for specific functions in the body, and

what happens when this process goes wrong in disease.

- If we understand stem cell development, we may be able to replicate this process to create new cells, tissues and organs[7].
- We can grow tissue and organ structures from stem cells, which can then be studied to find out how they function and how they are affected by different drugs[7]

RESEARCH ABSTRACT

Human mesenchymal stem cell-conditioned medium improves cardiac function following myocardial infarction

Astudy conducted by LeoTimmers et al, Recent studies suggest that the therapeutic effects of stem cell transplantation following myocardial infarction (MI) are mediated by paracrine factors. One of the main goals in the treatment of ischemic heart disease is to stimulate vascular repair mechanisms. Here, we sought to explore the therapeutic angiogenic potential of mesenchymal stem cell (MSC) secretions. Human MSC secretions were collected as conditioned medium (MSC-CM) using a clinically compliant protocol. Based on proteomic and pathway analysis of MSC-CM, an in vitro assay of HUVEC spheroids was performed identifying the angiogenic properties of MSC-CM. Subsequently, pigs were subjected to surgical left circumflex coronary artery ligation and randomized to intravenous MSC-CM treatment or non-CM (NCM) treatment for 7 days. Three weeks after MI, myocardial capillary density was higher in pigs treated with MSC-CM (645 ± 114 vs 981 ± 55 capillaries/mm2; $P = 0.021$), which was accompanied by reduced myocardial infarct size and preserved systolic and diastolic performance. Intravenous MSC-CM treatment after myocardial infarction increases capillary density and preserves cardiac function, probably by increasing myocardial perfusion.

Clinical Trials [9,10]

Regenerative treatment models

Stem cells are thought to mediate repair via five primary mechanisms: 1) providing an anti-inflammatory effect, 2) homing to damaged tissues and recruiting other cells, such as endothelial progenitor cells, that are necessary for tissue growth, 3) supporting tissue remodeling over scar formation, 4) inhibiting apoptosis, and 5) differentiating into bone, cartilage, tendon, and ligament tissue.

To further enrich blood supply to the damaged areas, and consequently promote tissue regeneration, platelet-rich plasma could be used in conjunction with stem cell transplantation. The efficacy of some stem cell populations may also be affected by the method of delivery; for instance, to regenerate bone, stem cells are often introduced in a scaffold where they produce the minerals necessary for generation of functional bone. Stem cells have also been shown to have a low immunogenicity due to the relatively low number of MHC molecules found on their surface. In addition, they have been found to secrete chemokines that alter the immune response and promote tolerance of the new tissue. This allows for allogeneic treatments to be performed without a high rejection risk.

Drug discovery and biomedical research

The ability to grow up functional adult tissues indefinitely in culture through Directed differentiation creates new opportunities for drug research. Researchers are able to grow up differentiated cell lines and then test new drugs on each cell type to examine possible interactions *in vitro* before performing *in vivo* studies. This is critical in the development of drugs for use in veterinary research because of the possibilities of species-specific interactions. The hope is that having these cell lines available for research use will reduce the need for research animals used because effects on human tissue *in vitro* will provide insight not normally known before the animal testing phase.

With the advent of induced pluripotent stem cells (iPSC), treatments being explored and created for the used in endangered low production animals possible. Rather than needing to harvest embryos or eggs, which are limited, the researchers can remove mesenchymal stem cells with greater ease and greatly reducing the danger to the animal due to noninvasive techniques. This allows the limited eggs to be put to use for reproductive purposes only.

Conservation

Stem cells are being explored for use in conservation efforts. Spermatogonial stem cells have been harvested from a rat and placed into a mouse host and fully mature sperm were produced with the ability to produce viable offspring. Currently research is underway to find suitable hosts for the introduction of donor spermatogonial stem cells. If this becomes a viable option for conservationists, sperm can be produced from high genetic quality individuals who die before reaching sexual maturity, preserving a line that would otherwise be lost.

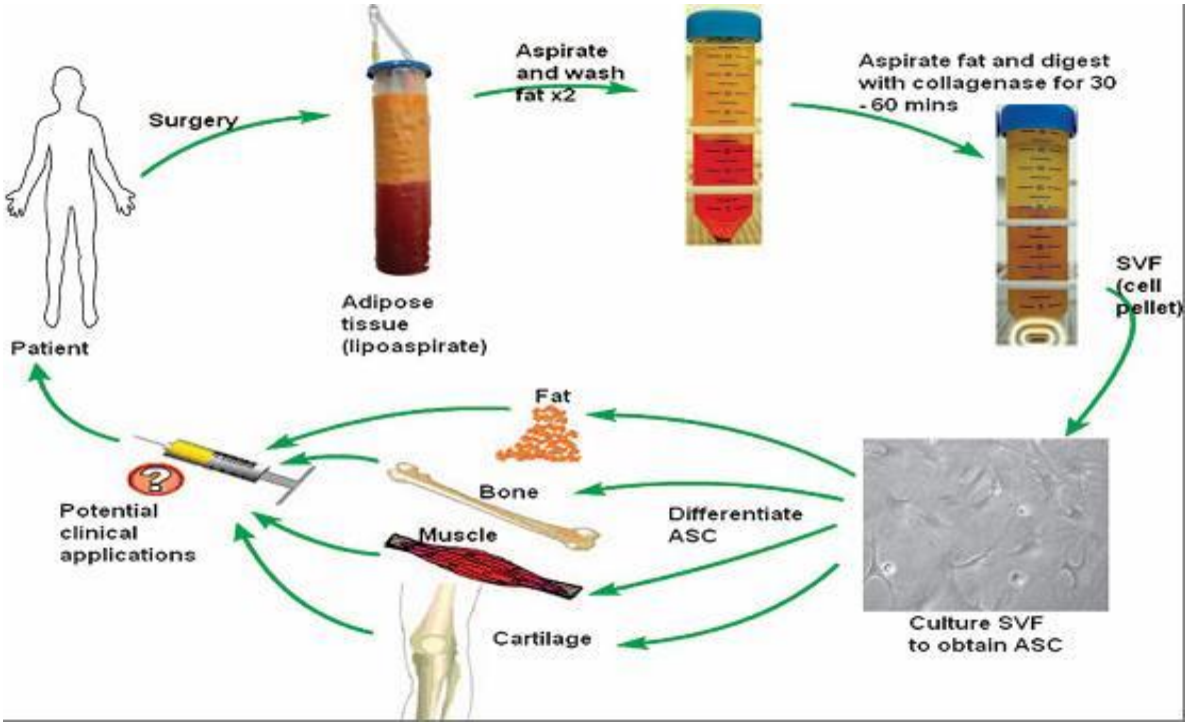

Figure 7: Stem cells - Clinical trials

CONCLUSION

Stem cell pose a bright future for the therapeutic world by promising treatment options for the diseases which are considered as noncurable now a days. However, because of significant peri & post-transplant morbidity &mortality further research & trials are required to refine & optimize conditioning regimens & modalities of supportive care.[9] By virtue of funding of stem cell research, we hope to see new horizon of therapeutics in the form of organ development & replacement of lost tissue such as hairs, tooth, retina, & cochlear cells.

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