International Standard Serial Number (ISSN): 2249-6807

*International Journal of Institutional Pharmacy and Life Sciences 2(2): March-April 2012*

*INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES*

**Life Sciences** **Review Article……!!!**

Received: 03-03-2012; Accepted: 10-03-2012

# STEM CELLS AND THEIR POTENTIAL IN REGENERATIVE MEDICINE

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## INTRODUCTION

The stem cells are undifferentiated cells that occur in all most all organs of the body. These cells show their signature characteristics namely; the ability to differentiate into other cell types and ability to regenerate symmetrically or non-symmetrically. Based on the source, these cells have been classified in two categories viz. embryonic and adult, although induced pleuripotent stem cells have recently been developed. Whatsoever be the source of the stem cell the time point of their development actually determines their potency. A totipotent stem cell (*e.g*. a single cells fertilized egg/ one cell embryo) can develop into all type of cell including the embryonic membranes. A pluripotent stem cell can develop into cells from all three germinal layers (*e.g*. cells from the inner cell mass). Other cells can be multipotent/oligopotent, bipotent or unipotent depending on their ability to develop into few, two or one other cell type(s). The ability of a stem cell to develop/ differentiate into another type of cell is referred as transdifferentiation or plasticity. Self-regeneration is the ability of stem cells to divide and produce more stem cells of its own type (symmetric cell division) or one of its own type and other as a differentiated different type of cell (non-symmetric cell division). During early development, the cell division is symmetrical i.e. each cell divides to gives rise to daughter cells each with the same potential. Later in development, the cell divides asymmetrically with one of the daughter cells produced also a stem cell and the other a more differentiated cell.

Throughout the world, for the majority of degenerative diseases, both chronic and acute, the therapeutic interventions are almost negligible, which amounts to substantial economic and physiological loss. Billions of rupees are spent on the management of diseases like diabetes Type-I (in children), autoimmune diseases, nervous system diseases (Parkinson's, Alzheimer's, Sclerosis, spinal cord injuries etc.), primary immuno-deficiency diseases (more than 70 different forms of congenital and inherited deficiency of immune system like Bubble Boy disease, Wiskott-Aldrich syndrome and Systemic lupus erythromatosus), diseases of bone and cartilages, cancers etc with variable success rates. In all these debilitating diseases, the use of stem cells (implantation) is about to enter a phase of research and development that could lead to unprecedented cures and palliative treatments. The current excitement over potential stem cells applications emanates from new understanding of genetic and developmental biology. The stem cells may allow scientists to investigate how early human cells become committed to the major

lineages of the body, how these lineages lay down the structure of rudimentary organs and differentiate to form the diverse functionally committed cells which perform and maintain normal functions in the adults. The stem cells are present in most of the organs whether rapidly proliferating tissues like gut, skin, bone marrow, cornea and skin, and so do the organs with slow turn over terms like liver, brain and pancreatic islet cells. However, their number is very small (one in $10^5$ bone marrow cells). These cells are entrusted to carry out long-term tissue maintenance and normal wear and tear in the resident organ. Stem cells are small spherical with a diameter of 6-8 μm and possess high nuclear to cytoplasmic ratio. They have no particular morphological features but bear on their surface receptors for most hematopoietic growth factors.

**Natural role of stem cell(s) in mammalian development**

Approximately 300 trillion cells that make up the body have completely specialized functions. Most of these differentiated cells are matured to perform the functions as per their lineage specificity. Stem cells, on the other hand, are immature cells that posses the potential to develop into majority of the cells types in the body. Moreover, unlike specialist cells, stem cells have the capacity to keep multiplying. The inherent capacity to both proliferate and form different types of cells makes stem cells ideal for replacing the tissues that are undergoing degenerative changes (Heidaran, 2000).

It is well known that the all the different cells in the body arise from a single cell, the fertilized egg. The zygote, a fertilized oocyte is a kind of stem cell. It is functionally totipotent with the ability to produce all the cell types of the species including the trophoblast and the embryonic membranes. Development begins when the zygote undergoes several successive cell divisions, each resulting in a doubling of the cell number and a reduction in the cell size. At the 32- to 64-cell stage each cell is called blastomeres. The blastomeres stick together to form a tight ball of cells called a morula. The outermost layer enclosing inner mass cells is called trophoectoderm that secretes important growth factor (Leukemia inhibition factor, LIF). LIF maintains pleuripotency of the inner mass embryonic stem cells. Each of these cells divides but retains pleuripotency. Till 8-cell stage the cells have the totipotency after which these cells divide to be pleuripotent, please check this out The next stage is the blastocyst, which consists of a hollow blown up mass of cells; trophoblast cells along the periphery develop into the embryonic membranes and placenta while the inner cell mass develops into the fetus. Beyond the blastocyst

stage, development is characterized by cell migration in addition to cell division. The gastrula is composed of three germ layers: the ectoderm, mesoderm and endoderm. The outer layer or ectoderm gives rise to the future nervous system and the epidermis (skin and associated organs such as hair and nails). The middle layer or mesoderm gives rise to the connective tissue, muscles, bones and blood, and the endoderm (inner layer) forms the gastrointestinal tract of the future mammal. Early in embryogenesis, some cells migrate to the primitive gonad or genital ridge. These are the precursors to the gonad of the organism and are called germinal cells. These cells are not derived from any of the three germ layers but appear to be set aside earlier.

