Review Article

Function of Stem Cells and Their Future Roles in Healthcare

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ABSTRACT

Stem cell biology has come of age. Stem cells are found in the early embryo, the foetus, amniotic fluid, the placenta and umbilical cord blood. After birth and for the rest of life, stem cells continue to reside in many sites of the body, including skin, hair follicles, bone marrow and blood, brain and spinal cord, the lining of the nose, gut, lung, joint fluid, muscle, fat, and menstrual blood, to name a few. A stem cell can reproduce itself over and over again (a special trick known as "self-renewal" or "self-replication"). Stem cells have enormous potential in health and medical research. For example, researching the differentiation processes and understanding to control stem cell differentiation in the laboratory is providing insights into how humans develop from embryo to adult. Directing the growth of cells or tissues can be used for specific purposes such as modeling diseases, drug screening or cell-based therapies. Research on adult stem cells has generated a great deal of excitement. This finding has led researchers and clinicians to ask whether adult stem cells could be used for transplants. Scientists now have evidence that stem cells exist in the brain and the heart. If the differentiation of adult stem cells can be controlled in the laboratory, these cells may become the basis of transplantation-based therapies. Despite the fact that stem cell research has been advancing fast, there are many challenges ahead to allow the use of stem cells for drug discovery or regenerative medicine.

Keywords: Stem cells, self-renewal, transplants, regenerative

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INTRODUCTION

Stem cells are cells that have not yet developed into specialized cells. They have the ability to differentiate, meaning that they can become cells of any organ of the body. They can also multiply to create new stem cells. Stem cells are generated in the bone marrow but are also present in our blood, as they are released from the bone marrow to the blood system. The regulators of stem cell growth at genomic and proteomic level were identified and we might be able to control stem cell in vitro [1].

The regenerative capability of a living creature was recorded as early as 330 BC although stem cell technology is just emerging; the regeneration of body parts is hardly a new concept, when Aristotle observed that a lizard could grow back the lost tip of its tail. From that time, there have been slow but steady attempts at understanding the regenerative capabilities of human being and it is only in the last 10 years that we have seen an information explosion in the area of stem cell research. Stem cells are likely to revolutionize the entire health care delivery [2].

Stem cell research is improving by leaps and bounds. These may soon become the basis for treating diseases such as Parkinson's disease, diabetes, heart failure, cerebral palsy, heart disease and host of other chronic ailments. Stem cells may also be used for screening new drugs and toxins and understanding birth defects without subjecting human volunteers to the toxins and drugs.

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 [3].

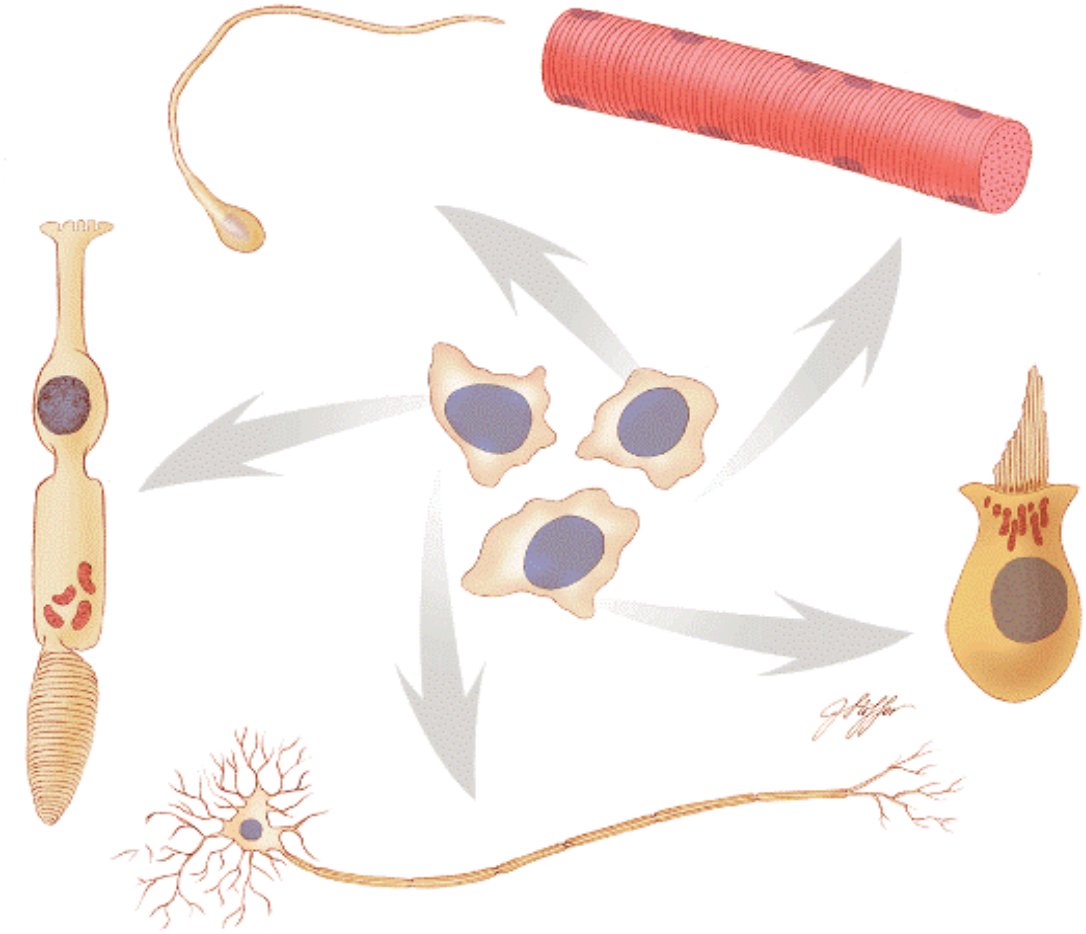

Fig. 1: Stem cells (center ones) can develop into any cell type. They are valuable as research tools and might, in the future, be used to treat a wide range of diseases

Stem cells can be used in cellular therapy to replace damaged cells or to regenerate organs. In addition, stem cells have expanded our understanding of development as well as the pathogenesis of disease. Disease-specific cell lines can also be propagated and used in drug development. We now know many of the genes involved in regulating development in different species and find remarkable conservation of genetic pathways across evolution [4].

Over the years, a number of preclinical and small clinical trials have shown that tissue regeneration can be induced when stem cells of various types – embryonic stem cells, stem cells from cord blood and bone marrow, and adult stem cells – are injected into injured or degenerated tissue. Several small clinical trials have reported varying degrees of functional improvement [5].

This article focuses on history, source and types of stem cells, stem cell application in various diseases and future enlightening communication.

❖ **The history of a medical sensation**

The potential of stem cells to revolutionize medicine [6] got a huge boost with news of an ultra-versatile kind of stem cell from adult mouse cells using a remarkably simple method [7]. This timeline takes us through the ups and downs of the stem cell rollercoaster.

Year	Title	Description
1981	Mouse beginnings	Martin Evans [8] of Cardiff University, UK, then at the University of Cambridge, is first to identify embryonic stem cells [9] – in mice.
1997	Dolly the sheep	Ian Wilmut [10] and his colleagues at the Roslin Institute, Edinburgh unveils Dolly the sheep [11], the first artificial animal clone. The process involves fusing a sheep egg with an udder cell and implanting the resulting hybrids into a surrogate mother sheep. Researchers speculate that similar hybrids made by fusing human embryonic stem cells with adult cells from a particular person could be used to create genetically matched tissue and organs.
1998	Stem cells go human	James Thomson of the University of Wisconsin in Madison and John Gearhart of Johns Hopkins University in Baltimore, respectively, isolate human embryonic stem cells [12] and grow them in the lab.
2001	Bush controversy	US president George W. Bush limits federal funding of research on human embryonic stem cells because a human embryo is destroyed in the process. But Bush does allow continued research on human embryonic stem cells lines [13] that were created before the restrictions were announced.
2005	Fraudulent clones	Woo Suk Hwang of Seoul National University in South Korea reports that his team has used therapeutic cloning – a technique inspired by the one used to create Dolly – to create human embryonic stem cells genetically matched to specific people [14]. Later that year, his claims turn out to be false [15].
2006	Cells reprogrammed	Shinya Yamanaka [16] of Kyoto University in Japan reveals a way of making embryonic-like cells from adult cells – avoiding the need to destroy an embryo. His team reprograms ordinary adult cells by inserting four key genes [17] – forming "induced pluripotent stem cells".
2007	Nobel prize	Evans shares the Nobel prize for medicine [18] with Mario Capecchi and Oliver Smithies for work on genetics and embryonic stem cells.
2009	Obama-power	President Barack Obama lifts 2001 restrictions on federal funding for human embryonic stem cell research [19].
2010	Spinal injury	A person with spinal injury becomes the first to receive a medical treatment derived from human embryonic stem cells [20] as part of a trial by Geron [21] of Menlo Park, California, a pioneering company for human embryonic stem cell therapies.
2012	Blindness treated	Human embryonic stem cells show medical promise in a treatment that eases blindness [22].
2012	Another Nobel	Yamanaka wins a Nobel prize for creating induced pluripotent stem cells [23] which he shares with John Gurdon of the University of Cambridge.
2013	Therapeutic cloning	Shoukhrat Mitalipov [24] at the Oregon National Primate Research Center in Beaverton and his colleagues produce human embryonic stem cells using therapeutic cloning [25] – the breakthrough falsely claimed in 2005.
2014	Pre-embyronic state	Charles Vacanti [26] of Harvard Medical School together with Haruko Obokata at the Riken Center for Developmental Biology [27] in Kobe, Japan, and colleagues announced a revolutionary discovery that any cell can potentially be rewound to a pre-embryonic state [28] – using a simple, 30-minute technique.
2014	Human trials	Masayo Takahashi [29] at the same Riken centre is due to select patients for what promises to be the world's first trial of a therapy based on induced pluripotent stem cells, to treat a form of age-related blindness.

