CENTRE FOR STEM CELL RESEARCH

(A unit of inStem, Bengaluru)

Christian Medical College Campus,
Bagayam, Vellore-632002

ANNUAL REPORT

2011-2012

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Centre for Stem Cell Research (CSCR),
Christian Medical College Campus, Bagayam, Vellore

The Beginnings …….2005-2010

The Center for Stem Cell Research (CSCR) was established in Vellore by the Department of Biotechnology (DBT) of the Ministry of Science and Technology, Government of India, in collaboration with the Christian Medical College, Vellore in December, 2005. It was envisaged to become the translation unit of the Institute for Stem Cell Biology and Regenerative Medicine (inStem), an autonomous institute of the DBT created in 2008. As of 2011, CSCR is integrated with inStem with focus on translation research and clinical studies with stem cells.

Mission

The Centre for Stem Cell Research stands for applying stem cell science to patient care. This will be achieved through collaborative multidisciplinary research of the highest quality that is relevant to the needs of this country. It will involve establishing intra and inter-institutional collaborations that will bring together basic scientists with different expertise and physicians to address clinical challenges. It will also aim to develop human resource for this field through a doctoral programs as well other training opportunities. An important goal will also be to share its facilities and expertise with other centre's and scientists working in this field in the country**.**

Governance – 2005 to 2010

Even though it was initiated as a project by the DBT, in view of the fact that it was envisioned to become an institution, CSCR was governed by a Governing Body, chaired by the Secretary DBT and also had a Finance Committee. There also was a DBT designated Scientific Advisory Committee that reviewed the work done at CSCR every year. In addition, there were two committees appointed by the CMC, Vellore to help with the management of CSCR on a regular basis both from the administrative as well as the scientific aspects. These included a Core Committee of scientists who would work with the Head, CSCR for all scientific issues and a Steering Committee, chaired by the Director, CMC, Vellore to provide policy guidance for CSCR in the early stages of its establishment.

Infrastructure

Within the funds available, the following physical infrastructure were established: about 34,000 sq ft of laboratory space, 5000 sq ft of small laboratory animal facility and about 4000 sq ft of cGMP space with four suites and space for further expansion. A core laboratory with all facilities for cell culture, light and fluorescent microscopy, flow-cytometry and molecular genetics was established. In addition, a laboratory animal facility with individually ventilated cages, a histopathology laboratory for routine staining, immunocytochemistry and FISH was also established. An autoclave facility for sterilization of laboratory ware has also been established. Five individual laboratories were also equipped for individual scientists to work with their teams. These details can also be viewed at the following url: www.cscr.in .

After construction of the building, installation of the scientific infrastructure and recruitment of suitable scientists over the first two years, scientific work began in 2008.

Milestones and Achievements 2008- 2010

- **2008**
  - Scientific work was initiated at CSCR

- **2009**
  - *Basic Science*
    - ✓ First dedicated centre for translational stem cell research established within an academic medical environment with several basic and clinical (physician) scientists involved in stem cell research.
    - ✓ A full GMP facility also created for generation of stem cells for clinical trials.
    - ✓ First report of generation of mouse induced pluripotent stem cells in India. (Dr. R.V. Shaji)

  - *Animal Models*
    - ✓ Regeneration of spinal cord injury in the rat with stem cells. (Dr. G. Tharion)
    - ✓ Recovery from traumatic brain injury using stem cell transplantation in the mouse. (Dr. R. Moorthy)
    - ✓ Repair of articular cartilage damage in joints and epiphysis (rabbits / goats), (Dr. V. Madhuri / Dr. Bhoopalan)
    - ✓ Treatment of inflammatory bowel disease in the mouse using mesenchymal stromal cells. (Dr. B.S. Ramakrishna)

  - *Human Stem Cell trials*
    - ✓ Bone marrow stem cells for the treatment of acute myocardial infarction (a DBT multicenter study). (Dr. S. Chandy / Dr. Paul George)
    - ✓ Mesenchymal stromal cells for treatment of steroid resistant graft versus host disease after allogeneic stem cell transplantation. (Dr. V. Mathews / Dr. A. Srivastava)

  - *Stem Cell Research – Policy and Regulation*

    Faculty involved with drafting the National Guidelines for Stem Cell Research, particularly the clinical aspects and standards for GMP facilities.

- **2010**
  - *Basic Science*

    - ✓ Human induced pluripotent stem cells generated from normal human fibroblasts (Dr. R.V. Shaji)
    - ✓ Work initiated towards developing engineered AAV vectors for gene transfer based on the previous work done at University of Florida, USA. (Dr. G. Jayandharan).
    - ✓ Pharmacogenetics of cytarabine and daunorubicin in the leukemic stem cell in acute myeloid leukemia. (Dr. B. Poonkuzhali)
    - ✓ Culture characteristics of human growth plate chondrocytes and effect of alteration of media characteristics. (Dr. V. Madhuri)
    - ✓ Cancer stem cells in squamous cell carcinoma of buccal cavity and tongue: Correlations with clinical behaviour and metastasis. (Dr. B. S. Ramakrishna)
    - ✓ .Isolation, cultivation and characterization of human corneal endothelial cells (HCECs). (Dr. Zia Pradhan / Dr. T. Kuriakose)

  - *Animal model*
    - ✓ Feasibility of urothelial cell culture and seeding on a decellularized scaffold. (Dr. Antony Devasia).
    - ✓ Role of mesenchymal stromal cell in modulation of microvesicular steatosis in the liver in the mouse. (Dr Uday Zachariah / Dr. C.E. Eapen / R. Sumathy / A. Srivastava).

  - *Human Stem Cell trials*
    - ✓ Autologous cultured chondrocytes from iliac crest in the treatment of physeal bars in children. (Dr. V. Madhuri / V. Mathews / A. Srivastava)
    - ✓ Treatment of large segmental bone defects in children treated with custom made triphasic hydroxyapatite ceramic coated scaffolds loaded with mesenchymal stem cells. (Dr. V. Madhuri / Dr. P. Nair / V. Mathews / A. Srivastava – SCTIMS)
    - ✓ Case control study to assess the benefit of transplantation of autologous bone marrow derived mononuclear cells in patients with nonreconstructable peripheral arterial disease presenting with critical limb ischemia. (Dr. I. Sen / Dr. S. Agarwal / V. Mathews / A. Srivastava)
    - ✓ Is the use of bone marrow derived mononuclear and mesenchymal stromal cells safe and feasible in prevention of wound breakdown in patients undergoing abdominoperineal excision after neoadjuvant

chemoradiotherapy for carcinoma rectum – a phase 1 study. (Dr. Johann Boaz / Dr. B. Perakath / V. Mathews / A. Srivastava)

- *Stem Cell Research – Policy and Regulation*
  - ✓ Dr. Alok Srivastava was appointed chairman of the National Apex Committee for Stem Cell Research and Therapy by the Secretary, Department of Health Research.

- *Training*
  - ✓ Initiated PhD programme in association with the Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum and Thiruvalluvar University, Vellore.
  - ✓ Training course of isolation, culture and characterization of adult stem cells
  - ✓ Short term student projects (Bi-annual)

CSCR – A unit of the Institute for Stem Cell Biology and Regenerative Medicine (inStem), Bengaluru

After completion of the term of CSCR in the project mode of the DBT in between Dec, 2005 and June, 2011, as envisaged in the approval of the Government of India for the establishment of the Institute for Stem Cell Biology and Regenerative Medicine (inStem) at Bengaluru, CSCR has integrated with inStem from 1.7.2011.

Core Scientific Activities and Initiatives: 2011 -2012

The current areas of collaborative research include gene therapy using the AAV vector for haemophilia, using induced pluripotent stem cells to create disease models as well as the possibility of developing safe iPS cells for potential clinical applications, studying endothelial precursors to understand vascular pathology in diabetes as well using them for creating vascular grafts, a program on evaluating the heterogeneity of mesenchymal stromal cells as well its clinical applications and a program on studying the haematopoietic stem cell niche and its applications in human disease. A few early clinical trials have also been undertaken utilizing the cell processing facility for such studies. Several clinical problems are also being evaluated in animal models of these diseases.