**Stem cells in regenerative medicine**

Stem cells also exist in adults and allow specific tissues to regenerate throughout life. They also have the ability for self-renewal and multi-lineage differentiation. In fact, the list for identifying adult stem cells and lineage specific progenitor cells (with limited self-renewal ability) is growing. Nutshell, stem cells have the potential to cure many human diseases because they are;

- Like naïve cells-they can become any cell in the human body when triggered by a specific growth factor/ inducer for differentiation.
- Enduring- embryos, in particular, can provide an endless supply of stem cells.
- Regenerative- can be used as source of self-repair in case of serious injuries.

The discovery of human embryonic stem (hES) cells, the primordial human cells that give rise to all specialized tissue in a developing fetus were a landmark event with vast biomedical potential. Mesenchymal stem cells (MSC) are stromal progenitors of all connective tissue cells and hold a promise for many therapeutic applications (Alhadlaq and Mao 2004).

Medical needs for tissue and organ substitute's results from trauma, age related diseases, degenerative conditions and end-stage organ failure (Langer and Vacanti 1993). Physicians treat organ or tissue loss by transplanting matched organs from one individual to another. Although these procedures have saved and improved lives, they remain an imperfect solution. Transplantation is severely limited by critical organ shortages, and by difficulties in overcoming immune responses to transplants received from unrelated donors. A true solution to this massive problem can be found through tissue engineering, an interdisciplinary field that applies the principles of life sciences and engineering to the development of biological substitutes that restore, maintain, and improve tissue functions.

The stem cells serve as source of donor cells to be used to replace cells in transplantation therapy. Stem cells can be obtained from several sources (Table 1). Embryonic stem cells must be obtained when an embryo is in an early development, which means when the fertilized egg has divided to form about ~200 cells. These cells are separated and maintained in a cell culture dish, thereby halting embryonic development towards creating an individual. This is why embryonic stem cell research is the subject of ethical considerations and debates. Utilization of adult stem cells poses less of an ethical dilemma; however, adult stem cells may not have the same potential as those derived from embryos for medical therapeutics.

**Table 1: Different sources of stem cells**

| Source of stem cells | Remarks |
|---|---|
| Spare embryos | Stem cells can be isolated from leftover embryos stored at fertility clinics that were not used by couples to have children. |
| Special purpose embryos | Embryos are generated by *in vitro* fertilization (artificially in the lab) for the sole purpose of extracting their stem cells. |
| Cloned embryos | Embryos are cloned in labs using somatic nuclear transfer method in order to harvest their stem cells. |
| Aborted fetuses | Stem cells ate obtained from fetuses in early development that have been aborted. |
| Adult tissue/ organ | Stem cells are obtained from the tissue or organs of living adults during surgery. |
| Umbilical cord | The placental cord after childbirth holds potential for research. |
| Amniotic fluid | Chorionic villi and amniotic fluid may be an excellent cell source of stem cells for therapeutic purpose. |
| Cadavers | Isolation and survival of neural progenitor cells from human post-mortem tissues (up to 20 h after death) has been reported and provides an additional source of human stem cells. |
| IPS | Induced pleuripotent stem cells generated by transfecting oct4, Kif, sox2 cmyc genes in differentiated cells to become pleuripotent stem cells. |

**Stem cell types**

**Embryonic stem cells (ESC)**

Several mammalian pluripotent embryonic stem cell lines derived from blastocyst-early stage embryos have been established. Human ESC lines express many markers that are common to

pluripotent and un-differentiated cells, such as CD9, CD24, octamer-binding protein (Oct-4), Nanog, alkaline phosphate, LIN28, Rex-1, Cripto/TDGF-1, DNMT3B, SOX2, EBAF and Thy-1; as well as SSEA-3(stage specific embryonic antigen), SSEA-4 and tumor-rejection antigen-1-60 (TRA) and TRA-1-80. Moreover, all ESC lines generally exhibit high levels of telomerase expression and activity for prolonged periods in culture. In this way, ESCs possess the dual ability to undergo unlimited self-renewal and to differentiate in all fetal and adult stem cells and their more differentiated progenitors. Therefore, they represent a useful source of stem cells for investigating the molecular events that are involved in normal embryogenesis and generating a large number of specific differentiated progenitors for cellular therapies.

**Amniotic epithelial cells**

Amniotic epithelial cells (AECs) derived from the amniotic membrane in human term placenta also express the markers that are present on pluripotent ESCs such as Oct-4, Nanog and alkaline phosphatase. They can also differentiate as ESC in the cell lineages from three germ layers, including pancreatic endocrine cells and hepatocytes (endoderm), cardiomycetes (mesoderm) and neural cells (ectoderm), *in vitro*. Of therapeutic interest, AECs do not express the telomerase and form no teratomas after transplantation *in vivo*. Therefore, AECs constitute a source of pluripotent stem cells that might be used in transplantation for tissue regeneration.