❖ **Sources of Stem Cells**

There are several sources of stem cells. Pluripotent stem cells can be isolated from human embryos that are a few days old. Cells from these embryos can be used to create pluripotent stem cell "lines" —cell cultures that can be grown indefinitely in the laboratory. Pluripotent stem cell lines have also been developed from fetal tissue (older than 8 weeks of development).

The two broad types of stem cells found in people are adult stems cells and embryonic stem cells.

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.

Embryonic stem cells

- Human embryos
- Fetal tissue [30]

Blood and Bone Marrow:

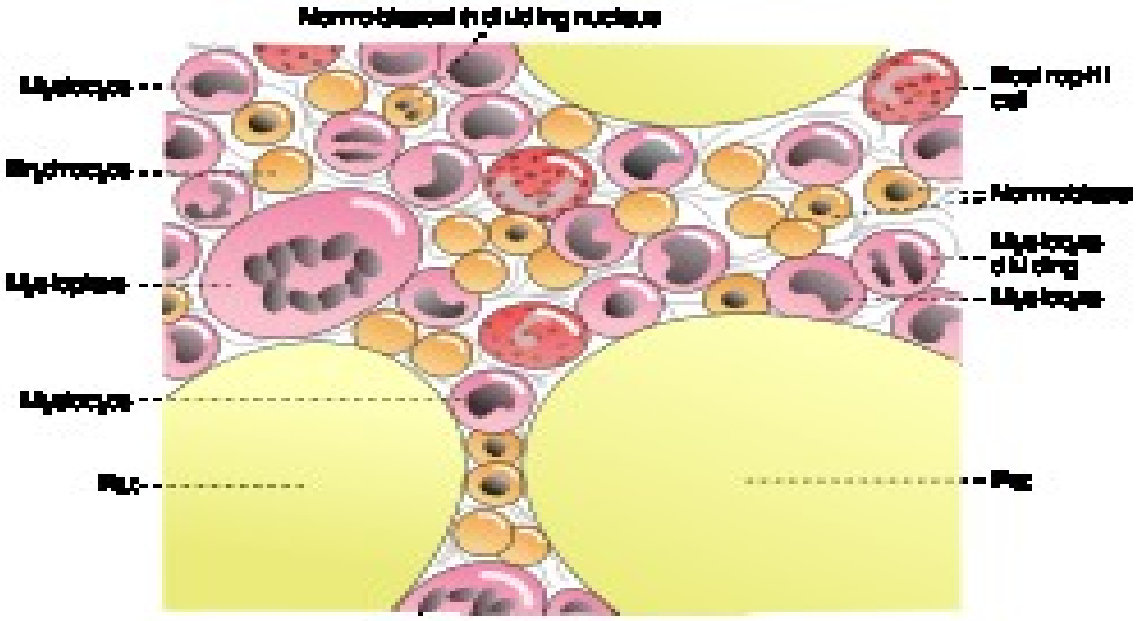

Fig. 2: Blood and Bone Marrow

Stem cells are found in bone marrow, a spongy tissue inside the bones. Stem cells develop into the three types of blood cells that the body needs:

- Red blood cells, which carry oxygen throughout the body
- White blood cells, which fight infections
- Platelets (PLATE-lets), which help the blood clot

Small numbers of stem cells also are found in the blood and in the umbilical cord (the cord that connects a fetus to its mother's placenta).

In autologous bone marrow transplantation, bone marrow is extracted from bone and given back to the same person to replenish the hematopoietic stems cells, for example in leukemia patients. For research use, stem cells from the blood are easier to obtain [31].

Bone marrow is the tissue that makes blood cells. When stem cells are needed from a bone marrow donor, the aspiration procedure uses a special needle inserted into the bone to collect the cells. Although the procedure is uncomfortable it can be tolerated by both children and adults. The collection site is often the back of the hip bone. For stem cell transplants, aspiration will likely be done in several locations to ensure plenty of stem cells are available for the transplant [32].

Cord Blood Cells:-

Autologous cord blood cells can be collected and stored at birth, and safely infused back into the individual at a later stage, without the risk of immune rejection. The use of

cord blood stem cells in treating conditions such as brain injury and type I diabetes is already being studied in humans [31].

Adipose Tissue:-

Adipose tissue is a rich source of adult stem cells. These multipotent stem cells can differentiate into many cell types, such as fat, muscle and bone cells, as well as cartilage and nerve [31].

Skin stem cells:-

Medical researchers have long studied the ability of stem cells, which can regenerate and form almost any cell type in the body, to treat numerous chronic diseases. Now skin-care brands like Lifeline and Origins are hoping that stem cells can deliver the powerful results in the cosmetics industry that they have in medicine.

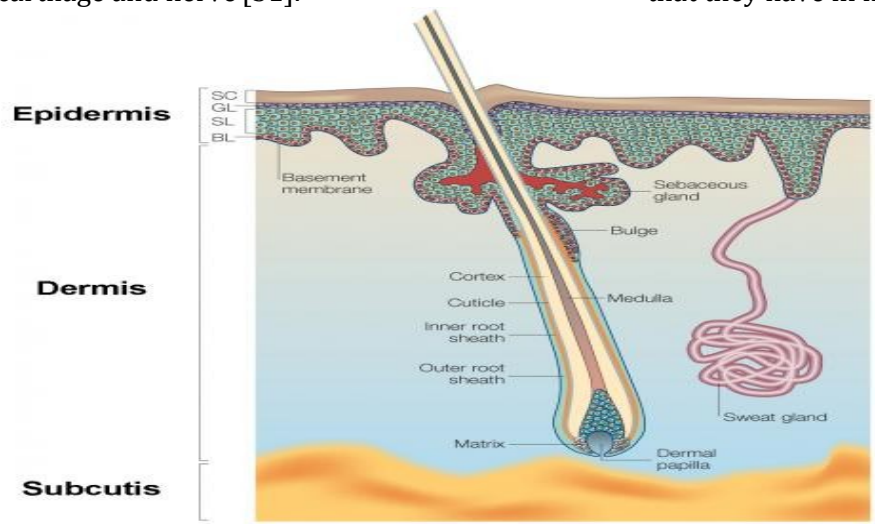

Fig. 3: Skin stem cells

Skin stem cells are responsible for constant renewal (regeneration) of your skin, and for healing wounds. So far, scientists have identified several different types of skin stem cell:

- Epidrmal stem cells are responsible for everyday regeneration of the different layers of the epidermis. These stem cells are found in the basal layer of the epidermis.
- Hair follicle stem cells ensure constant renewal of the hair follicles. They can also regenerate the epidermis and sebaceous glands if these tissues are damaged. Hair follicle stem cells are found throughout the hair follicles.
- Melanocyte stem cells are responsible for regeneration of melanocytes, a type of pigment cell. Melanocytes produce the pigment melanin, and therefore play an important role in skin and hair follicle pigmentation. It is not yet certain where these stem cells are found in humans.

Some studies have also suggested that another type of stem cell, known as mesenchymal stem cells, can be found in the dermis and hypodermis. This remains controversial amongst scientists and further studies are needed to determine whether these cells are truly mesenchymal stem cells and what their role is in the skin [32].

Other sources of human stem cells

Many scientists consider embryonic stem cells to be ideal to treat disease because they multiply extensively and can differentiate into all the cells and tissues of the body. However, to obtain them, five-day-old embryos have to be destroyed.

To avoid the ethical and political hurdles that surround stem cells taken from embryos, scientists are hunting for alternative sources.

Adult bone marrow

One promising source of stems cells might be from the bone marrow of an adult. Stem cells from adult bone marrow normally produce blood and bone marrow cells.

Bone marrow is either red or yellow, depending upon the preponderance of hematopoietic (red) or fatty (yellow) tissue. In humans the red bone marrow forms all of the blood cells with the exception of the lymphocytes, which are produced in the marrow and reach their mature form in the lymphoid organs. Red bone marrow also

contributes, along with the liver and spleen, to the destruction of old red blood cells. Yellow bone marrow serves primarily as a storehouse for fats but may be converted to red marrow under certain conditions, such as severe blood loss or fever. At birth and until about the age of seven, all human marrow is red, as the need for new blood formation is high. Thereafter, fat tissue gradually replaces the red marrow, which in adults is found only in the vertebrae, hips, breastbone, ribs, and skull and at the ends of the long bones of the arm and leg; other cancellous, or spongy, bones and the central cavities of the long bones are filled with yellow marrow.