This report will provide details of the work done in these areas of research.

Core Facilities and Instrumentation

1. <u>CSCR Core Facilities:</u>

All facilities are accessible not only to scientists working full time at CSCR but also to all other scientists in CMC, Vellore who require these technologies / platforms for their work.

a. ***Molecular Biology Core Facility:***

- Faculty In-Charge: **Dr. R.V. Shaji**, PhD.
- Technical Officer: **Mr. S. Senthilnathan.**
- Graduate Technician Trainee: **Ms. J. Saranya.**

The Molecular Core Facility under the supervision of Dr. Shaji, is actively involved in providing the high end molecular biology services for the users(in house and off campus users) with DNA Sequencing using Life Technologies – Model 3130, a 4 capillary sequencer and RT PCR service with 7500 Fast and Quanta Studio RT PCR machines.

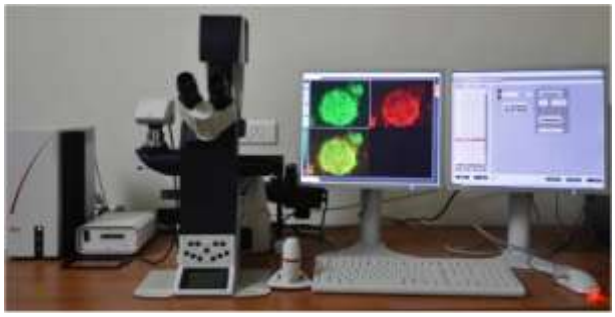
In addition to this Molecular core facility is also planning to conduct a workshop internally for the CSCR and CMC users to process the sample and also to analyze the same, the demo on operation of the software will also be given to the users on the same program.

b. ***Flow Cytometer Core Facility*:**

- Faculty In-Charge: **Dr. Sanjay Kumar**, PhD.
- Technical Officer: **Mr. S. Senthilnathan**.
- Graduate Technician Trainee: **Ms. J. Saranya.**

The Flow Core Facility is involved in providing Flow analysis and Flow Sorting services for the users (in house and off campus users) for Flow analysis using BD FACS – Calibur a 2 laser, 4+2 Parameter system and for sorting using BD FACS Aria-1, 3 laser, and 9+2 parameter system.

***c. Imaging Core Facility*:**



- Faculty In-Charge: **Dr. Rekha Samuel**, MD.
- Technical Officer: **Mr. S. Senthilnathan.**

The Imaging Core Facility is involved in providing Fluorescence imaging services for the users (in house and off campus users) using Leica DMI 6000B inverted fluorescent microscope can do 3 colors and one TL image at a time.

From January 2011 to June 2012 around 398 users were used this facility, with and without the technical assistance of our Core staffs.

d. Histopathology Core Facility:

- Faculty In-Charge: **Dr. Rekha Samuel**, MD.
- Technicians: **Mrs. Esther Rani**, DMLT and **Mr. Satish Perumal** , DMLT are the histopathology technicians responsible for the functioning of the histopathology laboratory at CSCR

New Developments

CSCR purchased a Shandon cytospin in 2012. The Histopathology core has standardized the following special stains:Perl's Prussian blue for Iron, Masson Trichrome, Periodic Acid Schiff, Gordon Sweet Reticulin, Alcian Blue.

Training

Mrs. Esther and Mr. Sathish received 4 days of training in immunohistochemistry, cytology smear preparation and cell block in the Department of Histopathology CMCH, in November 2011.

2. **CSCR Laboratory Animal Facility:**

- Faculty In-Charge: **Aparna Venkatraman**, PhD
- Scientific Officer: **Dr. R.Sumathy**, M.V.Sc
- Technicians: **Esther Rani and P. Sathish.**
- Graduate technician trainee: **R. Pavithra**.

The mandate given to the laboratory animal facility at CSCR is on "humane care, management and supply of small laboratory animals of quality" for scientific research activities at the institution.

The animal care facility is located in the basement of the building in a total floor space area of 5000 sq. ft with 6 animal rooms. The facility has got double corridor system to facilitate unidirectional movement of personnel. The 'clean' corridor is for the movement of the animal facility staff and animal users only. The 'dirty' corridor is for the movement of unsterile bedding, cages, and trolleys. This facility at CSCR is under the supervision of a full time veterinarian. It is registered with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests (Animal Welfare) Government of India (Reg. No.*88/1999/CPCSEA).* CSCR complies with the Government of India policy on animal welfare and all other applicable central, state and local laws. All protocols are submitted for review to the Institutional Review Board and Institutional Animal ethics Committee (IAEC)

Salient features of the facility:

- Barrier maintained facility with Individually Ventilated Caging system for animal breeding, holding and experimentation.
- Temperature and relative humidity of the animal rooms are maintained between 20 to 25 °C and 30 to 70% respectively throughout the year.
- Temperature and humidity monitored round the clock through individual room sensors.
- Photoperiod of 12 hrs light and 12 hrs dark maintained through automatic lighting system.
- Light intensity (300 lux) and noise level (< 65db) maintained as per CPCSEA regulations.
- Ad libidum supply of UV treated autoclaved R.O water and irradiated commercial diets to the animals.
- Regular health monitoring programs to ensure healthy animals to the experiments.

Species & strains available: The following 7 strains of mice namely,

1. BALB c/J mice
2. C57BL6/J mice
3. B6.129P2-F9tm1Dws/J
4. B6.CB17-Prkdcscid/SzJ
5. B6;129S4-Pou5f1tm1Jae/J
6. B6;129S4-F8tm1Kaz/J
7. CD-1 and
8. Sprague dawley rats

Protocols Established:

Genotyping protocols for the strains B6.129P2-F9^{tm1Dws}/J;B6.CB17-Prkdc^{scid}/SzJ and B6;129S4-Pou5f1^{tm1Jae}/J were standardised.

Man power:

A new graduate technician trainee Ms. Pavithra was recruited in April 2012.

Training:

Dr Sumathy attended the JAX Mouse Colony Management Workshop, conducted at NCBS, Bangalore, 7-9 March 2011

3. **CSCR Current Good Manufacturing Practice (cGMP) Facility:**

- Faculty In-Charge: **Dr. Vikram Mathews,** MD, DM
- Technical Officer: **Mr. Augustine Thambaiah**, MSc, P.G. Diploma
- Graduate Technician: **Ms. Aleya Tabasum,** BSc

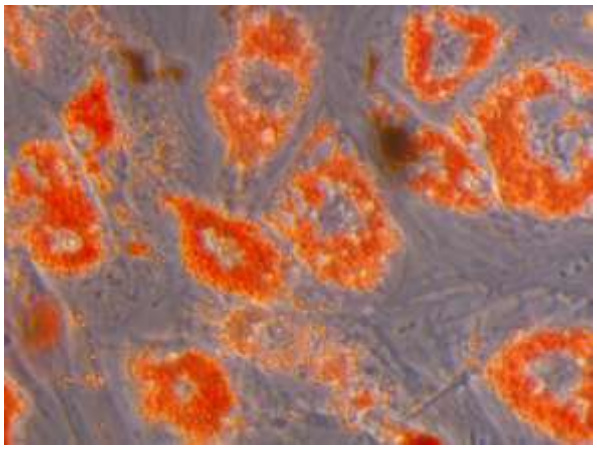
The Cell Processing Unit (cGMP Facility), under the supervision of Dr. Vikram Mathews, is actively involved in the large scale production of Clinical Grade Human Bone Marrow derived Mesenchymal Stromal Cells (CGMSC), which has therapeutic potential in various clinical settings. Expanded cells are cryopreserved and banked for future use in the liquid nitrogen storage facility.