**Fetal stem cells**

Multipotent fetal stem cells (FSCs) are generally more tissue specific than ESCs. Thus FSCs are able to generate a more limited number of progenitor types. One of the particular therapeutic advantages of FSCs as compared to ESCs is the fact that FSCs do not form teratomas *in vivo*. The FSCs obtained up to week 12 offer the possibility of transplanting these primitive stem cells without frequent rejection reactions in contrast to UCB and BM stem cell transplants. The cells from the growing fetus appear to be able to cross over the placenta and enter the mother's bloodstream and vice-versa (fetomaternal trafficking); the maternal cells can also pass into fetal circulation and persist into adult life, a phenomenon known as microchimerism. Hence, the fetal cells that are transferred to the mother during gestation can migrate to different damaged peripheral tissues, such as liver and skin, or can cross the blood-brain barrier to enter damaged areas of the brain, where they actively contribute to the mother's tissue repair by generating mature cell progenitors.

**Umbilical cord stem cells**

Fetal HSC (hematopoietic stem cell) and MSC (mesenchymal stem cells) can be isolated from the umbilical cord. This source of HSC has been used in clinic for nearly 20 years. Umbilical cord blood is a rich source of HSC and progenitors, comprising 0.54% of the leukocytes, containing on average 5 X $10^6$ $CD34^+$ cells per collection. Approximately 120 ml of blood can be collected from placenta and cord following delivery. The number of HSC per cord blood unit is less than that from bone marrow or mobilized peripheral blood; however, full haemopoietic reconstitution is achieved, although engraftment is slower because of lower numbers of transplanted HSC. It is preferred source of HSC for allogenic grafts in children and small adults since they are better tolerated than allogenic bone marrow transplants, and graft versus host disease is less severe.

MSC have also been identified in umbilical cord blood, although they are rare and difficult to isolate. They have been isolated from Wharton jelly and perivascular region of the umbilical cord. MSC from these sources have a differentiation potential similar to other fetal MSC *in vitro*. These cells bear MSC markers like CD13, CD29, CD90 and CD105. Haemopoietic stem cell ($CD34^+$) and haemopoietic lineage markers ($CD14^-$, $CD45^-$) are absent in these cells (Mouse HSC: $CD34^{lo/-}$, SCA-$1^+$, thy$1.1^{+/lo}$, $CD38^+$, C-$kit^+$ and $lin^-$; Human HSC: $CD34^+$, CD59+, Thy1/ $CD90^+$, $CD38^{lo/-}$, C-kit/ $CD117^+$ and $lin^-$).

**Adult stem cell(s)**

A population of adult stem cells (ASCs) resides within specific designated areas called niches in most of adult mammalian tissues/ organs, including BM (bone marrow), heart, kidneys, brain, skin, eyes, gastrointestinal tract, liver, pancreas, lungs, breasts, ovaries, prostate and testes. In fact ASCs appear to originate during ontogeny and persist in specialized niches within organs where they may remain quiescent for short or long periods of time. Although ASCs as observed for ESCs, FSCs and UCB stem cells, might generally show an uncontrolled growth in a specific microenvironment and enhanced telomerase activity, they generally show a more restricted differentiation potential and give rise to a more limited number of distinct cell progenitors.

**Immune rejection: a stumble block**

To overcome immune rejection, however, patients receiving a graft of the stem cells would probably be treated in much the same way that organ transplant recipients are treated (heavy

doses of immunosuppressive drugs). To counter such problems research focus has been to exploit stem cell of mesengial origin. These are the mesenchymal stem cells those seem to evade detection by the immune system. These cells are also pleuripotent in nature. Following their isolation from bone marrow these cell types would normally give rise to cartilage, bone or fat cells. And thus become ideal candidate for future treatments of bone and joint diseases or repairing the damaged heart muscle. Both placentas as well as the cord blood cells are important sources of mesenchymal stem cells to be used for future therapeutic applications. Reports are also now surfacing on identification of the mesenchymal stem cell from the adult's tissues as well. Adult stem cells are though essentially multipotent, exist in certain mature tissues and supply the tissue with replacement cells throughout life. For instance, our blood stem cells churn out 5 million cells per second! There is, however, a technical difficulty associated with isolated adult stem cells that once isolated and placed in the culture dish, they don't grow indefinitely as embryonic stem cells do. Thus, their number available for their therapeutic intervention becomes the hindering factor. The need is therefore to develop newer strategies in order to have ample supply of these stem cells in the patients receiving autografts (self stem cell) or otherwise. These strategies though would provide an answer to a key question of immune-rejection; however, it is still in the infancy before this is being realized as potential therapeutic candidate for the degenerative diseases. The focus is thus now on to look for the molecular markers that are important for deciding the stem cell niche, self renewal and controlled proliferation and more so of providing appropriate growth environment. This would help in maximally exploiting the use of the stem cells (embryonic and adult). In this regard the preliminary emphasis will be laid on characterizing the stem cells bound to synthetic or biodegradable supports (matrices). The stem cells are although best defined functionally, yet a number of molecular markers have been used to characterize various stem cell populations (Table 2).