Placental blood

The human placenta and cord blood are rich in hematopoietic progenitor and hematopoietic stem cells (HSCs), which give rise to all the blood cell types including myeloid (monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, and dendritic cells) and lymphoid lineage (T-cells, B-cells, and NK cells) cells. Since the first successful umbilical cord blood transplants in children with Fanconi anemia, cord blood banking and the therapeutic use of cord blood stem cells have grown quickly in the last two decades. Like stem cells from bone marrow, umbilical cord blood hematopoietic stem cells have been used to treat various genetic disorders including leukemia, certain cancers, and some inherited disorders.

These companies argue that in addition, the cord blood from the baby might provide a source of compatible stem cells for the baby's relations - brothers and sisters, parents and grandparents [33].

Sources of stem cells in dentistry:

Teeth are a great source of stem cells for banking. The most obvious reason is that it's easy to collect a baby tooth that's naturally falling out or a wisdom tooth being extracted.

More importantly, the dental pulp in your child's baby and wisdom teeth is an excellent source of mesenchymal stem cells.

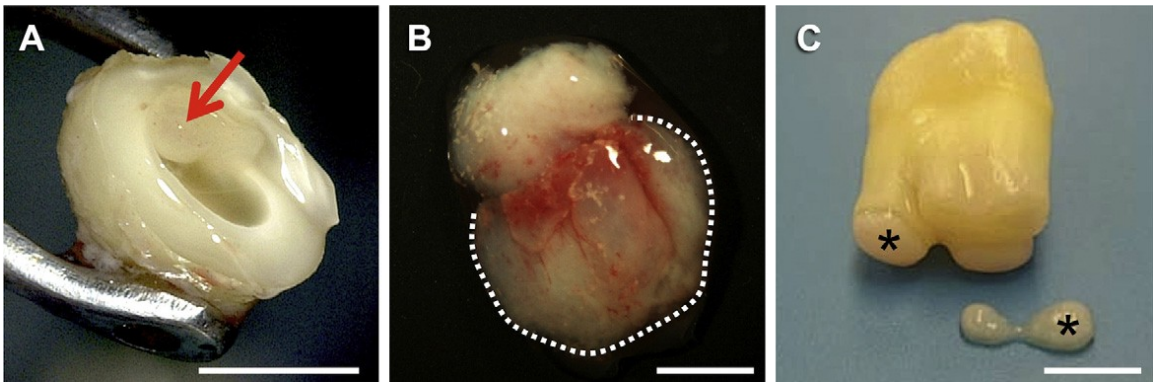

Fig. 4: shows Sources of adult stem cells in dental tissues. (A) After a tooth was cut horizontally, the pulp tissue (arrow) in the pulp chamber was exposed; this pulp provides dental pulp stem cells (DPSCs). (B) Extracted impacted third molar (10-year-old female) containing the dental follicle (dotted line) that provides dental follicle stem cells (DFSCs). Bar: 5 mm. (C) Extracted impacted third molar (18-year-old male) containing root apical papillae (asterisks) that are a source of stem cells from the apical papilla (SCAP). Bars: 5 mm [34]

Spermatogonial stem cells

The biological activities of spermatogonial stem cells (SSCs) are the foundation for spermatogenesis and thus sustained male fertility. Therefore, understanding the mechanisms governing their ability to both self-renew and differentiate is essential. Moreover, because SSCs are the only adult stem cell to contribute genetic information to the next generation, they are an excellent target for genetic modification. In this chapter, we discuss two important approaches to investigate SSCs and their cognate niche microenvironment in the mouse, the SSC transplantation assay and the long-term serum-free SSC culture

method. In the adult testis, only 0.03% of all germ cells are spermatogonial stem cells. They have the potential to self-renew and to differentiate in order to produce spermatozoa [35].

TYPES OF STEM CELL

Not all stem cells come from an early embryo. In fact, we have stem cells in our bodies all our lives. One way to think about stem cells is to divide them into three categories:

1. Totipotent stem cells: - are found only in early embryos. Each cell can form a complete organism (e.g., identical twins).
2. Pluripotent stem cells:- exist in the undifferentiated inner cell mass of the blastocyst and can form any of the over 200 different cell types found in the body.
3. Multipotent stem cells: - are derived from fetal tissue, cord blood and adult stem cells. Although their ability to differentiate is more limited than pluripotent stem cells, they already have a track record of success in cell-based therapies.

- Classification based on sources of stem cells:

1. Embryonic stem cells - are harvested from the inner cell mass of the blastocyst seven to ten days after fertilization.
2. Fetal stem cells - are taken from the germline tissues that will make up the gonads of aborted fetuses.
3. Umbilical cord stem cells - Umbilical cord blood contains stem cells similar to those found in bone marrow.
4. Placenta derived stem cells - up to ten times as many stem cells can be harvested from a placenta as from cord blood.
5. Adult stem cells - Many adult tissues contain stem cells that can be isolated [36]
6. Amniotic: Multipotent stem cells are also found in amniotic fluid.
7. Cancer stem cell: Cancer stem cells are a subpopulation of cancer cells that can self-renew, can propagate the cancer, and differentiate into the many types of cells that are found in a tumor.
8. Induced pluripotent: These are not adult stem cells, but rather adult cells (e.g. epithelial cells) reprogrammed to give rise to pluripotent capabilities.
9. Lineage: To ensure self-renewal, stem cells undergo two types of cell division. It is one of the divisions.

Embryonic

Embryonic stem (ES) cells are stem cells derived from the inner cell mass of a blastocyst, an early-stage embryo [37]. Embryonic stem cells (ESCs) are stem cells derived from the undifferentiated inner mass cells of a human embryo.

Embryonic stem cells are pluripotent, meaning they are able to grow (i.e. differentiate) into all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm.

In other words, they can develop into each of the more than 200 cell types of the adult body as long as they are specified to do so. Embryonic stem cells are distinguished by two distinctive properties: their pluripotency, and their ability to replicate indefinitely.ES cells are pluripotent, that is, they are able to differentiate into all derivatives of the three primary germ layers: ectoderm, endoderm, and mesoderm. These include each of the more than 220 cell types in the adult body. Pluripotency distinguishes embryonic stem cells from adult stem cells found in adults; while embryonic stem cells can generate all cell types in the body; adult stem cells are multipotent and can produce only a limited number of cell types.

Human ES cells are grown on a feeder layer of mouse embryonic fibroblasts (MEFs) and require the presence of basic fibroblast growth factor (bFGF or FGF-2) [38]. Without optimal culture conditions or genetic manipulation [39], embryonic stem cells will rapidly differentiate.

A human embryonic stem cell is also defined by the expression of several transcription factors and cell surface proteins. The transcription factors Oct-4, Nanog, and Sox2 form the core regulatory network that ensures the suppression of genes that lead to differentiation and the maintenance of pluripotency [40]. The cell surface antigens most commonly used to identify hES cells are the glycolipids stage specific embryonic antigen 3 and 4 and the keratan sulfate antigens Tra-1-60 and Tra-1-81. The molecular definition of a stem cell

includes many more proteins and continues to be a topic of research [41].

There are currently no approved treatments using embryonic stem cells. The first human trial was approved by the US Food and Drug Administration in January 2009 [42]. However, the human trial was not initiated until October 13, 2010 in Atlanta for spinal injury victims. On November 14, 2011 the company conducting the trial announced that it will discontinue further development of its stem cell programs [43,44]. However, these problems associated with histocompatibility may be solved using autologous donor adult stem cells, therapeutic cloning, stem cell banks or more recently by reprogramming of somatic cells with defined factors (e.g. induced pluripotent stem cells).

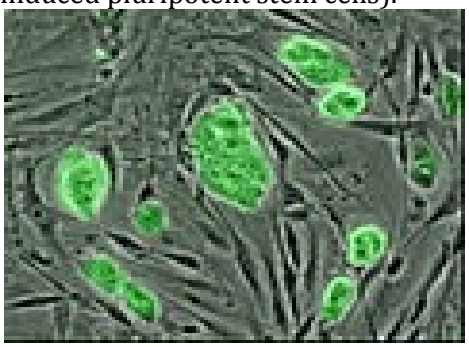

Fig. 5 A: Shows Mouse embryonic stem cells with fluorescent marker

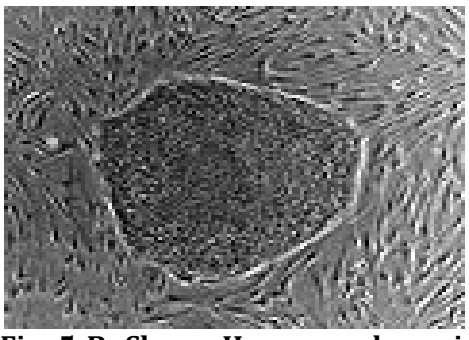

Fig. 5 B: Shows Human embryonic stem cell colony on mouse embryonic fibroblast feeder layer

Fetal

The primitive stem cells located in the organs of fetuses are referred to as fetal stem cells [45]. There are two types of fetal stem cells:

1. Fetal proper stem cells come from the tissue of the fetus proper, and are generally obtained after an abortion. These stem cells are not immortal but have a high level of division and are multipotent.
2. Extra embryonic fetal stem cells come from extra embryonic membranes, and are generally not distinguished from adult stem cells. These stem cells are acquired after birth, they are not immortal but have a high level of cell division, and are pluripotent [46].

