

Stem Cell Research in India: Emerging Scenario and Policy Concerns

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Abstract: In India, stem cell programmes have been initiated with the aim of promoting both basic and translational research in view of its potential applications. Strategy for promoting stem cell research (SCR) in the country is gradually taking shape. The key components of the strategy are: creation of centres of excellence (CoE); virtual network of centres; generation of adequate human embryonic stem cell (hESC) lines; human resource development through training, short and long-term overseas fellowships, etc. The short term goal is to study the biology of all types of adult stem cells and in parallel evaluate its safety and efficacy in animal models. The Government of India has provided support to establish a Centre for SCR to carry out basic and translational research; training centres to provide training for both embryonic and adult stem cells; facilities in medical schools to handle stem cells; clinical research on myocardial infarction and stroke for safety and efficacy study; etc. Over 30 institutions, hospitals and industry are involved in SCR in the country. The government has invested about 8.0 million US\$ for SCR in last two years. Support to small and large biotech companies is also being considered through the Research “Small Business Innovative Initiative (SBIRI)” scheme. Draft guidelines for SCR in the country have been formulated and the same is currently being placed for public debate.

Keywords: Stem Cell, India, Regulation

Introduction

Over the past few years, human stem cell research (SCR) has emerged as a new and exciting field in the life sciences being novel in its potential for clinical applications. Stem cells are precursor, unspecialized, undifferentiated cells capable of self proliferation, migration and differentiation. In the simplest form, the stem cell is an immature cell that has the capability to differentiate into any possible mature cell. Based on the sources, it is categorized into embryonic stem cells, adult stem cells and cord blood stem cells.

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The embryonic stem cell (ESC) is the 'mother' of all other cell types in the body and is derived from early stage of the human embryo, i.e. blastocyst. Blastocyst formation takes place after four days of fertilization. The blastocyst has an outer layer of cells while inside it has a hollow sphere with a cluster of cells called the inner cell mass. These inner mass cells are pluripotent in nature and may undergo further specialization into stem cells and give birth to cells having a particular function. These cells have the capability to turn into different types of tissues in the human body.

Adult stem cells may be derived from the bone marrow, peripheral blood, tissues, muscles, cardiac tissues, cartilage, brain tissues, etc. The adult stem cells are haematopoietic, non-haematopoietic and organ specific stem cells. Haematopoietic stem cells are blood-forming cells isolated from the bone marrow. Non-haematopoietic stem cells are mesenchymal stem cells (MSCs) and are present in many tissues of adult cells, i.e. bone marrow, cord blood, fat, bone, placenta, lung, liver, etc. These cells are pluripotent and non-immunogenic in nature. They are not patient specific and have a tendency to home to sites of inflammation.

The most important characteristics of MSCs is their ability to differentiate into several cell lineages such as cartilage, bone, active tissues, etc. to treat various diseases. Because of this unique characteristics, MSCs possess enormous potential for allogenic transplantation. MSCs may be isolated from various sources from normal healthy volunteer donors and manufactured under strict cGMP conditions. These cells may be characterized for research purpose as well as for its clinical applications in an allogenic setting.

In addition, stem cells may also be isolated from the umbilical cord blood. Much like the bone marrow, cord blood is one of the richest sources of stem cells. Cord blood stem cell research is being conducted for potential future use in the treatment of certain auto-immune disorders, neurological disorders, muscular/cartilage diseases, stroke, etc.

Any disease in which there is tissue degeneration may be a potential candidate for stem cell therapies, including conditions and disabilities as Parkinson's and Alzheimer's diseases, spinal cord injury, stroke, burns, heart disease, Type 1 diabetes, osteoarthritis, rheumatoid arthritis, liver diseases, retinal regeneration, limb ischemia, hair cell regeneration, etc. Stem cell therapies, like other tissue transplants, face the problem of immune rejection. Alternatively, using the patient's own cells and tissues

will overcome the issue of immune rejection. The researchers have explored four possible ways to use mesenchymal stem cells for clinical applications, i.e. implantation of MSCs at site for localized diseases, systematic transplantation, combining stem cell therapy with gene therapy and use of MSCs in tissue engineering protocols. Clinical trials (mostly phase I) are being conducted using MSCs for osteogenesis imperfecta, large bone defect, myocardial infarct, chronic non-healed skin wound, etc. A lot more basic information about stem cells and their behaviour are required before they can be used for treatment.