**Table 2. Stem-cell specific markers for their screening and identification**

| **Stem cell type** | ***Marker*: function** |
|---|---|
| **Embryonic** | ***Oct-4*:** A POU transcription factor (Scholer *et al*., 1990). It is expressed in embryonic stem and germ cells (Scholer *et al*., 1989; Rosner *et al*., 1990) and also required to sustain stem cell self-renewal and pluripotency (Niwa *et al*., 2000). ***Rex-1: Stage Specific*** |

| | |
|---|---|
| | ***Embryonic Antigens*** **(SSEAs)** Three isoforms are known that are expressed on 8 cell-staged murine embryos, surface of terato-carcinoma cells, membranes of oocytes, zygotes and early cleavage stage embryos. |
| **Haemopoietic** | ***CD34*****:** Found on surface of some bone marrow cells (Civin *et al*., 1984 and 1990) and most critical marker for Haemopoietic stem cells (HSCs). **CD133:** A 120-kDa-glycosylated protein identified by the AC133 mAB that recognizes a CD34+ subset of human HSCs (Mirraglia *et al*., 1997). ***ABCG2*****:** ATP-binding cassette super family G member 2 is found in a wide variety of stem cells (Kim *et al*., 2002). ***Sca-1*****:** Stem cell antigen-1 is an 18 kDa phosphatidylinositol-anchored protein (Van de Rijn *et al*., 1989), found in adult bone marrow, fetal liver and mobilized peripheral blood and spleen (Stanford *et al*., 1997). |
| **Mesenchymal/Stromal** | ***STRO-1*****:** Expressed by human $CD34^+$ bone marrow cells. Marker for the cells differentiating into multiple mesenchymal lineages (Dennis *et al*., 2002). |
| **Neural** | ***Nestin*****:** Class VI intermediate filament protein (Lendahl *et al*., 1990). Predominantly expressed in stem cells of central nervous system (Sawamoto *et al*., 2001) but also in non-neural stem cells viz. pancreatic islet progenitors (Lechner *et al*., 2002) and hematopoietic progenitors (Shih *et al*., 2001). ***PSA-NCAM*****:** Polysialic acid-neural cell adhesion molecule: expressed mainly in the developing nervous system. Important for self-renewal and differentiation into multiple neuronal phenotypes (Mayer-Proschel *et al.,* 1997). ***P75 Neurotrophin R*****:** A nerve growth factor receptor. $P75NTR^+$ cells generate neurons and glia both *in vitro* and *in vivo*, and also able to differentiate into neurons, smooth muscle and Schwann cell in culture (Mujtaba *et al*., 1998). They are also identified mesenchymal precursors as well as hepatic stellate cells (Cassiman *et al*., 2001). |

**Stem cells and tissue engineering**

Broadly tissue engineering essentially includes development of artificial implants, laboratory-grown tissues, cells and/or molecules to replace and support the function of defective or injured parts of the body. Motivated by the potential for curing diseases and the ability to design custom tissue for implantation, researchers are attempting to engineer virtually every human tissue that include skin, cartilage, bone, CNS tissues, muscles, liver and pancreatic islet cells. Although

cells have been grown outside the body *in vitro* but the possibility of 3-D grown tissues that replicate the design and function of actual human or animal tissue has been realized recently. The bio-engineered organ to reach clinic may be bladder that can be made by co-culturing various cells on a scaffold of synthetic or natural polymers (Morgan and Yarmush 1999; Atala 1999). While several tissue engineering breakthroughs have been made, there remains two important challenges to further progress in generating laboratory-grown tissues and organs: (a) refinement of polymer scaffolding that mimics the organ architecture, and also supports the growth of appropriate stem cells (Nerem and Sambanis, 1995); and (b) an abundant source of pluripotent stem cells that have the potential to proliferate and become fully specialized. Such cells can obviously thus form bone cartilage, muscle or fate depending on the exact nature of their environment (Pittenger *et al*., 1999). Currently most, if not all, organs and tissues made in the laboratory are generated using stem cells of animal or in some cases undefined human origin. The tissues/cell lines obtained in this way have very limited clinical success, primarily because they like the donor tissues/organs are frequently rejected by the recipient's immune system. A scientifically and rational cost effective strategy to circumvent this problem is to use stem cells isolated from the known and characterized tissue.

The formation/ regeneration of bone-like structure from the bone marrow derived adult human MSCs following preconditioning by 1 week of exposure to adipogenic-inducing supplement followed by photo encapsulation in *poly* (ethylene glycol) diacrylate (PEGDA) hydrogel in predefined shape and dimensions has been achieved (Alhadlaq *et al*., 2005; Hardy *et al*., 2010). Such a soft tissue augmentation is a wide spread practice in plastic and reconstructive surgery. The tissue engineered adipogenic constructs demonstrated specific staining as well as expressed PPAR-2 adipogenic gene marker *in vivo* in a mouse model. The recovered *in vitro* and *in vivo* constructs maintained their predefined physical shape and dimensions. This study demonstrated that adipose tissue engineered for human MSC could retain predefined shape and dimensions for soft tissue augmentation and reconstruction in case of fatal accidents with severe facial injuries requiring facial soft tissue/ adipose tissue reconstruction. Human term placenta has been found to be a good reliable source of mesenchymal stem cells that could be trans-differentiated into various functional differentiated cell lineages such as neurogenic, chondrogenic, osteogenic, adipogenic and myogenic (Portmann-Lanz *et al*., 2006).