Adult stem cells, also called somatic stem cells, are stem cells which maintain and repair the tissue in which they are found [47]. They can be found in children, as well as adults [48]. Pluripotent adult stem cells are rare and generally small in number, but they can be found in umbilical cord blood and other tissues [49]. Bone marrow is a rich source of adult stem cells [50], which have been used in treating several conditions including spinal cord injury [51], liver cirrhosis [52], chronic limb ischemia [53] and endstage heart failure [54]. The quantity of bone marrow stem cells declines with age and is greater in males than females during reproductive years [55]. Much adult stem cell research to date has aimed to characterize their potency and self-renewal capabilities [56]. In mice, pluripotent stem cells are directly generated from adult fibroblast cultures. However, mice do not live long with stem cell organs [57].

Most adult stem cells are lineage-restricted (multipotent) and are generally referred to by their tissue origin (mesenchymal stem cell, adipose-derived stem cell, endothelial stem cell, dental pulp stem cell, etc.) [58,59]. Adult stem cell treatments have been successfully used for many years to treat leukemia and related bone/blood cancers through bone marrow transplants [60]. Adult stem cells are also used in veterinary medicine to treat tendon and ligament injuries in horses [61].

The use of adult stem cells in research and therapy is not as controversial as the use of embryonic stem cells, because the production of adult stem cells does not require the destruction of an embryo. Additionally, in instances where adult stem cells are obtained from the intended recipient (an autograft), the risk of rejection

is essentially non-existent. Consequently, more US government funding is being provided for adult stem cell research [62].

Adult

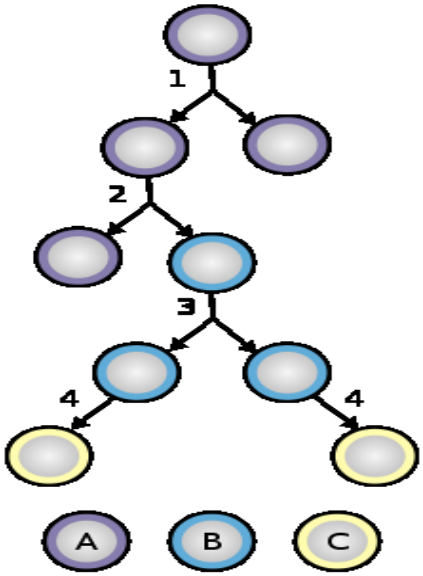

Fig. 6: Shows stem cell division and differentiation. A: stem cell; B: progenitor cell; C: differentiated cell; 1: symmetric stem cell division; 2: asymmetric stem cell division; 3: progenitor division; 4: terminal differentiation

Amniotic

Human amniotic fluid cells have been used as a diagnostic tool for the prenatal diagnosis of fetal genetic anomalies for more than 50 years. Evidence provided in the last 5 years, however, suggests that they can also harbour a therapeutic potential for human diseases, as different populations of fetal-derived stem cells have been isolated from amniotic fluid. Mesenchymal stem cells were the first to be described, which possess the higher proliferation and differentiation plasticity of adult mesenchymal stem cells and are able to differentiate towards mesodermal lineages. Amniotic fluid stem cells have more recently been isolated. They represent a novel class of pluripotent stem cells with intermediate characteristics between embryonic and adult stem cells, as they are able to differentiate into lineages representative of all three germ layers but do not form tumours when injected in vivo.

Roman Catholic teaching forbids the use of embryonic stem cells in experimentation; accordingly, the Vatican newspaper "Osservatore Romano" called amniotic stem cells "the future of medicine"[64]. It is possible to collect amniotic stem cells for donors or for autologuous use: the first US amniotic stem cells bank [65,66] was opened in 2009 in Medford, MA, by Biocell Center Corporation [67-69] and collaborates with various hospitals and universities all over the world [70].

Cord blood

After a baby is born, cord blood is left in the umbilical cord and placenta. It is relatively easy to collect, with no risk to the mother or baby. It contains haematopoietic (blood) stem cells: rare cells normally found in the bone marrow.

Haematopoietic stem cells (HSCs) can make every type of cell in the blood – red cells, white cells and platelets. They are responsible for maintaining blood production throughout our lives. They have been used for many years in bone marrow transplants to treat blood diseases.

There have been several reports suggesting that cord blood may contain other types of stem cells which can produce specialised cells that do not belong to the blood, such as nerve cells. These findings are highly controversial among scientists and are not widely accepted.

A certain kind of cord blood stem cell (CB-SC) is multipotent and displays embryonic and hematopoietic characteristics. Phenotypic characterization demonstrates that (CB-SCs) display embryonic cell markers (e.g., transcription factors OCT-4 and Nanog, stage-specific embryonic antigen (SSEA)-3, and SSEA-4) and leukocyte common antigen CD45, but that they are negative for blood cell lineage markers (e.g., CD1a, CD3, CD4, CD8, CD11b, CD11c, CD13, CD14, CD19, CD20, CD34, CD41a, CD41b, CD83, CD90, CD105, and CD133)[71,72].

Additionally, CB-SCs display very low immunogenicity as indicated by expression of a very low level of major histocompatibility complex (MHC) antigens and failure to stimulate the proliferation of allogeneic lymphocytes [71,73]. They can give rise to three embryonic layer-derived cells in the presence of different inducers [71,74]. More specifically, CB-SCs tightly

adhere to culture dishes with a large rounded morphology and are resistant to common detaching methods (trypsin/EDTA) [71,73,74]. CB-SCs are the active agent in stem cell educator therapy, which has therapeutic potential against autoimmune diseases like type 1 diabetes according to studies by Yong Zhao [72,75-77].

Induced pluripotent

These are not adult stem cells, but rather adult cells (e.g. epithelial cells) reprogrammed to give rise to pluripotent capabilities. Using genetic reprogramming with protein transcription factors, pluripotent stem cells equivalent to embryonic stem cells have been derived from human adult skin tissue [78-80]. Shinya Yamanaka and his colleagues at Kyoto University used the transcription factors Oct3/4, Sox2, c-Myc, and Klf4 [78] in their experiments on cells from human faces. Junying Yu, James Thomson, and their colleagues at the University of Wisconsin–Madison used a different set of factors, Oct4, Sox2, Nanog and Lin [78], and carried out their experiments using cells from human foreskin.

As a result of the success of these experiments, Ian Wilmut, who helped create the first cloned animal Dolly the Sheep, has announced that he will abandon somatic cell nuclear transfer as an avenue of research [81].Frozen blood samples can be used as a source of induced pluripotent stem cells, opening a new avenue for obtaining the valued cells [82].

Lineage

To ensure self-renewal, stem cells undergo two types of cell division (see Stem cell division and differentiation diagram). Symmetric division gives rise to two identical daughter cells both endowed with stem cell properties. Asymmetric division, on the other hand, produces only one stem cell and a progenitor cell with limited self-renewal potential. Progenitors can go through several rounds of cell division before terminally differentiating into a mature cell. It is possible that the molecular distinction between symmetric and asymmetric divisions lies in differential segregation of cell membrane proteins (such as receptors) between the daughter cells [83].

An alternative theory is that stem cells remain undifferentiated due to environmental cues in their particular niche. Stem cells differentiate when they leave that niche or no longer receive those signals. Studies in Drosophila germarium have identified the signals decapentaplegic and adherens junctions that prevent germarium stem cells from differentiating [84,85].

Challenging the terminal nature of cellular differentiation and the integrity of lineage commitment, it was recently determined that the somatic expression of combined transcription factors can directly induce other defined somatic cell fates; researchers identified three neural-lineage-specific transcription factors that could directly convert mouse fibroblasts (skin cells) into fully functional neurons. This "induced neurons" (iN) cell research inspires the researchers to induce other cell types. It implies that all cells are totipotent: with the proper tools, all cells may form all kinds of tissue [86].

Cancer stem cell

The discovery of normal adult stem cells, able to self-renew and regenerate damaged tissue, has led to the idea that cancer may either originate from adult stem cells or contain stem cell-like cells that are self renewing, resistant to therapy and able to seed new tumour growth. 'Identifying the stem cell-like cells within cancers and understanding their properties will be crucial if effective anti-cancer therapies are to be developed' says Colin Goding who works on melanoma, one of the most aggressive types of cancer.

John Dick at the University of Toronto identified the first cancer stem cell in 1997. Michael Clarke, then at the University of Michigan, later found the first cancer stem cell in a solid tumor, in this case breast cancer. Now at Stanford University School of Medicine, Clarke and his group have now found cancer stem cells in colon cancer and head and neck cancer [87].

❖ **Stem cell therapy**

Cell based therapy

The concept of cell-based therapy (or simply cell therapy, as it is sometimes

called) is to repair, replace or supplement damaged or diseased cells with healthy cells. The work of Stem Cells scientists has already generated the means to supply stem cells, which upon transplantation, can differentiate into healthy new cells or tissues, and which may thereby be capable of alleviating or potentially even curing a broad array of intractable conditions [88].

Stem cell transplantation

Stem Cell Transplantation is one of the innovative treatments offered at Apollo Hospitals. This facility is provided by Apollo Hospitals Ahmedabad and has shown steady growth since inception.

What is Stem Cell Transplantation?

Stem Cell Transplantation is an exciting area of medicine. It is a well established treatment for several cancers and diseases of blood since the last few decades.