A significant proportion of cell therapy based clinical trials that are underway involve Adult Stem Cells (mostly Mesenchymal Stromal Cells (MSC)). At our centre we have an ongoing clinical trial for treating steroid refractory acute Graft vs. Host Disease (aGvHD) with MSC. There are numerous published studies showing the utility of MSC in aGvHD and most experts in the world consider this an acceptable therapeutic option even outside the setting of clinical trial.

The cGMP facility has been functioning from December 2008. Over this period 51 bone marrow samples (total yield of ~ 5.3 billion MSC) and 2 placenta samples (total yield of ~550 million MSC) have been processed for clinical grade MSC expansion in vitro. During this period 43 patients with steroid refractory aGvHD have been treated with MSC infusion in an ongoing clinical trial.

From January 2011 to June 2012 around 11 Bone Marrow samples, with an average yield of 337 million MSC per sample was cryopreserved in a total of 37 bags with ~100 million cells per bag. Further 2 placenta samples were processed, with an average yield of 225 million cells per sample. The cells (both bone marrow and placenta derived MSC) were used to treat patients with aGVHD. We plan to expand MSC yield from placenta samples, which will be cryopreserved for future clinical trials at other centers.

In addition to MSC expansion, the CGMP facility is also involved in the culture and expansion of autologous chondrocytes. This is an ongoing clinical trial headed by Dr. Vrisha Madhuri (Department of Paediatric Orthopaedics, CMC, Vellore), titled "Autologous cultured chondrocyte from iliac crest in the treatment of physeal bars in children – A pilot study". They have successfully transplanted the cultured cells for 5 patients with no report of any adverse reaction.



***Ongoing research work*:**

Human platelet lysate as a substitute for Fetal Bovine Serum in the culture and expansion of bone marrow derived Mesenchymal Stromal Cells (Institution Fluid Research Funding)

New initiative 2011-12

1. **Establishment of Vector Core facility**

Establishing an Virus (Adeno-associated virus, Lenti-virus, Retro-virus) Vector Core facility at the Centre for Stem Cell Research (CSCR), Vellore.

The concept of treating human diseases by introducing normal alleles of genes into appropriate target cells has become a clinical reality. This is achieved by either direct transfer of the normal gene copy (*in vivo* gene therapy) or by gene modified cells such as by using stem cells (*ex vivo*- Cellular therapy) in the recipients. Although a variety of physical and chemical methods have been developed for introducing the defective genes, viruses have generally been proven to be much more efficient for this purpose. Despite its potential, gene and gene-modified stem cell therapy in general is an area untouched in India.

We at the Centre for Stem Cell Research (CSCR), Vellore have active interest in developing safe and efficacious gene transfer protocols for their potential use in patients with haematological disorders. To achieve this, several individual projects are being initiated and some are in active pursuit by different investigators. These require a large quantity of viral vectors to be generated and tested. We recognize that the main requirement for initiating and setting up such a gene and cell therapy research group is to develop an infrastructure for vector production. To facilitate this, we propose to establish an vector core within the existing infrastructure at CSCR, Vellore. The present initiative to set up a vector core will be the first of its kind in the country. The objective of this facility is to provide shared resources to investigators with a wide variety of relevant expertise to promote multifaceted approach to gene transfer and gene correction research. This will enable investigators to conduct their funded individual research projects more efficiently and/or more effectively. This core may not only serve the needs of a single Institution [CMC, Vellore] but will provide services on a Regional/National/level as elaborated further in this proposal.

The proposed vector core will have dedicated space and equipments for large scale plasmid generation, Cell culture, Vector purification and Quality control testing for seamless work-flow as well as to adhere to quality assurance/ containment requirements for such a BSC Level 2 recombinant DNA vector generation facility. The immediate capabilities of this core will include the rapid case construction and characterization of plasmid DNA constructs, and the production of both research and pre-clinical grade viral vectors useful for the genetic modification of mammalian cells or in their testing *in vivo*. The Core will provide its expertise in the design and construction of vectors useful for obtaining the tissue-specific expression of inserted genes, and vectors in which expression can be regulated by exogenous means. A rather unique feature of the Core is that in addition to providing vector production services and general advice on matters of gene transfer, members of the staff will become directly involved in the development and optimization of transduction protocols for a number of the specific cell

types that are the focus of studies by clinical and research investigators from CMC and from rest of the country. These investigators may have had limited experience with virus-derived gene transfer vector technologies, but may wish to utilize such technologies for efficient functional expression of genetic sequences of interest for a variety of genetic disorders seen in their practice or of their interest.

Long-term, we expect that the availability of a central vector core facility with a common principle of generating high quality vectors and technical expertise will significantly improve the pace and output of research and development in the field of gene and cellular therapy in India.

2. ***In vivo*** **imaging facility**

An upright multiphoton laser scanning microscope and confocal imaging systems have been sanctioned by the DBT and will be installed in CSCR. One of the proposed applications of the multiphoton laser scanning microscope system is to allow Dr. Rekha to continue vascular tissue engineering work at CSCR. The confocal laser-scanning microscope has been a much-anticipated system that will find a variety of applications with scientists working at CSCR.

3. **Small Animal Imaging**

Bioluminescent and fluorescent imaging by a single-view 3D optical imaging system combined with anatomical imaging by CT substantially improves the quantitative outcomes of *in vivo* imaging. In addition, it facilitates non-invasive longitudinal monitoring of disease progression, cell trafficking and gene expression patterns in living animals. With recent strict guidelines adopted by animal ethics committee's on the number of animals/alternative models proposed to be used, technologies that enable the scientists to track their research (such as the use of luciferase tagged cells/ vectors) on animals that facilitate serial monitoring of their interventions, have become an absolute necessity. This is more crucial in a Centre such as CSCR that has scientists working on diverse areas of stem cell biology and gene therapy. E.g. Luciferase tagged stem cells are required to track transplanted cells for understanding the complex nature of mesenchymal stem cell homing and engraftment in the murine bone marrow as well as in bones of morphogenetic disorders. It is also crucial to document (Dr. Sanjay). A similar approach is required for scientists who have interests in leukemic stem cell biology to understand the *in vivo* fate of subsets of cellular populations of interest (Dr. Aparna), identifying various hematopoietic stem cell and niche factors contributing to stem cell homing and engraftment characteristics in specific knock-out strains of mice, tracking the fate of tagged induced pluripotent stem cells *in vivo* (Dr. Rekha, Dr. Shaji), tracking the fate of gene delivered ScaI+/c-kit+ murine progenitor cells during primary and secondary transplants *in vivo* (Dr. Jayandharan).

The small animal imaging system will be a boon for researchers working in the field of gene therapy (Drs. Jayandharan, Sanjay). Most of the novel plasmid constructs developed in the CSCR for the potential gene therapy of various diseases such as haemophilia will have to be tested for their efficiency for gene expression through a reporter such as Luciferase or Green fluorescent protein *in vivo* in normal C57BL/6J mice. The availability of an imaging system capable of fluorescent and bioluminescent live imaging will be crucial for this purpose, as it dramatically reduces the number of animals required for monitoring. E.g. for just 1 novel gene therapy vector to be compared with its control for a period of 1 year, the requirement of animals normally would be 120 mice [5 animals per group X 2 vectors x 12 months (monthly sacking)] to monitor gene expression. However, the same experiment with a luciferase construct that allows for serial monitoring over a period of time rather sack groups of mice at individual time points would be just 10 mice [5 animals per group x 2 vectors]. This not only reduces the number of animals that are required for conducting research but also saves significantly on researchers time and resources' [flow cytometry costs] spent on their research.

The Xenogen IVIS Spectrum/CT imaging system that we wish to procure is the state of the art system that will fit most of these bioluminescent and fluorescent and CT imaging requirements at CSCR. Key features on the IVIS Imaging include, 3D optical tomography for fluorescence and bioluminescence, the most sensitive detection technology ideal for: Bioluminescence, Multispectral fluorescence and spectral unmixing and ultra fast micro CT. This system will be located at the CSCR animal facility for use by the full-time and adjunct scientists at CSCR.