Advances in cellular and molecular biology have created a range of opportunities for the successful isolation of pluripotent stem cells from embryonic tissue (Brustle *et al*., 1999; Thomson *et al*., 1998), adult bone marrow (Pittenger *et al*., 1999; Petersen *et al*., 1999), peripheral and umbilical cord blood (Boyer *et al*., 2000) and various human placental tissues (Portmann-Lanz *et al*., 2006). The advantages of using fetal tissues as a source of stem cells include noninvasive cell collection, low risk of complications, and immunological native-ness. Stem cells like other mammalian cells can be stored using conventional cryo-preservation techniques for long periods of time. *In vivo*, the specialized cells are organized with in a complex structural and functional framework called extra cellular matrix (ECM). The great diversity observed in ECM composition contributed enormously to the properties and functions of each organ and tissue: the rigidity and tensile strength of bone, and elasticity of skin, are examples of how different ECM compositions contribute to tissue function. The ECM also provides a diffusible reservoir for soluble signaling molecules, and through its own dynamic composition, a source of additional signals to migrating, proliferating and differentiating cells. Artificial substitutes for ECM called scaffolds can consist of natural or synthetic polymers, or both, and have been used successfully alone and in combination with cells and soluble factors to induce tissue formation or promote tissue repair.

However, the scaffolds that are synthetic will be known for its compositions and character can be tailor-made to exploit such scaffolds for optimal cell binding and to obtain desired functions, optimally. The complexity of biological systems suggests the manufacture of tissue-engineered products will be complicated, and that their operating conditions, design and specifications are intrinsically multi-factorial. If the biodegradable matrix-supported MSCs or bone marrow stem cells transdifferentiate *in vitro*, the scaffold supported mesenchymal stem cells for implantation *in vivo* in a mouse model, may be transdifferentiate *in situ* into functional beta-pancreatic cells in diabetic (streptozotocine treated) mouse model under the influence of specific growth factor/ inducer. The scaffold-supported cells might be able to overcome immunological rejection there by promoting the sustenance of the implanted cells *in vivo*. Moreover, covalently bound hormone (say insulin) to an extra-cellular matrices (ECM) like collagen, fibronectin etc may be used to affinity bind specific type of stem or differentiated cells (a step towards niche recognition). The application of information and principles obtained through years of research in several

disciplines to the goals of tissue engineering has already resulted in several promising medical advances such as tissue engineered heart valve leaflets, bio-artificial skin (Morgan and Yarmush 1999), artificial blood vessels (Niklason *et al*., 1999), urinary bladders (Atala 1999) and bone (Liu *et al*., 1999).

Human stem cell research holds enormous potential for contributing to our understanding of fundamental human biology. The ability to isolate tissue-restricted progenitor/ stem cells has important implications for transplantation medicine and to learn tissue repair mechanisms during normal and disease condition. Embryonic stem cells derived from blastocysts inner cell mass, the embryonic ancestors of all adult tissues represent a potentially unlimited source for generating tissues progenitor cells *ex vivo*. Embryonic stem cells can be sourced from leftover embryos (not used in fertility clinics), aborted fetuses in early development, umbilical cords as well as adult stem cells are available sources of stem cells (Table 1). Embryonic stem cells are flexible, immortal and easily available but involve ethical controversies/ implications. Adult stem cells are already specialized, easy to induce, immune hardy, flexible but low in number, finite life span and may carry genetic mutations. Chorionic villi and amniotic fluid has been suggested to be an excellent source of cells for therapeutic purpose (Copi *et al*., laboratory of Cellular therapeutics and tissue engineering, Children Hospital and Harvard Medical School, NMSC Abstracts 2003). Clones of cells cultured in different conditions could form *in vitro* and *in vivo* fully differentiated muscle, adipogenic and bone tissues. Furthermore, they were also able to *in vitro* differentiate in endothelial, neurons and hepatocytes. On other hand, easy availability of abundant umbilical cord tissue by non-invasive approach makes it a most suitable and attractive source of stem cells.

**S**ynthetic supports such as *poly* glycolic acid (PGA), *poly* lactic acid (PLA), poly glyco- and lactic-acid) PGLA, hydroapatite, as well as *poly* ethylene glycol methacrylic acid (PEGMA), *poly* acrylic acid, *poly* propylene fumerate, polyarylates supports or natural materials such as collagen and alginate or patented polystyrene matrix Alvetex$^{R}$ (Knight *et al*., 2011) have been reported to immobilize mammalian cells for tissue engineering (Levenberg *et al*., 2005). The degradable matrices may be sometimes activated/ made biocompatible to enhance the adherence of inoculated cells by exposing them to collagen, fibronectin, poly D-lysine, condrionectin 6 sulfate, laminin, Matrigel$^{R}$ etc.