Extensive basic research is required for standardization of methods for the isolation of embryonic and adult stem cells from various sources. Future prospects for embryonic stem cell research include the following: generation of therapeutic grade cell lines; identification of human embryonic stem cells (hESC) growth factors; controlled differentiation, i.e. generation of specific cell population; study of fundamental changes in cell cycle control that occurs during embryonic stem cells differentiation; maintenance of stem cell in undifferentiated stage; regulation of differentiation of ESC; pluripotency and differentiation of established cell lines; standardization of animal free defined culture conditions; developmental potential of human versus mouse ESC; standardization in use of specific stem cells to specific organ systems, etc. In addition, ESC could also be used for toxicology tests and may be valuable tools for traditional drug discovery.

Clinical research using stem cells in animals and humans is an emerging science. Use of *ex-vivo* expanded stem cells has been identified as new use of drug as per FDA, USA, i.e. investigational new drug (IND). IND covers issues related to cells, animal studies and clinical trial. The cell issues include among other: the source, number, purity, appropriate stage, optimum condition and criteria for harvesting stem cells; mechanisms of harvesting, standardization of procedures for harvesting, purification and characterization, also standardization of doses in terms of concentration and number of stem cells for each application and minimal manipulation of cells for clinical use. The animal studies issues include small vs. large animal, human to rat or rat to rat or both, length of safety studies, whole animal histopathology, defining delivery, dosage study, etc. Clinical trial issues cover the number of patients to be enrolled in rare diseases, inclusion and exclusion criteria, randomization, non invasive tracking of cells, lack of definitive guidance, etc. Good animal models are required to address the issues of

Table1: Institutes, Hospitals and Industries involved in Stem Cell Research

Embryonic stem cell research	<ul style="list-style-type: none"> ● National Institute for Research in Reproductive Health, Mumbai ● National Centre for Biological Sciences, Bangalore ● National Centre for Cell Science, Pune ● National Brain Research Centre, Manesar ● Rajiv Gandhi Centre for Biotechnology, Thiruvanthapuram ● Centre for Human Genetics, Bangalore ● Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore
Heamatopotic stem cells and bone marrow mononuclear cells	<ul style="list-style-type: none"> ● Christian Medical College, Vellore ● Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow ● Post Graduate Institute of Medical Education & Research, Chandigarh ● Manipal Hospital, Bangalore ● All India Institute of Medical Sciences, New Delhi ● National Centre for Cell Science, Pune ● National Institute of Immunology, New Delhi ● Indian Institute of Science, Bangalore ● Indian Institute of Technology, Chennai ● Research & Referral Hospital, New Delhi
Limbal stem cells	<ul style="list-style-type: none"> ● L.V. Prasad Eye Institute, Hyderabad ● R. P. Centre, AIIMS, New Delhi ● Regional Institute of Ophthalmology, Kolkata
Neural stem cells	<ul style="list-style-type: none"> ● National Brain Research Centre, Manesar ● National Institute of Mental Health and Neurosciences, Bangalore ● National Centre for Cell Science, Pune ● University of Hyderabad, Hyderabad
Mesenchymal stem cells	<ul style="list-style-type: none"> ● Christian Medical College, Vellore ● Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow ● Manipal Hospital, Bangalore
Liver stem cells	<ul style="list-style-type: none"> ● Centre for Liver Research and Diagnostics, Hyderabad ● Centre for DNA Fingerprinting and Diagnostics, Hyderabad
Pancreatic progenitor cells	<ul style="list-style-type: none"> ● National Institute of Nutrition, Hyderabad ● National Centre for Cell Science, Pune
Cardiac stem cells	<ul style="list-style-type: none"> ● Sree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram

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Muscle stem cells	<ul style="list-style-type: none"> ● Centre for Cellular and Molecular Biology, Hyderabad
Cancer stem cells	<ul style="list-style-type: none"> ● Indian Institute of Science, Bangalore
CMC-DBT Centre for Stem Cell Research	<ul style="list-style-type: none"> ● Christian Medical College, Vellore
Stem cell research facilities	<ul style="list-style-type: none"> ● Postgraduate Institute of Medical Education and Research, Chandigarh
Clean room facilities for stem cell research	<ul style="list-style-type: none"> ● Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow ● KEM Hospital, Mumbai ● L.V. Prasad Eye Institute, Hyderabad
Cord blood bank	<ul style="list-style-type: none"> ● Reliance Life Sciences, Mumbai ● Life Cell, Chennai

Source: Compiled by Author.

safety and efficacy before attempting clinical applications of stem cells. The basic requirements for clinical trial are: adequate infrastructure, i.e. Good Manufacturing Practices (GMP), clinical grade reagents, trained manpower, proper documentation, Standard Operating Procedures (SOPs), quality control, etc. In clinical application for most of the diseases, the basic issues such as which cells are best for which purpose, homing of cells, mechanisms of action, etc. involved in clinical trials are also not well known yet.

SCR in India: Emerging scenario

In India, several science agencies of the government are promoting SCR. Priority areas for research have been identified by means of thorough discussion in various fora on basic and applied research for specific diseases and various programmes have been supported on embryonic and adult stem cells. The major programmes include among others: establishment of hESC lines, use of limbal stem cells to repair corneal surface disorders caused by limbal stem cell deficiency; isolation, purification and characterization of haematopoietic, mesenchymal and liver stem cells; differentiation of stem cells into neural, cardiac, β cell lineages, etc. In addition, studies have been supported to explore the potential applications of adult stem cells in stroke, cardiac, pancreatic, spinal cord injury, use of lectins for haematopoietic stem cell preservation, etc. Apart from the government, some industry research organizations are also involved in SCR. For example, Reliance Life Science, Mumbai

has characterized 10 stem cell lines including two neuronal cell lines, Dopamine producing neurons and neurons for patients of stroke. One cell line has been deposited in the National Centre for Cell Science (NCCS), Pune. Their research focus is on ESC; haematopoietic stem cells; treatment of leukaemia; sickle cell anaemia; and skin and tissue engineering.

Interaction between clinicians-basic scientists already exists in several centres in India such as the Christian Medical College (CMC), Vellore; L.V. Prasad Eye Institute (LVPEI), Hyderabad; National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore; All India Institute of Medical Sciences (AIIMS), New Delhi; Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS), Lucknow; Manipal Hospital, Bangalore, etc. Limbal stem cells are being routinely used at LVPEI, Hyderabad to repair corneal surface disorders caused by limbal stem cell deficiencies. More than 300 patients suffering from severe limbal stem cell deficiency have been treated using limbal stem cells. At CMC, Vellore, a technology has been established for collection, isolation and purification of HSC for haploidentical haematopoietic stem cell transplantation. The first, haploidentical haematopoietic transplantation was carried out at CMC, Vellore in April 2003. NCCS, Pune and Indian Institute of Science (IISc.), Bangalore have shown that banana lectins may be used to preserve HSC and a patent has been filed for this finding. Phase I multi-centric clinical trial and a pilot study using bone marrow mononuclear cells have been initiated in the country on myocardial infarction and stroke, respectively.