Research Profiles

G. R. Jayandharan, PhD. Adjunct scientist, Jan 2010-present

RESEARCH PROGRAM:

ADENO-ASSOCIATED VIRUS MEDIATED TRANSLATIONAL GENE THERAPY

The collective experience with Adeno-associated virus (AAV) vector mediated human gene therapy trials so far, have clearly pointed to the need for substantial improvement in the efficiency of AAV mediated transgene expression as well as the need to attenuate the capsid-or transgene specific innate or adaptive immune responses against these vectors to achieve successful long-term gene transfer. My research is thus focused on dissecting out the biology of AAV life-cycle by understanding the interactions between AAV and various host cellular proteins, use this knowledge to design strategies to either augment the efficiency of gene transfer or intervene with (immune response) processes which are detrimental to AAV's survival, yet maintain the safety of these interventionist strategies to the host cellular environment and finally authenticate their use in therapeutic models such as pre-clinical animal models of hemophilia (Fig. 1). My individual grants were conceived to achieve this, albeit in parts, for an efficacious and safe gene therapy approach for hemophilia.

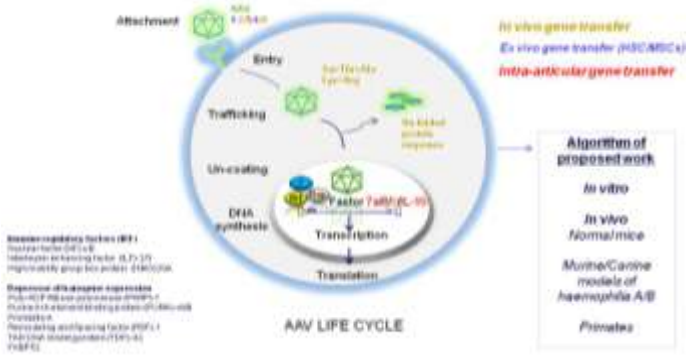


Figure: 1. Research overview: Understanding the biology of adeno-associated virus- host cellular interactions at each step of the virus life-cycle to improve the outcome of gene therapy for diseases such as haemophilia

LABORATORY HIGHLIGHTS OF YEAR 2011-12.

a. Manpower:

Name	Designation	Qualification/University
Dr. Dwaipayan Sen	Post-doctoral Fellow	PhD/ State University of Newyork, Binghamton, USA
Dr. Ruchita Selot	Post-doctoral Fellow	PhD/ Mysore University
Dr. Sabna Cheemadan	Post-doctoral Fellow	PhD/ Nanyang Technological University, Singapore
Mr. Nishanth Gabriel	Senior Research Fellow	Msc/ Cochin University of Science and Technology, Kochi
Mrs. Sangeetha Hareendran	Senior Research Fellow	Msc/ Cochin University of Science and Technology, Kochi
Mr. Balaji B	Junior Research Fellow	M.Phil/ Madras veterinary college, Chennai
Ms. Akshaya Krishnagopal	Junior Research Fellow	M.Phil/ Madras University, Chennai
Ms. KalaiVani	Technician	BSc/Thiruvalluvar University, Vellore

b. Awards

2012-16 **Swarnajayanti Fellowship Award**, Department of Science and Technology (DST), GoI **(Jayandharan GR)**
2010-13 **Innovative Young Biotechnologist**, Department of Biotechnology (DBT), GoI **(Jayandharan GR)**
2010-12 **Bayer Hemophilia Early Career Investigator Award**, Bayer Inc, USA **(Jayandharan GR)**
2011-**Eberhard Mammen Young Investigator** award, Thieme Publishers. **(Jayandharan GR)**
2012 - **Young Investigator Award**, SSC meeting of the International Society on Thrombosis and Haemostasis, Liverpool, United Kingdom **(Dwaipayan Sen)**
2012 **CSIR Senior Research** fellowship **(Sangeetha H)**
2011- **DST Travel Award** to attend American Society of Gene and Cell Therapy meeting **(Sangeetha H)**
2011- **Best Poster Award**, CMC Annual Research Day **(Sangeetha H)**
2012 **UGC Junior Research** fellowship **(Balaji B)**

c. Publications in 2011-12 (****corresponding author***):

1. **Giridhara R Jayandharan*,** Arun Srivastava, Alok Srivastava. Application of molecular genetics in haemophilia: from diagnoses to therapy. ***Sem Thromb Haemost*** 2012; 38: 64-78.
2. **Giridhara R. Jayandharan*,** Alok Srivastava. Hemophilia: disease, diagnosis and management. ***J Genet Syndr Gene Ther;*** 2012. doi: 10.4172/2157-7412.S1-005.
3. Li Zhong , **Giridhara R Jayandharan,** George Aslanidi, Sergei Zolutukin, Roland W Herzog, Arun Srivastava. Development of Novel Recombinant AAV Vectors and Strategies for the Potential Gene Therapy of Hemophilia ***J Genet Syndr Gene Ther;*** 2012. doi: 10.4172/2157-7412.S1-008.
4. George Aslanidi, Angela E Rivers, Luis Ortiz, Lakshmanan Govindasamy, Chen Ling, **Giridhara R Jayandharan,** Sergei Zolotukhin, Maevis Agbandje-McKenna, Arun Srivastava. High-efficiency transduction ofhuman monocyte-derived dendritic cells by capsid-modified recombinant AAV2 vectors. ***Vaccine*** 2012; 30: 3908-17.
5. **Giridhara R Jayandharan,** George Aslanidi, Ashley T Martino, Stephan C Jahn, George Q Perrin, Roland W Herzog, Arun Srivastava. Activation of the NF-kB pathway by AAV vectors and its implications in immune response and gene therapy ***Proc Natl Acad Sci USA*** 2011; 108: 3743-8.
6. **Geoffrey L Rogers, Ashley T** Martino AT, George Aslanidi, **Giridhara R Jayandharan,** Arun Srivastava, Roland W Herzog. Innate immune responses to AAV vectors. ***Front Microbiol,*** 2011; 2: e194.
7. Wen Qin Ma, BaoZheng Li, Chen Ling, **Giridhara R Jayandharan,** Arun Srivastava, Barry J Byrne. A simple method to increase the transduction efficiency of single-stranded AAV vectors *in vitro* and *in vivo*. ***Hum Gene Ther*** 2011; 22: 633-40.
8. Hilda Petrs-Silva, Astra Dinculescu, Qiuhong Li, Wen-Tao Deng, Ji-jing Pang, Seok-Hong Min, Vince Chiodo, Andy W Neeley, Lakshmanan Govindasamy, Antonette Bennett, Maevis Agbandje-McKenna, Li Zhong, BaoZheng Li, **Giridhara R Jayandharan,** Arun Srivastava, Alfred S Lewin, William W Hauswirth. Novel Properties of Tyrosine-mutant AAV2 Vectors in the Mouse Retina. ***Mol Ther*** *2011; 19: 293-301.*

ONGOING RESEARCH SUPPORT

1. Swarnajayanti Fellow (*PI: Jayandharan*) Dec 2011- Dec 2016, Department of Science and Technology, India- **Optimized AAV serotype 2 and 5 vectors by bioengineering surface exposed motifs to improve the efficacy of therapeutic gene transfer in hemophilia B.**
2. Early Career Investigator (*PI: Jayandharan*) June 2010-June 2012 Bayer Inc, USA, **AAVvectors for the potential gene therapy of hemophilia B: Modulation of the host immune response *via* the NF-□ B pathway.**
3. Innovative Young Biotechnologist Award (*PI: Jayandharan*) April 2011- March 2013 Department of Biotechnology (DBT), India, **Modulation of Adeno-associated virus (AAV) replication by host cell transcriptional repressors: Pharmacologic and RNA interference to improve AAV vector delivery during gene therapy.**
4. Research grant (PI: *Jayandharan, Co-I: Alok Srivastava, Sukesh C Nair*) June 2011 June 2014 DBT, India. **Efficacy of bio-engineered adeno-associated virus serotype 8 vectors for the potential gene therapy of hemophilia A.**