We have successfully attempted isolation of stem cells from human term placenta chorionic villi as well as human placental cord blood mesenchymal stem cells and were able to obtain 12-30 colonies of cells *in vitro* in Dulbecco's' Modified Eagle's Medium (DMEM supplemented with Fetal calf serum and antibiotics (penicillin 100 U/ml, streptomycin 100 µg/ml and amphotericin 0.25 µg/ml) in a period of 14-21 days (Fig 1 and 2). Moreover, we are synthesizing and employing a variety of tailor made synthetic hydrogels for immobilization of biomolecules to perform various applications in aqueous and organic media. Our studies have shown the stable nature of the synthetic hydrogels. We are also attempting to use these hydrogels to entrap/support the growth of transformed/ established mammalian cells such as Vero, Hep 2 and RD-cells. Osteogenic, hepatic, chondrogenic and pancreatic cell lineage may be produced from the human placental mesenchymal stem cells. If the scaffold supported stem cells transdifferentiate *in vitro*, they may be trans-differentiated to β-pancreatic cells for implantation *in vivo* in a diabetic mouse model.

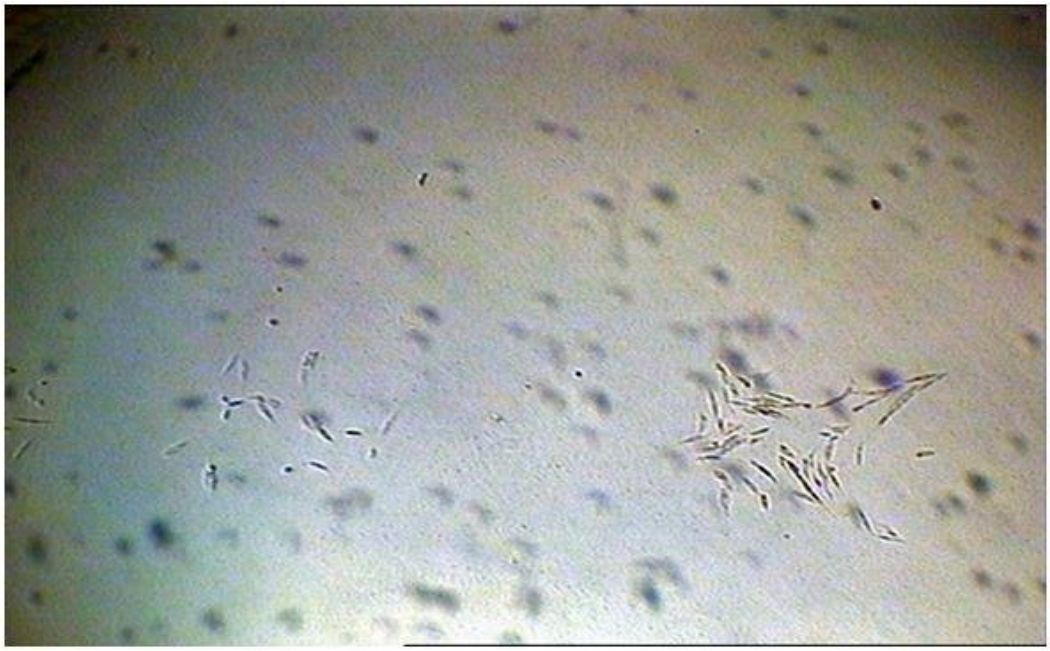

Fig. 1: Placenta derived stem cells obtained from human chorionic villi on day 14 of *in vitro* culture in DMEM.

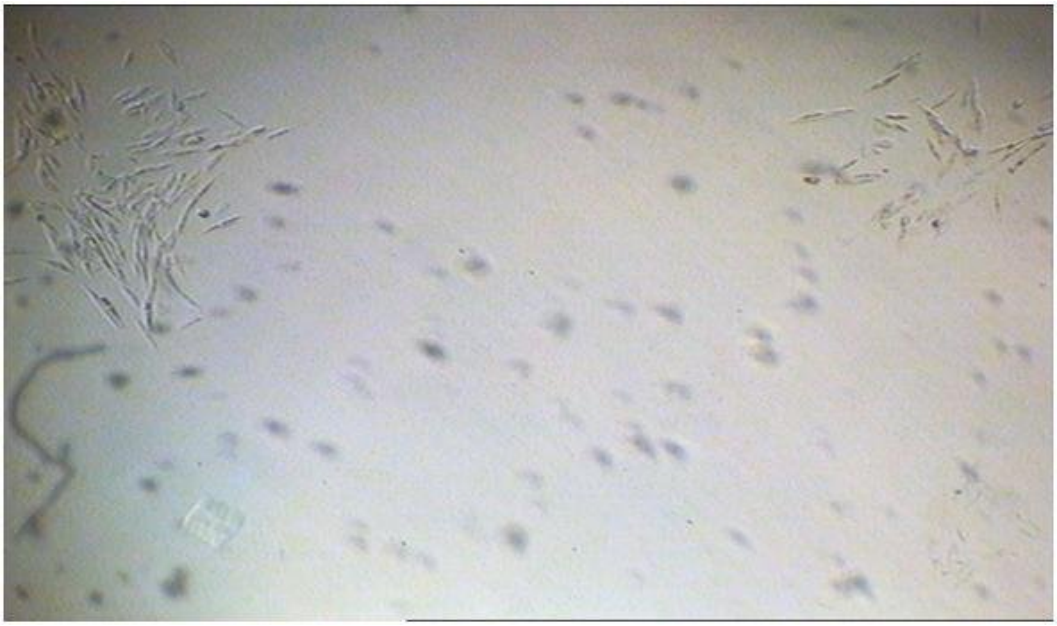

Fig. 2: Placenta derived stem cells obtained from human chorionic villi on day 21of *in vitro* culture in DMEM