A 'CMC-DBT Centre for stem cell research' has been supported at CMC, Vellore. SCR facilities are also being created at the Post Graduate Institute of Medical Education & Research (PGIMER), Chandigarh. The training centre for embryonic and adult stem cells has been supported jointly at the National Centre for Biological Sciences (NCBS) and Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASAR), Bangalore. Clean room facilities for SCR are being established at SGPGIMS, Lucknow; KEM Hospital, Mumbai; and LVPEI, Hyderabad. Dedicated short and long-term overseas fellowship programmes have been initiated by the Government of India for providing training to twenty five fellows every year in niche areas including stem cells. It has been decided to support both clinical and basic research on stem cells simultaneously. To consider the clinical trial proposals, four separate committees have been constituted: (i) Human Studies Committee for

evaluation and guidance for clinical research particularly for the development of clinical research protocols; (ii) Ethical Committee for Stem Cell Research to ascertain rigid ethical guidelines being followed while conducting research on human beings; (iii) Task Force to evaluate basic research and also recommend the funding for clinical research based on the evaluation of the above committees; and (iv) Programme Advisory Committee to consider the proposals received for the Centre of Excellence. For clinical application of stem cells, the initial focus could be on the phase-I clinical trial in cardiology, stroke, orthopaedics, limb ischemic disease, bone marrow transplantation for leukemia, spinal cord injury, liver, etc. As the experience and success is gained, this may be expanded to other clinical applications also.

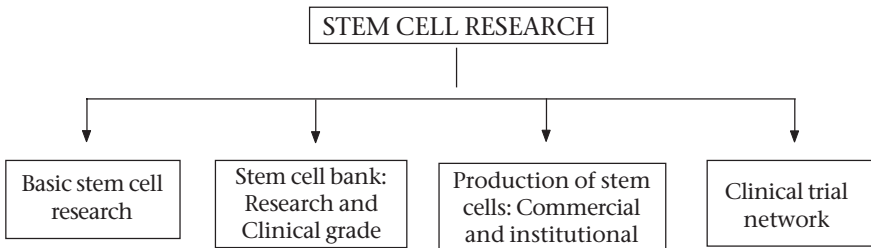
Would Stem Cell Bank be a Reality?

Thorough research is needed to understand exactly how stem cells work and how their potential can be harnessed for treatments of various diseases. Good quality of research materials are needed to carry out detailed basic research that may lead to clinical applications. A stem cell bank may be a useful repository for all types of stem cells, i.e. cord blood, adult and embryonic which may resolve the issue of standardization of cells/research materials as it is an important criteria for comparison and the reproducibility of results. The bank may hold stem cell lines derived originally from embryonic, foetal and adult tissues. This would also reduce the use of surplus embryos for the development of stem cell lines by individual teams. There is a need to develop genetically and biologically more diverse stem cell lines with better technologies in order to speed research for therapeutic purposes. To set up a stem cell bank it requires proper networking among hospitals for supply of cord blood and a public-private partnership model may work. Stem cells obtained from the bank may be used to examine and to better understand the process of all development, to learn how specific cell types and specific tissues and organs are formed. It may also be useful in enabling an understanding of the properties and behaviour of stem cells to determine their usefulness for future cell therapies; to understand what goes wrong in cells to cause various diseases. One may also study their ability to form different types of cells that can be used to restore or replace damaged tissue in patients with disease or injury; may compare hESC lines for their potential in tissue repair with adult stem cells; and confirm whether stem cells

from adult tissues or umbilical cord blood are pluripotent as compared to hESCs.

Parameters for quality control procedure in the stem cell bank are: checking of chromosomal abnormalities, testing of infectious diseases such as HIV I&L, HTLV, HCV, HBS Ag, CMV ability of stem cells to undergo freeze-thawing processes, immune compatibility of the stem cells with patients potentially requiring the cells, presence of viruses within the stem cells that may cause disease, ability of the stem cells to give rise to the required adult cell types when required, and ability of the stem cell numbers to be increased to useful amounts. In India, some companies have started establishing the repositories of cord blood banking. Reliance Life Sciences, Mumbai has a repository of 3,000 cord blood samples. These samples have been processed and tested for infectious diseases and stored at 196°C. Life Cell is a Chennai-based company and has a licence agreement and knowledge-sharing tie up with Cryo-Cell International, USA. They have a repository of 1,000 cord blood samples and are offering to preserve stem cells for 30 years.