PENDING RESEARCH SUPPORT

1. Research grant (*PI: Jayandharan, Co-I: Alok Srivastava, Sukesh C Nair, Noel Walters, Viju Daniel*) Sep 2012- Sep 2015. DBT, India **Dissecting the molecular regulators of blood induced joint damage to develop targeted gene transfer strategies for haemophilia.**
2. Research grant (*PI: Jayandharan, Co-I: Alok Srivastava, Sukesh C Nair*) Sep 2012- Sep 2015. Indian Council for Medical Research, India. **Targeted delivery of human coagulation factor VII in myeloid compartment of haematopoietic stem cells for gene therapy of hemophilia B by Adeno-associated virus (AAV) vectors.**

PATENTS

1. Indian Provisional patent-1714/CHE/2012. Nucleotide sequence, recombinant vector, methods and kit there of. *Sangeetha H, Nishanth G, Dwaipayan S, Rupali G, Sudha G, Srinivasan N, Srivastava A, Jayandharan GR.*

2. Indian Provisional patent-1911/CHE/2012. A vector, recombinant cell, methods and kit there of. *Dwaipayan S, Aaron C, Noel W, Viju Daniel, Srivastava A, Jayandharan GR.*

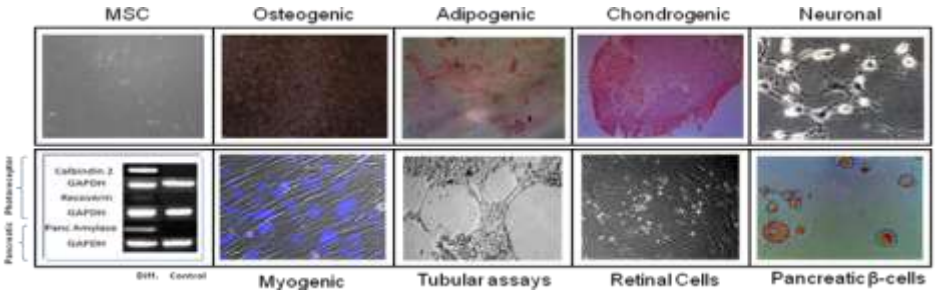
3. Indian Provisional patent-2231/CHE/2012. Nucleotide sequences, recombinant vectors, methods and kit there of. *Dwaipayan S, Ruchita S, Balaji B, Akshaya K, Srivastava A, Jayandharan GR.*

Sanjay Kumar, PhD, Ramalingaswamy Fellow, October 2010- present

RESEARCH PROGRAMS:

I. Therapeutic Applications of human term-placenta derived Mesenchymal Stem Cells (MSC) in mouse models.

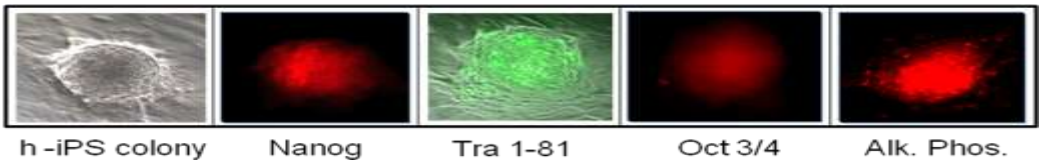
Mesenchymal stem cells (MSCs) are an alluring therapeutic resource because of their plasticity, immunoregulatory capacity and ease of availability. Human bone marrow derived MSCs have limited proliferative capability consequently it is challenging to use in tissue engineering and regenerative medicine applications. Hence, Placental MSCs of maternal origin, which serves as one of richest sources of MSCs, were chosen to establish long-term culture from the cotyledons of full-term human placenta. Flow cytometry analysis studies established bonafide MSCs phenotypic characteristics, staining positively for CD29, CD73, CD90, and CD105 and negatively for CD14, CD34, and CD45 markers. Pluripotency of the cultured MSCs was evidenced by induced differentiation towards not only intra-lineage cells like adipocytes, osteocytes, chondrocytes, myotubules, and endothelial cells but also trans-lineage cells such as pancreatic islet cells, neural cells and retinal cells displaying plasticity in their differentiation potential.



These cells did not significantly alter cell cycle or apoptosis pattern while maintaining the normal karyotype; also have limited expression of MHC-II antigens and are naïve for stimulatory factors CD80 and CD 86. Further soft agar tests revealed that placental MSCs do not have the ability to form invasive colonies in a soft agar assay. Taking together all these characteristics into consideration, it indicates that placental MSCs could serve as good candidates for development and progress of stem cell based therapeutics.

II. Generation of viral integration free human induced pluripotent (iPS) cells.

Results: Human induced pluripotent stem (iPS) cells generated from human term placenta-derived mesenchymal Stem Cells (MSC). Ongoing work: We have derived iPS like colonies from human placental MSC and currently characterizing and validating the basic features of induced pluripotent (iPS) cells.



LABORATORY HIGHLIGHTS OF YEAR 2011.

a. Manpower: Recruitment and training of Junior research Fellows (2), Short-term trainees and project fellows (6) and technical staff (1).

b. Awards:

Ramalingaswami Fellowship (Sanjay Kumar)

DBT Travel Award to attend Cold Spring harbour laboratory meeting on Cellular Reprogramming Shou Chou, China (Sabapathy V).

c. Publications: (Published / Manuscript in Prep.): 2 (CSCR) + 3 (CSCR + UAB)

Publications from CSCR

i. Long-term cultured human term placenta-derived mesenchymal stem cells of maternal origin displays plasticity. **Stem Cells Int.** 2012:174328.Mar 26. Sabapathy V, Ravi S, Srivastava V, Srivastava A, **Kumar S**.

ii. Isolation of human placenta-derived Mesenchymal Stem Cells (MSC) from maternal side of term-Placental tissue (Manuscript in preparation)

Publications from CSCR/UAB:

1. Bone healing by endogenous stem cell mobilization. **Bone**2012.Sep; 51(3): 635.Kumar **S**, Ponnazhagan S.

2. Mobilization of bone marrow mesenchymal stem cells *in vivo* augments bone healing in a mouse model of segmental bone defect. **Bone.** 2012 Apr; 50(4): 1012-8. **Kumar S**, Ponnazhagan S.

3. LL-37 as a therapeutic target for late stage prostate cancer. **Prostate**.2011. May; 71 (6): 659-70. Hensel JA, Chanda D, **Kumar S**, Sawant A, Grizzle WE, Siegal GP, Ponnazhagan S.

Abstracts:

i. Enrichment of bone marrow stem cells *in vivo* by dissociation- and proliferation-inducing compounds augments bone growth in a mouse segmental bone defect model. Sanjay Kumar and Selvarangan Ponnazhagan *(American Society of Gene and Cell Therapy, Seattle, WA 2011 published in* Molecular Therapy, ASGT, 2011)

ii. Long-term cultured human term placenta derived Mesenchymal Stem Cells of maternal origin displays plasticity *in vitro*

Vikram Sabapathy, Vivi Srivastava, Vikram Mathews, Alok Srivastava, Sanjay Kumar

(Poster presented at Cellular programs and Reprogramming meeting, 2011 organised by Cold Spring harbour laboratory (CSHL) and ISSCR at Shou Zhou China).

ONGOING RESEARCH SUPPORT:

i. Research grant (PI: Sanjay Kumar, Sept.2011-2013; Department of Biotechnology (DBT) India

Project Title: "Site-specific excisable AAV-based vector technology for consistent and reliable generation of virus-free patient-specific induced Pluripotent Stem (iPS) Cells"

ii. Research Grant Support from Ramalingaswami Fellowship, Oct. 2010-2015.