Indications

» Autologous Transplant (Stem Cells collected from one's own body)

- Hodgkin's & Non Hodgkin's Lymphoma: For relapsed / refractory cases, it is standard therapy and in most such cases, it is the only curative option.
- Myeloma: Although not curative, it is standard treatment as a part of initial therapy, as it prolongs survival substantially.
- Leukemia: Acute Myeloid Leukemia as part of consolidation therapy, to increase chance of cure in this disease.

» Allogenic Transplant (Stem Cells collected from someone else's body)

- Thalassemia
- Several other genetic disorders, especially with single gene defects
- Aplastic Anemia
- Chronic Myeloid Leukemia
- High Risk AML & Relapsed AML
- Relapsed ALL (Acute Lymphocytic Leukemia)
- As an option in several advanced or refractory haematological malignancies eg. follicular lymphoma, CLL, myeloma etc

What the procedure achieves

The procedure serves mainly 3 purposes-

1. Replacing a missing gene e.g. in Thalassemia, Sickle cell disease and many genetic disorders. These are diseases where the person is otherwise normal, except for one missing gene, and replacing that gene is curative.
2. Allows use of high doses of anticancer therapies, which may lead to loss of bone marrow. Without stem cells support, marrow will recover only after a long time, resulting in high complication rate from infections or bleeding. Infused stem cells provide early recovery of blood cells. It considerably lowers the risk of low blood counts due to marrow suppression. Thus it is one form of "Supportive Therapy" and not a treatment of cancer by itself. This is the case in Autologous (self) transplant and in majority of Allogeneic transplants.
3. Some "Graft versus Disease activity", more commonly known as "Graft v Leukemia effect", in Allogeneic transplant, especially evident in chronic myeloid leukemia.

Process of Transplant - How is it done?

There is no surgery involved in Transplant, for the patient or donor. It is very safe procedure for a donor. Nothing is lost permanently in the body e.g. as in kidney Transplant. Stem cells regenerate in few days. For the same reason there are over 1 crore (10 million) volunteer donors for stem cell transplant in USA. Stem Cells are infused into the patient through a live Blood Transfusion.

Types of Transplant

The Stem Cells can be collected from patient's own body or can be harvested from another person. This other person is known as donor.

Autologous transplant

Stem cells are taken from the patient either by bone marrow harvest or apheresis (peripheral blood stem cells) and then given back to the patient after conditioning treatment.

Allogeneic transplant

The donor has the same HLA type as the patient. Stem cells are taken either by bone marrow harvest or aphaeresis (peripheral blood stem cells) from a HLA matched donor, usually a brother or sister. Other donors for allergenic bone marrow transplants include the following:

- An identical twin - A syngeneic transplant is an allergenic transplant from an identical twin. Identical twins are

considered a complete genetic match for a transplant.

- Unrelated transplants (UBMT or MUD, for matched unrelated donor) - The HLA matched stem cells are from an unrelated donor, usually found through the national registries.
- Umbilical Cord Blood transplant - Stem cells are taken from an umbilical cord immediately after delivery of an infant. The stem cells are tested, typed, counted and frozen until they are ready to be transplanted.

Stem cell transplant is an exciting area of medicine. It is a well established treatment for several cancers and diseases of blood, for the past few decades.

1. **Diabetes (Type 1 & 2)**

Stem Cell therapy is most effective treatment for diabetes mellitus because scientists have already proved that mesenchymal stem cells can be differentiates into pancreatic beta-islet cells after in-vitro culturing. Diabetes is metabolic and autoimmune disease means our body attacks to our own pancreatic cells as foreign cells so the treatment with autologous bone marrow derived mesenchymal stem cells (MSCs) provide immune-regulatory properties and stops the immune attack by secreting anti-inflammatory cytokines (IL-10, TGF-beta and IL-1). Stem cell treatment shows good improvement in diabetes patient because bone marrow derived stem cells concentrate (CD44+, CD31+, CD34+) has the capacity to regenerate the beta islets cells [89-91].

2. Acute/Chronic Liver Disease

A lot of clinical trials have shown that Stem cell therapy is successful in liver related disorder like acute liver failure or end stage liver disease (ESLDs). So the concept is that stem cell has the potential to diffrentiated into hepatocyte cells after transplantation in liver disease and show improvement for all the evaluations in liver related disorder [92,93].

3. Muscular Dystrophy

There is no cure for Muscular Dystrophy but stem cell therapy mainly focuses on the inflammatory response in Muscular Dystrophy as a target for mesenchymal stem cell (MSC) therapy. In contrast to other cell based therapies attempted in DMD, Mesenchymal Stem Cells have the advantages to treat Muscular Dystrophy [94,95].

4. Kidney diseases

Currently the CKD patients are increasing worldwide and there is no appropriate treatment available for chronic kidney disease. So based on successful experiment on the animals, now researchers are hopeful of its application on human being. Chronic kidney disease causes dysfunction of the cells from vascular, interstitial, glomerular and tubular compartments, which triggers the release of fibrogenic cytokines and recruitment of inflammatory cells to injured kidneys. The rapid interposition of scar tissue probably confers a survival advantage by preventing infectious microorganisms from invading the wound, but prevents subsequent tissue or renal cell regeneration.
After Stem cell transplantation into CKD patient provides improvement like reduced creatinine level because stem cells like mesenchymal stem cell has the property to differentiate into renal specific cells also like nephron, tubular epithelial cells and others. After infusion of stem cells into the CKD patients via intravenously then these stem cells automatically migrate to the injured/inflammated site and then start to regenerate the damaged/injured renal tissue and also induce/activate resident stem cells in the renal via para/autocrine signaling. So mesenchymal stem cells therapy provides better environment to regenerate the damaged cells via differentiating into the renal specific cells and also induce the resident stem cell [96].

5. Lung Disease

Types of lung disease treated by stem cell therapy:-

1. Idiopathic Pulmonary Fibrosis (IPF):- IPF is an interstitial inflammatory disease with unknown etiology characterized by scarring and fibrosis of the lungs that ultimately result in terminal pulmonary insufficiency. Mesenchymal stem cell transplantation in IPF disease provides

A) Secrete anti-inflammatory cytokines so stop or reduce further inflammation and collagen deposition (Neuringer et al.; 2006, Ortiz et al., 2003) on lung cells.

B) Regenerate the lung injured/damaged cells via differentiating into lung progenitor cells.
C) Induce/activate resident stem cells in the lung via para/auto-crine signaling.
D) Mesenchymal Stem Cells transplantation provides anti-apoptic, immune modulation, anti-inflammatory and anti- fibrosis properties so this environment may accommodate the cells indefinitely and control their self-renewal and progeny production in vivo or refine their ability to induce host reparative processes.
2. Chronic obstructive pulmonary disease (COPD):- COPD is a lung disease which includes both chronic bronchitis and emphysema. COPD is one of the leading causes of death and disability in all over the world and which results predominantly from cigarette smoking.
Forms of COPD:
There are two main forms of COPD
"Chronic bronchitis" is a chronic inflammation of the bronchi (medium-size airways) in the lungs which involves a long-term cough with mucus.
"Emphysema" which is called an obstructive lung disease because the destruction of lung tissue around smaller sacs, called alveoli, makes these air sacs unable to hold their functional shape upon exhalation. Emphysema is most often caused by tobacco smoking and long-term exposure to air pollution involves destruction of the lungs over time [97].

Fig. 7: Lung Disease

6. Ischemia:

Currently available surgical or endovascular revascularization like angioplasty and coronary artery bypass graft (CABG) and drug based method are available treatment for Myocardial Infarction and coronary arterial disease.
But the stem cells transplant with angioplasty and CABG induces neo-myogenesis and neo-vascularization. Because neo-myogenesis or muscle regeneration is very difficult task in heart attack patient by its self even after angioplasty and CABG. In case of angioplasty, stem cell transplantation performs into the infarct-related coronary artery and induces both new blood formation and muscle regeneration and also decrease the further chance of heart attack. But in case of bypass surgery (or CABG), stem cell transplantation perform into the transplanted artery and directly into the infracted myocardium and induce new muscles regeneration and also decrease the further chance of heart attack [98].

7. RHEUMATOID ARTHRITIS (RA)

Currently, RA is treated with immune suppressive agents such as steroids, methothrexate, cyclosporine, gold, and more recently infliximab (Remicade). Despite inducing temporary improvement, these approaches possess long-term adverse effects due to non-specific inhibition of immune responses.
Stem cell therapy induces healing activity with various forms of arthritis. Besides healing activity of damaged tissues in RA related patients, stem cells provide immunomodulation (to shut off

pathological responses) and also start producing anti-inflammatory cytokines (especially Mesenchymal stem cells) to stop the immune attack on synovial fluid of joints.

Physical therapy is one of the parts of stem cell therapy because physical therapy stimulates muscles, bones, and joints through exercise or other methods. The result is more strength, tone, and overall fitness [99].