In the face of extraordinary advances in the prevention, diagnosis and treatment of human diseases, devastating illnesses such as heart diseases, diabetes, cancer and diseases of the nervous system such as Parkinson's disease and Alzheimer's disease continue to deprive people of health, independence and well being. Research in human developmental biology has led to discovery of human stem cells including embryonic stem cells, and adult stem cells. Recently, techniques have been developed for the *in vitro* culture of stem cells thus providing unprecedented opportunities for studying and understanding human embryo biology. As a result, scientists carried out experiments aimed at determining the mechanisms, understanding the conversion of a single, undifferentiated cell, the fertilized egg into the different cells comprising the organs and tissues of the human body.

***Cell differentiation markers and functional properties of differentiated cells***

The nature of differentiated cells can be determined by performing biochemical analysis, *in situ* hybridization using cell marker specific fluorescence tagged or enzyme (Horse radish peroxidase/ Alkaline phosphatase) monoclonal antibodies (Table 3). The issue of the adherent and non-adherent cells shall arise in case of the cord blood because the adherent cells (on culture

plate) are of the mesenchymal lineage while the non-adherent cell type represents a hematopoietic lineage. The fluorescent activated cell sorter (FACS) analysis is employed for isolating the adherent (mesenchymal) stem cell population. The isolated stem cells are expanded further in an appropriate tissue culture medium and characterized on the basis of molecular markers expressed by the cells at passage 2 to 3. The molecular markers often used to analyze stem cells include hematopoietic lineage markers (CD7, CD14, CD34, CD38, CD45, AC133, HLA-DR), matrix receptors (CD44, CD50, CD54, CD58, CD62E, CD62L, CD62P, CD105), integrins (CD29, CD49b, CD49d, CD51, CD61), factor receptors (tumor necrosis factor receptor 1, TGFßIIR, EGFR, PDGFR), osteogenic precursor markers (STRO-1, alkaline phosphatase, osteonectin, osteocalcin), CD90 (Thy-1), and MSC markers (SH2, SH3).

**Table 3: Lineage-specific markers for stem cell(s) derived differentiated cells.**

| **Cell type** | **Marker** |
|---|---|
| Osteogenic | Biochemical markers: Alkaline phosphatase and osteocalcin can be bio-chemically assayed in the cells and their presence indicates osteogenic nature of differentiated cells. |
| Chondrogenic | Periodic Acid Staining (PAS) for presence of muco-polysaccharides, and presence of Collagen Type 1 & 2. |
| Myogenic | Presence of tubular structures and desmin. |
| Cardiomyocytes | GATA 4 and Nkx-2.5 analysis and presence of alpha-myosin. |
| Neurogenic | MAP 2 and NSE |

The adherent and non-adherent stem cells or their derivatives/ differentiated cells may be further characterized on the basis of surface exposed markers or staining for XX and XY chromosomes (Table 4). The following markers are commonly used to identify the cell type(s) broadly mesengenic and hematopoietic cells.

**Table 4: Markers for identifying mesenchymal and haemopoietic stem cells.**

| **Markers/ *Staining*** | **Cell Type** | |
|---|---|---|
| | **Adherent** | **Non-adherent** |
| | Mesengenic | Haemopoietic |
| CD14 & CD45 | – | + |
| Stro-1 | + | – |
| ASO2 (fibroblast) | + | – |
| CD9, CD29, CD73 & CD105 | + | – |
| CD13, CD34 & CD 117 | – | + |
| *Maternal* | + *for* XX | |
| *Fetal (male)* | + *for* XY | |

**Trans-differentiation: A wonderful property of adult stem cells**

Adult stem cells were thought to be restricted to produce differentiated cells, which were specific to the organ from which they were isolated. Recently, several examples have been reported which demonstrated that these stem cells; under certain conditions can be induced to form other cell types (trans-differentiation). For example;

- Neural stem cells (NSC) can give rise to blood and skeletal muscle
- Bone marrow cells can give rise to muscle, liver and astrocytes.

When NSCs were used to form muscle, no inducers were needed other than co-culturing them with muscle progenitor cells (myoblasts) or injecting them into muscle. This holds promise for cell transplantation therapies in that the experiment suggests that host tissue can instruct transplanted cells to a desired result. Scientists, then, can instruct whether it is best for stem cells to be differentiated in vitro prior to transplantation or by transplanting them directly into the defective tissue. Some experiments have shown that naturally transplanted stem cells were able to migrate to regions where cells had died due to stroke (called ischaemia).

**Stem cell therapies**

Stem cells offer the opportunity of transplanting a live source for self-regeneration. Bone marrow transplants (BMT) are a well-known clinical application of stem cell transplantation. BMT can repopulate the marrow and restore all the different cell types of the blood after high doses of chemotherapy and/ or radiotherapy, our main defense used to eliminate endogenous cancer cells. The isolation of additional stem and progenitors cells is now being developed for many other clinical applications. Several applications are described below.