PATENT:

We are in the process to submit a request to C-CAMP Bangalore for initiation of a due diligence test on possible IPO application.

Project Name: **"**A novel vector technology for generation of virus-free patient-specific induced Pluripotent Stem (iPS) Cells".

Dr V. Madhuri, MS Orth, MCh Orth, Prof and Adjunct scientist,
Dr Abhay Gahukambale, Asst Prof and Adjunct scientist (on Leave) MS Ortho,
Dr Vivek Dutt, Asst Prof and Adjunct scientist, MS Ortho,
Dr B Balakumar, Asst Prof and Adjunct scientist, MS Ortho

(Paediatric Orthopedics Stem Cell Research Team)

RESEARCH PROGRAMS:

1. Musculoskeletal Stem Cells for tissue regeneration

Current collaborative efforts between India and Denmark in the area of stem cell biology have focused on interdisciplinary collaboration in the fields of stem cell research and scaffold engineering as well as technical expertise in the use of in vitro and in vivo models for musculoskeletal disorders. The PI for India for this project is Dr Prabha Nair and PI for CMC Vellore is Dr Vrisha Madhuri The project involves IIT Kanpur NCBS Banglore and Srichitra Tirunal Instiute of Medical Sciences, Trivandrum. Under this project multiple studies are under process.

Osteoarthritis (OA) model of rat knee has been successfully created in our laboratory by doing the surgical intervention which involved the medial meniscectomy and medial collateral ligament severance. Three drugs with properties of Anti VEGF, Wnt agonist and BMP antagonist are being evaluated in these models of rat OA knee. The initial results are promising with 2 months histopathology showing maintenance of cartilage integrity as assessed by Mankin scoring.

In second project, the discarded cartilage from the hip joint of the children with the Perthe's disease (4 patients), Idiopathic chondrolysis (2 patients) and slipped capital femoral epiphysis(1 patient) have been procured. The cartilage tissue is undergoing characterization using the markers like autotoxin, CD44, collagen II and collagen X.

In third project, the in vivo model of mice pseudarthrosis of tibia will be created using the mesenchymal stem cells (MSCs) isolated from the patient sample of congenital pseudarthrosis tibia CPT. MSCs from 2 patients with CPT have already been isolated and characterized. The animal ethics committee approval is awaited.

The study is awaiting the approval from animal ethics committee in which the polycaprolactone scaffold created using the electrospinning technique will be used with mesenchymal stem cells (MSCs) loaded over it. The large animal model (caprine) of articular cartilage over the femoral head will be created. The MSCs with this novel scaffold will be used for articular cartilage regeneration.

As a separate project, the cancer stem cells from the osteosarcoma tissue samples are being isolated and characterized. The chemosensitivity for routinely used drugs for these cancer stem cells will be tested which will be the first step towards selection of the targets for therapy for osteosarcoma.

2. Autologous cultured chondrocytes from iliac crest in the treatment of physeal bars in children

This pilot study is being carried out to treat the children with physeal bars (Physeal arrests and Physeal bridge) with autologous cultured chondrocytes from Iliac crest apophysis transplanted into the defect created after excision of the bar.

GMP (good manufacturing practices) protocol has been standardized as a first step as this study involves the human intervention. Recruitment for the study was started in March 2012. Total four children with mean age 4.5 years (range from 2 to 10) have been operated. Two children have undergone physeal bar excision and chondrocyte transplant at distal end of femur while one child is operated for physeal bar at proximal tibia. Fourth child is operated on both distal femur and proximal tibia. Fifth child has undergone the cartilage harvest and chondrocytes are under multiplication.

A mean 687.5 mg (range 450 to 800 mg) cartilage was harvested from ipsilateral iliac crest which yielded mean $2.32x10^6$ cells (range 0.5×10^6 to 7.1×10^6cells). A mean 217.75×10^6 chondrocytes (range 30×10^6 to 426×10^6) were transplanted into the physeal defect created after the bony bar excision.

Average duration of follow up following the index surgery is 7.7 weeks (range 1 to 14 weeks). One child has come for the follow up thrice while rest 3 children have followed up once following the physeal bar excision. Till date none of the children has faced any complication related to either cartilage harvest or the index surgery. Further follow up is required to analyze the longterm outcome.

LABORATORY HIGHLIGHTS OF YEAR 2011-12

a. **Manpower:** Recruitment and training adjunct scientist (3), Research associates(2), Senior research fellows (1), Project assistant (1), Research nurses and technical staff (4) and short-term project students (1).

Name	Designation	Qualification/University
Dr Sanjay Chilbule	Research associate	MS Ortho, Mumbai university. Postdoctoral fellowship paed ortho CMC Vellore
Mr Karthikeyan	Senior Research Fellow	Bharthiyar University Coimbatore
Ms Legasree	Project assistant	MSc VIT Vellore
Ms Soumya	Short-term project student (approved)	Shastra University Chennai

b. Awards:

1. Selected as member of International Paediatric Orthopaedics Think Tank
2. Invited speaker for the British Orthopaedic Association meeting 2012 Manchester - Indian summer session on "Cell therapies and scaffolds in articular and Physeal cartilage tissue engineering".
   Presentation on"The role of capital realignment and osteoplasty in slipped capital femoral epiphysis" at the Pre BOA meeting- London, UK.
3. Invited as guest speaker to Israel Orthopaedic Association meeting June Ramot 2012 Talks on 1) Acquired Physeal defects 2) Slipped capital femoral epiphysis An Indian experience3) Idiopathic chondrolysis in India and 4) congenital pseudarthrosis of tibia
4. Annual conference of Paediatric Orthopaedics Society of India (POSICON) 2012, Pune Best paper award – Author – Dr Balakumar, Dr Vrisha Madhuri
5. Annual conference of Paediatric Orthopaedics Society of India (POSICON) 2012, Pune Author- Dr Sanjay Arora, Dr Vrisha Madhuri
6. ICMR and International cartilage research society travelling fellowships to Dr Vivek Dutt for poster presentation at International Cartilage Research Society (ICRS) Congress at Montreal Canada in May 2012.

c. Publications in 2011-12: (***published/ in review/in revision.*corresponding author***):

1. Rajagopal K, Dutt V, Manickam AS, Madhuri V. Chondrocyte source for cartilage regeneration in an immature animal: Is iliac apophysis a good alternative? Indian J Orthop. 2012 Jul;46(4):402-6.
2. Chilbule SK, **Madhuri V.** Complications of pamidronate therapy for paediatric osteoporosis. J Child Orthop 2012;6(1):37-43, DOI: 10.1007/s11832-012-0383-5.
3. **Madhuri V** *et al.* Function Following Total Calcanectomy for Malignant Tumor in a Child: Is Complex Reconstruction Necessary? J Foot Ankle Surg. 2012;51(1):71-5.
4. **Madhuri V**, Dutt V, Samuel K, Gahukamble AD. Intra-operative femoral head vascularity assessment: An innovative and simple technique. Indian J Orthop 2011;45:231-5.
5. Mathew SE, **Madhuri V**, Alexander M, Walter NM and Gibikote S. Florid reactive periosteitis of the forearm bones in a child. J Bone Joint Surg Br 2011;93:418-20.
6. Bhalwani C and **Madhuri V**. Ultrasound profile of hips of south Indian infants. Indian Pediatr. 2011; 48(6):475-7.