Future Strategy for SCR



In the country, the main features of a strategy for SCR include the promotion of both basic and translational research using adult and embryonic stem cells as well as other more readily available sources such as bone marrow, peripheral blood and umbilical cord blood cells. The strategy also includes the creation of good infrastructure to handle stem cells. Human Resource Development (HRD) is the most important component of the strategy as creation of a critical mass of stem cell researchers in the country is a priority. This will include extensive training programmes, close interactions between basic scientists and clinical researchers, international collaboration including personnel exchange programmes, annual conferences,

workshops, etc. There is also a necessary focus on stem cell banks to resolve the issue of good quality research materials. Public-private partnership may be an ideal model for large-scale production of MSCs under cGMP conditions which will enable development of advanced technologies and products in this area.

Awareness must be created in industry which will be a stakeholder in the market scope of stem cell research. Once the process is standardized and the product is established, industry may take up further production/scale up for commercialization.

Global Regulations on SCR

Though SCR is one of the most exciting areas of the life science today, it has created lot of controversies and raised various ethical and moral issues on the use of embryonic/foetal stem cells in research. The

Table 2: Global Regulations of Human Embryonic SCR

Country	Policy
Australia	Approved SCR on human embryo isolated from supernumerary embryo after getting consent from the donors.
Canada	Assisted Human Reproductive Act allowing researchers to derive embryonic stem cell lines from left over embryos.
China	Human embryonic stem cells used for research purpose can only be derived from surplus IVF embryo, embryos created from fully-donated gametes and by nuclear transplantation.
France	France permitted research on embryo-derived cells in July 2004. French decree authorizing import of embryonic stem cells derived from supernumerary IVF embryos with the consent of the donors and research on the imported cells.
Germany	Prohibits the derivation and use of human embryonic stem cells from blastocysts.
India	Establishment of new hESC lines from spare, supernumerary embryos is permissible with prior approval of the Institutional Committee for Stem Cell Research and Therapy (IC-SCRT) and Institutional Ethics Committee (IEC) provided appropriate consent is obtained from the donor as per the draft guidelines.
Ireland	Prohibition of the creation of human embryos for research purposes and for the procurement of stem cells from human embryos by law.

Table 2 continued

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Country	Policy
Singapore, Israel, Italy	Allow the creation of human embryos for research purpose with somatic cell nuclear transfer technique as well as use of supernumerary embryos for procurement of human embryonic stem cells.
South Korea	Guidelines set by the Ministry of Health and Welfare issued after the South Korean Parliament in January 2004 banned human cloning but left room for stem-cell research for curing diseases.
Spain, Sweden, Denmark, Finland Greece, The Netherlands United Kingdom and Belgium	Allow the procurement of human embryonic stem cells from supernumerary embryos . Allow the creation of human embryos for research purpose by <i>in-vitro</i> fertilization, with somatic cell nuclear transfer technique as well as use of supernumerary embryos for procurement of human embryonic stem cells.
United States	Allow surplus frozen embryos from <i>in vitro</i> fertilization clinics for SCR with the permission of donors

Source: Compiled by Author.

controversy is not because of its goals, but rather because of the means of obtaining cells. There is no controversy in the research involving stem cells derived from adult tissues and umbilical cord blood. The crux of the debate centres around derivation of embryonic stem cells which require the destruction of an embryo.

Legislation governing hESC research varies from country to country. Some countries like India, Israel, Singapore, Sweden, Australia, United Kingdom and other European countries have relatively liberal and research-favourable regulatory policies, while others are still struggling to draft regulatory policies. Draft guidelines for SCR in India have been formulated jointly by the Department of Biotechnology, Ministry of Science and Technology and Indian Council of Medical Research. The same is currently being placed for public debate.

Conclusion

To explore the possibilities of clinical applications using stem cells, thorough basic research on all types of stem cells i.e. embryonic, adult and tissue is essential. National agencies are pro-active in supporting and promoting this area. However, there are many challenges in current stem

cell research such as non-availability of human resources of adequate expertise; very few indigenous hESC lines generated; inter disciplinary structure is yet to be created; enabling regulatory mechanisms is still evolving, etc. There is an enhanced awareness among the scientists and clinicians in the country about SCR and the process has gained momentum.

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