**Skin replacement**

The knowledge of stem cells has made it possible for scientists to grow skin from a patient's plucked hair. Skin (keratinocyte) stem cells reside in the hair follicle and can be removed when a hair is plucked. These cells can be cultured to form an epidermal equivalent of the patients own skin and provides tissue for an autologous graft, bypassing the problem of rejection. It is presently being studied in clinical trials as an alternative to surgical grafts used for venous ulcers and burn victims.

**Brain cell transplantation**

Neural stem cells were only until recently thought to be strictly embryonic. Many findings have proved this incorrect. The identification and localization of neural stem cells, both embryonic and adult, has been a major focus of current research. Potential targets of neural stem cell transplants include stroke, spinal cord injury and neuro-degenerative diseases such as Parkinson's disease. Parkinson's disease involves the loss of cells, which produce the neurotransmitter dopamine. The first double-blind study of fetal cell transplants for Parkinson's disease reported survival and release of dopamine from the transplanted cells and a functional improvement of clinical symptoms. However, some patients developed side effects, which suggested that there was an over-sensitization to or too much dopamine. Although the unwanted side effects were not anticipated, the success of the experiment at the cellular level is significant. Again, further studies are needed and are in progress. Over 250 patients have already been transplanted human fetal tissue. Several biotechnology companies are developing different strategies of stem cell therapies.

1. Diacrin has been developing xeno-transplants using fetal pig cells. Clinical trials for chronic stroke patients have begun. Presently, stroke patients require treatment within 24 hours after stroke for effective therapeutic results. Many patients do not receive treatment in time because the symptoms are not initially obvious. Diacrin's therapy could be applied weeks to months after the initial trauma.
2. NeuroNova's strategy is to culture adult human cells from donors, differentiate them in culture to produce the cell type (dopaminergic neurons), which is lost in Parkinson disease, and to transplant them into the brain of patients.
3. Neurotech is using genetically altered brain endothelial cells (engineered to produce human Interleukin-2) as immuno-therapy for gliomas. Results from experiments in rats showed that these cells "mopped up" the tumor cells and as a result a clinical study has commenced.

**Current status of stem cell research at national level**

Amongst various sources of stem cells such as early human embryos, aborted fetuses, adult stem cells and umbilical cord cells, the latter are readily available in plenty as the discarded material that holds a good potential source of stem cells. Such cells have properties of stem cells and may offer possibility for treatments and ultimately for cures for many diseases for which adequate therapies do not exist at present. In India various groups at NCCS, Pune; CMC, Vellore, NCBR, Manesar; CCMB, Hyderabad; CV Prasad Eye Hospital and Research Center, Hyderabad, AIIMS, New Delhi; NDRI, Karnal; National Institute of Nutrition, Hyderabad; IITM, Chennai etc. have successfully developed methods to isolate and culture embryonic and adult stem cells from human placental cord blood, bone marrow, mouse/ rat embryos,and diverse sources such as animal spermatogonardial cells.Chitra Institute,Trivandrum has successfully used hydroxyapatite for supporting mammalian cells. In India, over the last couple of years the technology for culture implantation of human corneal and myocardiocytes has been well established and continuously being refined to obtain higher success rates. However, reports on use of human term placental tissue(s) to obtain placenta derived mesenchymal stem cells are scanty.

**Future directions**

The science of stem cells dated to mid 1960s, and many articles have been published on the isolation and laboratory manipulation of stem cells from animal models. While these models

were imperfect, they are accepted in the scientific community as good initial predictions of what happens in human beings. The studies in animals have shown that stem cells could be differentiated into cells of choice and that these cells functioned well in the transplanted environment. In human, transplants of haemopoietic stem cells for cancer treatment have been done for years. Somewhat cruder experiments (transplantation of fetal tissues into the brain of Parkinson's patient's cardiac tissue) indicated that such stem cell therapies could provide robust treatments for many human diseases are only a reasonable one. It is only through controlled scientific research that the true promise will be understood and functional aspects of the transplanted cells could be studied.

There is an emergent need to research on human stem cells derived from all sources (embryonic, fetal and adult) should be conducted in order to contribute to the rapidly advancing and changing scientific understanding of the potential of human stem cells. The study of human stem cells is at an early stage of development, it is difficult to predict outcomes and findings at this point in time. Thus systematic research is essentially required to see the full developmental potential of different kinds of stem cells. However, the normal cells (non-haemopoietic origin) are functional and synthesize cytokines/ biological activities when they are attached. Thus it shall be interesting to combine and study the interactive role of newly synthesized biodegradable matrices, concepts of tissue engineering including effects porosity, swelling behavior of synthetic matrices in physiological buffers, effect of hydrophobicity/ hydrophilicity on the expansion pattern of stem cells as well as their ability to modulate the effect of mitogenic/ differentiation inducing biomolecules need to be studies extensively.

The human stem cell research carries immense potential for enhancing our understanding of mechanisms involved in multiplication of stem cell(s) and their differentiation into variety of end stage cells. The stem cells have a strong parallel to recombinant DNA and monoclonal antibody technologies both of which have amplified rare and precious biological entities. Like those technologies, use of adult stem cell technology may well be transformative in opening scientific areas that today appear obscure.

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