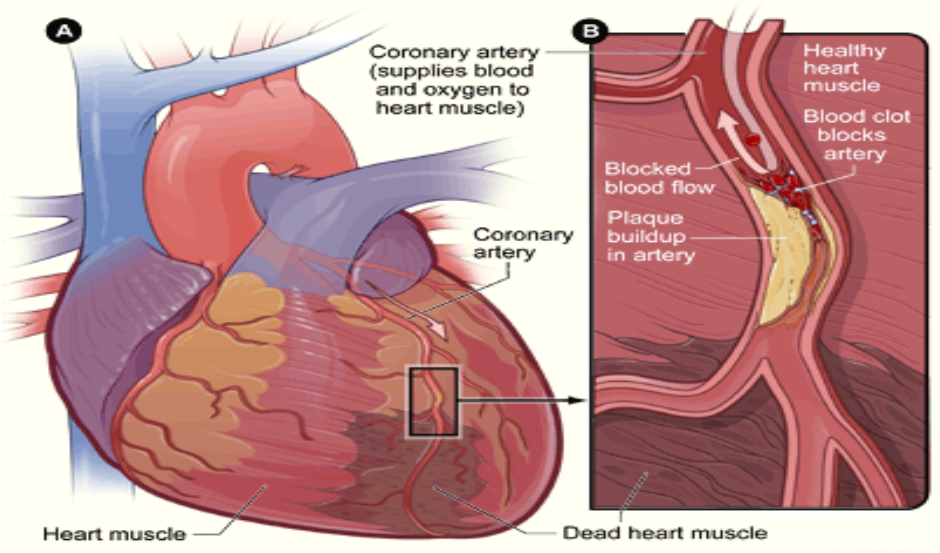

Fig. 8: Coronary Arterial Disease

8. Coronary Arterial Disease

Currently available surgical or endovascular revascularization like angioplasty and coronary artery bypass graft (CABG) are the main treatments for coronary arterial disease and myocardial infarction. Coronary artery disease involves not only the blockage of major arteries, but also the death of small blood vessels or capillaries and heart muscle. But the stem cells transplant with angioplasty induces the neo-vascularization and also reduces frequency of angina episodes. Transplantation of Bone Marrow Stem Cells into the infarct-related coronary artery via an angioplasty (by using with balloon catheter) provides neoangiogenesis (new vessel formation) in ischemic coronary artery. Bone Marrow Mononuclear cells (BM-MNC or Bone Marrow stem cells concentrate) is more useful in coronary arterial disease to get more re-vascularization because it contains all needed regenerative cells and also containing angiogenic growth factors such as vascular endothelial growth factor (VEGF) which improves endothelium-dependent vasodilation in patients with coronary arterial disease [100,101].

9. Peripheral Arterial Disease

Currently available surgical or endovascular revascularization treatment cannot be done in those patients who could not have arterial bypass graft surgery due to poor run-off or poor general medical condition. There may be some side effect by using the conventional method for peripheral arterial disease.

Bone Marrow derived Stem cells transplantation via intra-muscular and intra-arterial (by using with balloon catheter) provide neoangiogenesis (new vessel formation) in ischemic tissue. It is the only most effective treatment option for Critical Limb Ischaemia patients (severe peripheral arterial disease) and to reduce the major amputation. For obtaining a high local stem cell concentration in the ischemic leg, we use a new route of transplantation by combining both intra-arterial (by using with balloon catheter) and intra-muscular injections so that high concentration of stem cells can reach into all ischemic muscles. Bone Marrow Mononuclear cells (BM-MNC or BM stem cells concentrate) is

more useful in peripheral arterial disease to get more re-vascularization because it contains all needed regenerative cells and the hematopoietic stem cells (HSCs) in the BM-MNC release angiogenic growth factors such as vascular endothelial growth factor (VEGF) which improves endothelium-dependent vasodilation in patients with peripheral arterial disease. Autologous BM-MNC implantation improves ankle-brachial index (ABI) measurements, SaO_2 relief of rest pain, and ulcer healing and also to reduce major amputations [102,103].

10. Adult stem cells as therapeutic agents for wound treatment

A wide range of evidence from animal studies indicates that engrafting within multiple tissues in the body gives rise both to tis- sue-specific cells and releasing soluble factors that trigger the tissue's own endogenous repair pathways. It is now well known that stem cells can significantly stimulate the regeneration of endogenous cells and promote tissue recovery, which has undoubtedly shown cu- taneous stem cells to be superior as powerful healing agents for wound regeneration.

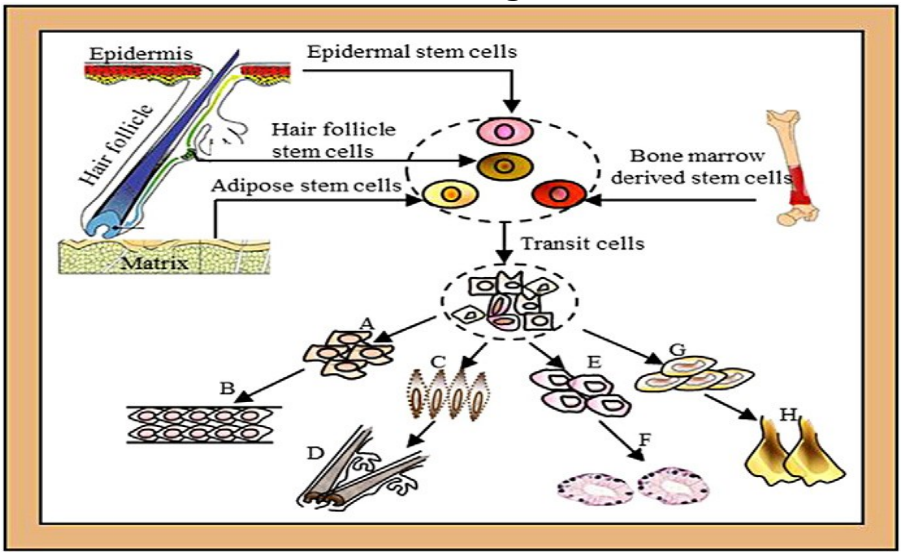

Fig.9: shows the differentiation characteristics of several tissue-specific adult stem cells for wound healing, because of their potential, demonstrated by several studies [104], to generate "skins"containing components observed in natural tissues, such as sweat gland and hair follicle. Hitherto, the implantation either of au- tologous or allogenic bone marrow-derived stem cells (BMSCs) has been demonstrated to accelerate wound healing and increase the for- mation of new vessels and granulation tissues [105,106]. The main dis- advantage of BMSCs, however, lies in the obvious impairment of their differentiation abilities with increasingage [107].

11. What might stem cells offer People with Parkinson's disease?

As the symptoms of PD are caused by the death of brain cells that produce the chemical dopamine, researchers have suggested that stem cells might be coaxed into developing into new dopamine-producing neurons which could replace those dying in the PD brain. There are two ways this might be done. Firstly, stem cells in the brain might be encouraged to reproduce in greater numbers and to travel to the pertinent areas of the PD brain where they would be encouraged to develop specifically into mature dopamine-producing neurons. This would likely be achieved by manipulating the chemical environment of stem cells to encourage them to multiply, to migrate within the brain and to develop into dopamine cells. Secondly, stem cells could be used to produce new dopamine cells outside the body harvested from human embryos or from a variety of body tissues during life,

such as from the umbilical cord or the nose. Recently it has also become possible to produce stem cells in a laboratory by genetically manipulating other body cells, such as skin cells. Stem cells from any of these sources would then be multiplied and grown into dopamine-producing neurons within a laboratory, before being surgically reintroduced back into the brain. Either of these approaches would aim to replace brain cells dying in PD with new dopamine-producing cells, thus increasing the amount of dopamine in the brain's motor circuits and restoring normal movement [108].

12. MSCs for the treatment of cancer

One of the other exciting research areas that utilize MSCs for cellular therapies is in the field of cancer. Two recent studies examine ability of MSCs to home to tumors to treat metastatic cancer [109,110]. Progress of this kind would be a major breakthrough in the treatment of solid tumors. The first study from the London Research Institute reported that MSCs engineered to produce and deliver tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) home to and kill cancer cells in a lung metastatic cancer model. This study was the first to show a significant reduction in metastatic tumor burden with frequent eradication of metastases using induced TRAIL expressing MSCs. The researchers concluded that this method 'would have a wide potential therapeutic role, including the treatment of both primary tumors and their metastases, possibly as an adjuvant therapy in clearing micro-metastatic disease following primary tumor resection' [110]. TRAIL has also been used with umbilical cord blood [111]. A second study takes advantage of MSC homing capacities by combinatorial treatment with intraperitoneal (IP) injections of 5fluorouracil (5-FU) and targeted interferon beta (IFNb) gene therapy in UC-MSCs to treat metastatic human breast cancer in SCID mouse lung cancer [109].UC-MSCs were found in the lung and not in other observed tissue, although this is not surprising as there is overwhelming evidence that intravenous injected cells initially home to the lung [112]. Although both treatments alone, significantly resulted in reductions in lung tumor area, the combined treatment of IFNb transduced UC-MSCs and 5-FU resulted in greater lung tumor reduction, compared to each treatment alone [109] .Although only two cancer studies utilizing MSCs are highlighted here, the use of MSCs to combat tumors continues to progress toward therapeutic utilization.

13. MSCs for the treatment of Heart disease

There are many different heart conditions for which stem cell treatments are potentially valuable. The rationale to use MSCs to treat heart conditions is based on the ability of MSCs to home to areas of injury/inflammation and/or on their ability to down regulate the immune response and support the tissue repair process. Stem cells may be shown to reduce the amount of scar tissue and increase the pumping strength of the heart in myocardial infarcted patients. According to the National Heart, Lung and Blood Institute, 1.1 million people suffer heart attacks in United States annually.