Completed projects: Externally funded -

1. Role on Project: Principal Investigator Proposed duration: 36 months Total cost : 32,81,000 INR **Funding Agency:** Department of Biotechnology, India
   Project Title: Efficacy of autologus chondrocyte transplantation for physeal injuries in goat

Internally funded-

2. Efficacy of mutilayered biomimetic scaffold loaded with cultured chondrocytes in the articular cartilage regeneration in rabbit knees. Collaboration with IIT Kanpur
   PI- Dr Abhay Gahukambale CO-I – Dr Vrisha Madhri
3. Efficacy of cultured chondrocyte loaded on scaffolds Monolayer vs PVA-PCL-IPN vs Biphasic in the articular cartilage regeneration in rabbit knees – Looking at the long term effects. A collaborative project using scaffolds generated at Sri Chitra Tirunal Institute of Medical Sciences.
   PI- Dr Vivek Dutt CO-I – Dr Vrisha Madhri
4. Evaluation of culture characteristics of human growth plate chondrocytes.
   PI- Dr B Balakumar CO-I – Dr Vrisha Madhri
5. Culture characteristics of cryopreserved human growth plate chondrocyte
   PI- Mr Karthikeyan R CO-PI- Dr Vrisha Madhuri

ONGOING RESEARCH SUPPORT-

Internally funded projects-(fluid research grant)

1. Autologous cultured chondrocytes from Iliac crest in the treatment of Physeal bars in children. PI- Dr Vrisha Madhuri

Externally funded projects-

1. **Project - Musculoskeletal stem cell in tissue regeneration**.

Funding agency – Danish council for strategic research and Department of Biotechnology, India.

Fund - 100,000 Euro

Projects under Indo- Danish funding-

a. Cartilage regeneration in an osteoarthritis rat knee model.
   The drugs being evaluated are
   a. Anti VEGF
   b. BMP antagonist
   c. WNT-agonists
   PI- **Dr Vrisha Madhuri**

b. Biology of Cartilage regeneration in childhood articular disorders.
   PI- **Dr Vrisha Madhuri**

c. Isolation and characterization of cancer stem cells from human osteosarcoma tissue
   PI- **Dr Sanjay Chilbule CO-PI- Dr Vrisha Madhuri**

d. Generation of a mouse model of congenital pseudarthrosis of tibia (CPT) by

injection of Mesenchymal stem cells (MSCs) derived from human lipofibromatosis tissue isolated from CPT biopsies in children.
PI- Dr Vrisha Madhuri

e. Efficacy of cultured mesenchymal stem cells (MSCs) loaded over the PCL electrospun scaffolds for regeneration of articular cartilage defects in goat hip joint.
PI- Dr Vrisha Madhuri

2. **In vitro and in vivo testing of a layered 3-D composite scaffold for articular cartilage tissue engineering**
Funding agency-Dept of Science and Technology, Government of India.
Budget - Rs 49.02Lakhs
Role- Principal Investigator
In collaboration with IIT Kanpur with Dr Dhiren Katti as the the PI for the IIT Kanpur part of the project.

3. **Design of a compliance monitored clubfoot brace for management of clubfoot deformity in children**
Funding agency- Dept of science and technology, Government of India.
Budget - Rs 33.6482 Lakhs
Role- Principal Investigator

4. **Assessment of incidence of DDH in south Indian population using ultrasonographic technique**
Funding agency- Indian Council for Medical Research,Government of India.
Budget - Rs 37.71 lakhs
Role – Principal Investigator

5. **Use of nano-bead implants in infected open fractures, failed implant surgeries and osteomyelitis for defect management and local delivery of appropriate antibiotics.**
Funding agency – Sri Chitra Tirunal Institute of medical Sciences,Trivandrum
Role – Principal Investigator
Budget – Rs 6 Lakh

PENDING RESEARCH SUPPORT

1. **Treatment of large segmental bone defects with custom made triphasic hydroxyappetite scaffolds loaded with mesenchymal stem cells in children.**
Funding agency- Dept of Biotechnology Government of India.
Budget – 50 lakhs
Awaiting ethics clearance

Dr. B. S. Ramakrishna, MD, DM, PhD (Gastroenterology)

RESEARCH PROGRAM:

CANCER STEM CELLS IN GASTROINTESTINAL CANCER

My laboratory is interested in identifying and elucidating the biology of cancer stem cells. We are essentially using the model of oral squamous cell cancer which has many similarities with squamous carcinoma of the esophagus. We harvest tumor cells from resected specimens of oral cancer, and through a combination of flow cytometry, *in vitro* clonogenicity assays, anchorage-independent spheroid formation assays, and *in vivo* injection into SCID mice, we have identified populations of cells that have characteristics of cancer stem cells in oral squamous carcinoma. Studies are ongoing to identify common mechanisms that may confer cancer stem cell properties on these cells. The biological properties of these cells will be correlated with the clinical behavior of the tumors from which these cells were isolated and these clinical correlations will hopefully be of use in identifying prognostic markers for targeted therapies. In addition to the oral cancer model, we have also been examining pancreatic neoplasms for these stem cells and their signaling pathways.

LABORATORY HIGHLIGHTS OF YEAR 2011-12.

a. Manpower: Ongoing training of Senior Research Fellows (1), technical staff (1) and short-term project students (4).

Name	Designation	Qualification/University
Sam Vijay Kumar	Senior Research Fellow	MSc, Sri Ramachandra University, Chennai
Santosh	Short-term project student	
Femina M.K.	Short-term project student	M.Sc. Biotechnology, VIT University
Faby Alexander	Short-term project student	M.Sc. Biotechnology, VIT University
Sunu Joseph	Short-term project student	M.Sc. Biotechnology, VIT University

b. Awards: None relevant to stem cells

c. Publications in 2011-12 None relevant to stem cells

ONGOING RESEARCH SUPPORT: (0)

Murugan Ramalingam, PhD., FIoN., FRSC., Associate Professor (Scientist G), June 2012- present

RESEARCH PROGRAM:

1. COMBINATORIAL NANOFIBER LIBRARIES FOR HIGH-THROUGHPUT SCREENING OF STEM CELL BEHAVIOR AND THEIR INTERFACE TISSUE REGENERATIVE CAPACITY AND MECHANISMS:

This project involves the development of combinatorial nanofiber libraries suitable for high-throughput screening of stem cell-material interaction. Scaffolds play a key role in tissue engineering wherein they provide structural support for cells to adhere, grow and guide them to synthesize tissue. Scaffolds made of multiple biomaterials are typically required to mimic the structural and compositional features of native extracellular matrix (ECM). Screening the effect of scaffold compositions and properties toward stem cell behavior that optimize tissue regeneration is the key for the selection of scaffolds for use in tissue engineering. Although previous approaches for rapid screening have used biomaterial libraries in the form of 2D surfaces or films, biomaterials are commonly used in a 3D scaffold format and cells behave more physiologically when cultured in a 3D-matrix environment. Therefore, this project aims to develop a 3D combinatorial gradient nanofiber scaffold libraries suitable for high-throughput screening of cellular responses such as adhesion, proliferation and differentiation that optimize tissue growth and continued evaluation of their interface (soft-to-hard) tissue regenerative capacity and mechanisms.

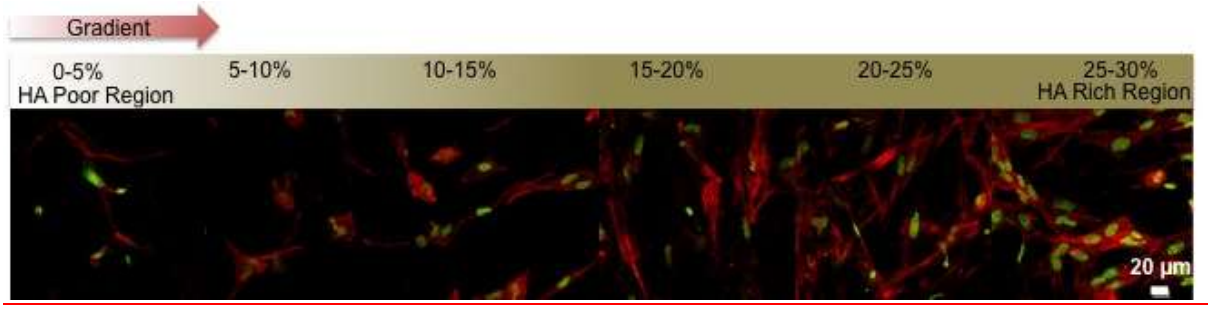


Figure 1 A representative confocal image of stem cell response to gradient ECM. HA denotes hydroxyapatite, a major bone mineral substance.