Coronary heart disease, which causes heart attacks and angina, is a leading cause of death in the United States with nearly 450,000 related fatalities in 2005, according to the American Heart Association. As such, cellular therapies to treat cardiac disease are aggressively being pursued, and cardiac stem cell therapies could be commonplace within several years. BM-MSCs have been shown to benefit patients early after myocardial infarction by exhibiting lower incidence of arrhythmias [113-115]. A recent study using a mesenchymal stem cell therapy presented by Osiris Therapeutics, Inc. showed that an intravenous injection of bone marrow-derived mesenchymal stem cells314 repaired heart damage in patients who had experienced heart attacks within 10 days [113,114]. The trial now has moved to a phase II study in 50 hospitals in the United States. However, one of the limitations of BM-MSCs cell therapies is the difficulty to expand

early-passage BM-MSCs to sufficient numbers and doses required to have a therapeutic benefit in the patient [116,117]. This is due primarily to increased senescence of BM-MSCs when expanded in vitro (unpublished data).

❖ **Non-therapeutic applications of stem cell research**

Given the promise of utilizing stem cells to treat or cure many illnesses plaguing humanity, there are numerous companies and academic institutions enthusiastically exploring using stem cells for non-therapeutic applications. The types of applications considered are designed to minimize the risk of harmful side effects such as tumor formation and to avoid some of the complicated medical problems associated with diseases such as cancer and diabetes.

Adult stem cells are being used for a not-quite-surgical procedure that re-contours faces using a mixture of the patient's own fat and stem cells. This procedure is reported to enable the implanted fat cells to better "take hold" in its new location and become part of the face. In addition, these added stem cells appear to increase the blood supply to the skin, which enhances its appearance and may increase the removal of aged collagen which is reported to contribute to wrinkle formation.

Maintaining a healthy life with a younger look has become increasingly important to many people as they age. The ability to achieve a younger look, however, is no longer such a difficult feat as stem cell therapy offers an “ideal solution.” Stem cells are thought to have the ability to enhance the removal of aged collagen, promote the proliferation of elastin synthesis, and bolster the production of new skin cells to remove wrinkles [118].

Hair Restoration

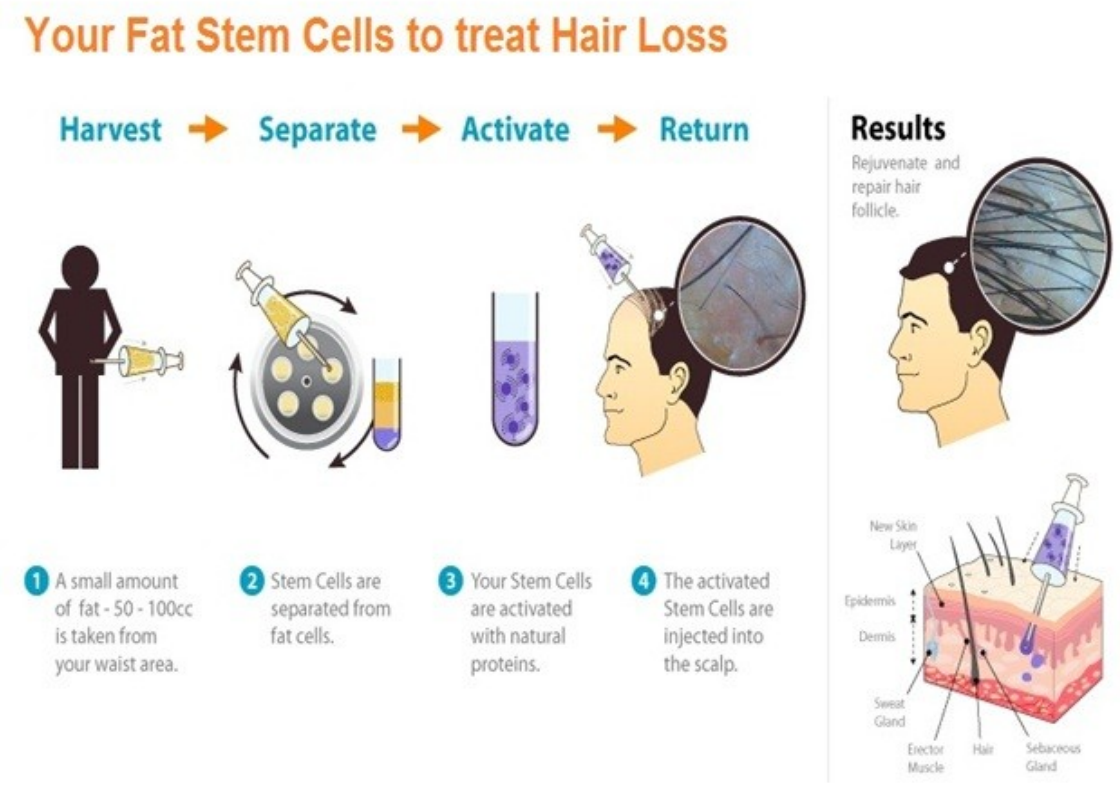

Fig. 10: Hair Restoration

Histogen, Inc., a SC company working on hair restoration among other applications, presented clinical evidence at the International Society of Hair Restoration Surgeons (ISHRS) Annual Scientific Meeting in Amsterdam July 22-26, 2009. They reported the use of adult stem cell technology to stimulate hair growth. According to Histogen, its Hair Stimulating Complex contains naturally secreted

embryonic proteins and growth factors that induce new follicle formation, and as well, hair growth and thickening of the hair when injected into the scalp.

Cotsarelis and colleagues reported [119] that scalps of men with male-pattern baldness (whose hair follicles are present but abnormally small) had a normal quantity of stem cells, but lack sufficient progenitor cells that generate hair. A treatment that stimulates stem cells to produce a normal complement of progenitor cells might be a solution to typical male-pattern baldness. If this approach proves viable, the commercial pressure and social demand to develop and sell this treatment would be very great. While baldness is seen as a cosmetic problem, treating it may not be without risks. Stimulating progenitor cells to grow where something has shut them off could conceivably produce tumors [119].

Tooth Restoration

Maintaining strong teeth and a healthy mouth is increasingly seen as a key component of health as we age. Dental medicine is advancing on many fronts, and scientific development to regenerate natural tooth material and structure would be a very valuable new therapeutic approach. Research has shown that the mesenchymal stem cells in bone marrow are a rich source of adult stem cells. Future studies are planned to examine whether these stem cells can be used in tooth regeneration and repair [120]. Dental pulp stem cells form vascularized pulp-like tissue and are surrounded by a layer of odontoblast-like cells expressing dentin proteins similar to those found in natural dentin. When seeded onto human dentin surfaces and implanted into immune-compromised mice, dental pulp stem cells created dentin-like structures that were deposited on the dentin surface [121].

❖ **Stem cells and tissue engineering**

Since stem cells are highly regulated by their microenvironment or the niche in which theyreside, efforts are on to provide constructs that can mimic the cell milieu through development of tissue-engineered scaffolds [122], These scaffolds also temporarily provide biomechanical support for cells until they are able to produce their own extra-cellular matrix [122]. Better control of the tissue formation process is an additional advantage. Scaffolds are typically fabricated by natural materials, which are inherently bioactive but lack mechanical strength, or synthetic materials, which lack inherent bioactivity but could be mechanically strong and can be fabricated with the desirable macro- (shape) and micro architecture (pore size, porosity). Numerous types of biomaterials both man-made or from natural sources are continually being discovered [123]. Efforts are being carried out to modify the surface of these materials, to guide, and enhance stem cell differentiation. Initially, scaffolds were designed to be bio inert. Currently, biomaterials are made to interact with the cells that release growth factors, genes, or other signals in a time-dependent manner [123–125]. Based on these active bio-materials, the conventional two dimensional (2-D) culture models have now paved the way for three-dimensional (3-D) culture environments that mimic the in vivo environments more closely and hence are more conducive to regulating stem cell proliferation and differentiation [126]. Elements of the extracellular matrix and stoma MSCs have gained increasing attention as potentially crucial mediators in developing and maintaining the characteristics of 3-D cell cultures. Fibrin alone or in combination with other materials has emerged as an important biological scaffold for stem cells to regenerate adipose tissue, bone, cardiac tissue, cartilage, liver, nervous tissue, ocular tissue, skin, tendons, and ligaments [127]. Culture on fibrous biodegradable scaffolds that mimic basement membrane texture has resulted in an increased expansion of both HSCs and ESCs [122]. Similarly, the immobilization of cell associated Notch ligand has shown to increase the self-renewal of HSCs [128]. A perfect tissue engineered scaffold is elusive at present. The scaffold should not only support attachment, spreading growth and differentiation of cells but also control inflammation and foreign body reaction. It should be biodegradable into non-toxic products, sterilizable and manufacturable.

It should offer options to deliver drugs, cytokines and genes. The set of criteria would appear demanding, but has to be met for the tissue-engineered scaffolds to be effective.

❖ **Stem cell research in India**

Stem cell research has gained considerable impetus in India in the recent years. Draft guidelines for stem cell research in the country have been formulated jointly by the Department of Biotechnology and Indian Council for Medical Research. Several groups are actively and enthusiastically pursuing the field with reasonably good results. At Christian Medical College (CMC), in Vellore, a total of 626 transplants have been performed in 595 patients, with 28 patients having more than one transplant from October 1986 to December 2006 [129]. Besides, CMC Vellore, autologous and allogenic bone marrow or blood stem cell transplantation is being performed at other hospitals such as All India Institute of Medical Sciences (AIIMS), New Delhi and Tata Memorial Hospital, Mumbai [130–132]. AIIMS has also set up the country's first cord blood bank for isolation of cord blood stem cells for in-house patients. At the L V Prasad Eye Institute, Hyderabad, transplantation of autologous cultivated limbal stem cells in patients with limbal stem cell deficiency, has shown a successful outcome with a stable ocular surface without conjunctivalization [133]. Small scale phase-I clinical trials using bone marrow stem cells have been reported for the treatment of diabetes at Dr. H L Trivedi Institute of Transplantation Sciences, Ahmedabad [134], acute myocardial infarction at Nizam's Institute of Medical Sciences, Hyderabad [135], Sir H N Hospital and Research Centre, Mumbai [136] and no ischemic dilated cardiomyopathy at AIIMS, New Delhi [137]. At Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Trivandrum, Procedures for the isolation and expansion of EPCs from peripheral blood of patients with CAD have been optimized [138]. Recent strategies are now directed towards augmenting the angiogenic potency of these cells by modulation with endothelial nitric oxide synthase gene transfer. Besides EPCs, ckitpositive stem cells have been isolated from atrial biopsies of CAD patients and also induced to differentiate into beating cardiospheres [139]. At the biomedical technology wing of SCTIMST, recent studies have reported that platelet rich plasma in combination with goat bone marrow-derived MSCs cultured on bioactive ceramic scaffolds leads to a much faster sequence of healing events in large segmental bone defects in a goat femur model [140]. Stem cell research at the Centre for Cellular and Molecular Biology, Hyderabad has been focusing on the genetic and epigenetic mechanisms governing the transient dormancy and activation of satellite cells, the stem cells in adult muscle tissues [141,142]. Vanikar et al have reported the generation of 30 healthy hESC lines from 33 voluntary oocyte donors using a donor somatic cell nuclear transfer technique on 190 oocytes [143] Researchers at National Brain Research Centre, Gurgaon and National Centre of Cell Sciences, Pune are working towards the differentiation of hESCs into neural stem cells [144–146]. Very recently, Jagatha et al have demonstrated the potential of FGF2-induced ES cell derived neural progenitors (ES-NPs) to generate retinal ganglion-like cells in vitro upon differentiation [147]. At the Reliance Life Sciences, Mumbai, functional dopaminergic precursor neurons from human embryonic stem cells (hESCs) have been recently reported. Transplantation of these precursor neurons into the lesioned rat model of Parkinson's disease has also shown to elicit significant reversal of lesion induced motor deficits sustained up to the end of 1 yearlong study period [148] Researchers at the Reliance Life Sciences have also demonstrated the generation of spontaneously beating cardiomyocytes using FGF from ESCs [149] .Studies at the Manipal Institute of Regenerative Medicine, Bangalore are directed towards the optimization of culture conditions of human MSCs with an attempt to obtain large numbers, preserve their characteristics and multilineage differentiation potential for therapeutic uses [150]. They have also reported the derivation of FGF2 expressing germ layer derived fibroblast cells from hESC lines for use as a feeder layer for culture of hESCs.

These feeders could support the pluripotency, karyotypes and proliferation of hESCs with or without FGF2 in prolonged cultures as efficiently as that on mouse embryonic fibroblasts [151].

❖ **Current challenges and future Possibilities**

Besides the overwhelming promise of stem cells in various cellular therapies, their clinical and practical use is constrained by several technical and ethical issues. The biggest hurdle for the clinical use of adult stem cells is the small number of cells that can be isolated from any adult tissue. The identification of cells and factors in the so called 'stem cell niche' affecting the growth and differentiation of resident adult stem cells may be one possible answer. For example, the bone marrow stromal cells are known to promote proliferation and differentiation of HSCs in long-term cultures [152]. The other approach is based on introduction of genes in the supporting feeder layer of cells that inhibits differentiation of target cells. The upregulation of notch ligands such as Jagged-1 and Delta in the stromal cells by gene modification strategies has been demonstrated to promote the expansion of stem cells without inducing differentiation. Another technique actively pursued is the usage of modified stem cells. Based on our understanding of the molecular pathways responsible for self-renewal and proliferation of stem cells as well as discoveries of new genes that control stem cell proliferation and differentiation, novel strategies have come up. For example, HOX genes that are expressed during early development and which govern various processes including bodypart patterning have been shown to increase the self-renewal potential of HSCs [153]. Destruction of life in the form of an embryo has been a major ethical objection in embryonic stem cell derivation and research in several western countries. One way that has been suggested to circumvent the objection is to fuse existing hESCs with an adult somatic cell, generating a cell line that retains ESC specific properties and yet has the genotype of the somatic cell [154]. There is however no technology available at present to selectively remove all the ESC chromosomes while retaining the somatic cell chromosomes. Development of such a technology is potentially expensive and will presumably take many more years. Other approach is the generation of induced pluripotent cell lines from induced somatic cell dedifferentiation. In this method, the adult somatic cells are genetically modified and reprogrammed to undergo a process of dedifferentiation. Availability of methods for growth and maintenance of ESC in culture present another major obstacle to their potential clinical use. Conventionally, hESC lines are grown in a medium containing animal serum as a source of nutrients and growth factors and then on mouse-derived fibroblast as feeder layers. The use of any cell based therapeutic agent in humans must however be free of animal contamination. In this direction, some laboratories have successfully cultured hESCs in a serum-free defined medium on human cellderived feeders or even in feeder free conditions [155,156].

The risk of tumor formation following transplantation of hESC is another factor to be considered. Studies with both ESCs and ES derived differentiated cells have shown that they can form teratocarcinomas in adult mice if injected subcutaneously, intramuscularly or into the testis [157,158]. The suitability of ESCs for transplantation purpose has also been skeptical because of the observed genetic instability of cloned cells and extreme inefficiency of the process [159]. Allergrucci et al recently reported that hESCs could undergo epigenetic changes over time in culture [160]. All these observations indicate the need for optimization of procedures and periodic monitoring of the cell lines to ensure their genetic stability and hence suitability for in vivo applications. Finally, immunological issues are a major concern for allogenic stem cell transplantations with both adult and embryonic stem cells from non-autologous sources. Rejection can be inhibited by the use of immunosuppressive drugs, which can have serious side effects. Technologies to develop individual-specific stem-cell lines through somaticcell nuclear transfer or cell fusion may allow engineered stem cells containing the individual's own

genetic material to be used for treatment [161].

The development of a bank of MHC-compatible hSC lines is also a lucrative option, though it also carries with it several ethical and technical problems. Another possible way to overcome immune rejection is to over express into the stem cells, genes such as fas-ligand that can suppress the immune system [162]. It has also been suggested that elimination of certain immunologically reactive cell surface molecules like B7 antigens or CD40 ligands from the stem cells prior to transplantation could also contain the immune rejections to some extent [163].

Stem cell banking

Stem cell "banks" could serve as a valuable resource for emerging treatments in the field of regenerative medicine, though challenges remain to making them a reality.

Stem Cell derived from Umbilical Cord is the hope for cure for two specific groups of diseases - blood cancers and Thalassemia.

Awareness about marrow or blood stem cell transplantation, widely recognized in the developed countries as the treatment of choice for leukemia, lymphoma and 70 other related diseases, is slowly spreading to the developing world, including India.

About 3 million Indians are diagnosed with cancer, between 5% and 10% of them with blood cancers. Similarly approximately 10,000 children are born every year with Thalassemia in India. For many such patients, peripheral blood stem cell or umbilical cord derives stem cell transplantation offers the only hope.

Cord blood is the blood that remains in the umbilical cord after a delivery of the baby. This blood is usually discarded. This blood has been identified as one of the richest sources of stem cells that could be used to effectively treat a variety of diseases and help research in regenerative medicine.

CONCLUSION

Medical researchers believe that stem cell treatments have the potential to change the face of human disease and alleviate suffering.A number of current stem cell treatments already exists, although they are not commonly used because they tend to be experimental and not very cost-effective.

A silhouette of the potential use of stem cells for treatment of human disease is now perceptible. The coming years will undoubtedly usher in new developments and technologies that would translate the envisioned therapeutic potential of stem cells to bedside medicine for patients suffering from devastating and debilitating diseases. The challenge in stem cell therapy is not simply to arrest organ dysfunction but is to achieve cell engraftment with functional integration into the organ, arrest adverse tissue remodeling and improve function of the diseased organ. To understand underlying mechanisms and to answer the many unknown questions related to regenerative therapy requires the knowledge and expertise of many disciplines. Be that as it may, stem cell therapy for regeneration has undoubtedly arrived.

In the future, medical researchers anticipate being able to use technologies derived from stem cell research to treat cancer, spinal cord injuries, and muscle damage, amongst a number of other diseases and impairments.

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