2. BIOINSPIRED CORE-SHELL NANOPARTICLE FORMULATION FOR SYSTEMIC DELIVERY OF SMALL INTERFERING RNAs AGAINST HEPATITIS C VIRUS:

This project aims to develop a biodegradable core-shell ceramic-polymer nanoparticle-based non-viral vector for gene silencing therapy. Hepatitis C virus (HCV) is one of the major human hepatic RNA viruses, which causes chronic liver diseases and affects over 300 million people worldwide. Treatment options for chronic viral hepatitis are limited. RNA interference represents an emerging technology that could have therapeutic applications for the treatment of HCV infections. The HCV genome is a single-stranded RNA that functions as both an mRNA

and a replication template, making it an attractive target for therapeutic approaches using short interfering RNA (siRNA). The delivery of siRNA in mammalian cells to target specific disease-causing genes represents a potential strategy for the treatment of HCV infectious diseases. A major limitation for the success of therapeutic siRNA-based strategies is systemic delivery of synthetic RNA to its target cells. Therefore, to effectively delivery the siRNA, the project aims to develop a new core-shell ceramic-polymer nanoparticle-based biodegradable non-viral delivery system and validate its efficacy in gene silencing therapy.

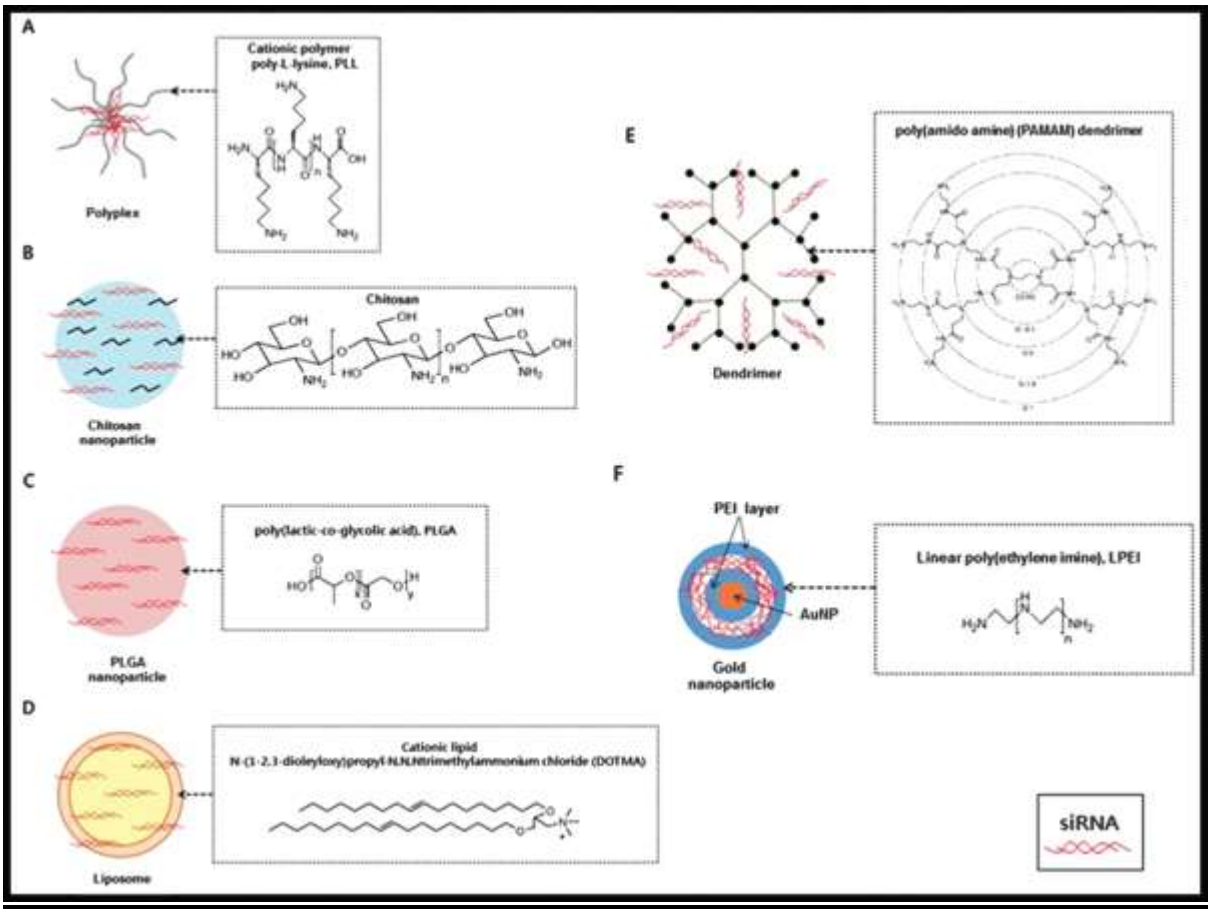


Figure 2. siRNA delivery carrier systems

LABORATORY HIGHLIGHTS OF YEAR 2011-12:

Dr. Ramalingam has recently joined the centre, CSCR, and is in the process of setting up "Stem Cell Nanotechnology" Laboratory to initiate interdisciplinary research on how nano-features and nano-structured assembly impact the clinically relevant stem cell functions and to study their mechanisms to develop disease models and artificial tissues and organ.

Dr. Rekha Samuel, MD, Professor, July 2011-present

Rekha Samuel returned to CSCR, in July 2011 from a 3-year postdoctoral fellowship in Dr. Rakesh K. Jain's laboratory at Harvard Medical School, Boston. This was funded by the fellowship- Associate ship in niche areas in biotechnology, Department of Biotechnology, Government of India, Ministry of Science and Technology. During her postdoctoral period, Rekha worked on the biology of endothelial and perivascular stem and progenitor cells. Some of the specialized techniques that she has worked on during her vascular tissue engineering training in Boston include chronic *in vivo* imaging using multiphoton laser scanning microscope, and derivation of human induced pluripotent stem cells (hiPS) from healthy and diseased models and novel differentiation protocols and characterization of endothelial and perivascular stem cells from hIPS cells. This project was a collaboration between the laboratories of Dr. Rakesh K. Jain, PhD (Director of the Edwin L Steele Laboratory for tumour biology, Massachusetts General Hospital), Dr. David Scadden, MD (Co-Director, Harvard Stem Cell Institute and Director, MGH Centre for Regenerative Medicine), and Dr. Laurence Daheron, PhD (Research Manager and Head of the Harvard Stem Cell Institute iPS Core facility).

RESEARCH PROGRAMME:

Dr. Rekha's vascular biology laboratory at CSCR is interested in the cellular and molecular mechanisms involved in human endothelial progenitor and perivascular cells in forming functional stable vasculature.
Besides fundamental insights into pathogenesis of vascular disorders, it is hoped that this knowledge will be applicable to optimization of clinically relevant treatment options such as targeting specific cytokines, autologous vascular cell therapy, and vascularization of engineered tissues. Current projects in Dr. Rekha's lab include Microvascular dysfunction in Type 2 Diabetes and biology of vascular progenitor/ stem cells from adult tissue.

a. Manpower: (0)

b. Awards during fellowship:
Harvard Stem Cell Institute Imaging Contest, November 3rd, 2010, in conjunction with the Stem Cell Salon, "New Technologies for Imaging Cells".
(http://www.hsci.harvard.edu/announcements/winners-%0Bhsci-imaging-contest)

c. Publications during fellowship: (Published 2-Harvard medical school)

1. Direct evidence that Bevacizumab, an anti-VEGF Antibody up regulates SDF-1 Alpha, CXCR4, CXCR6 and Neuropilin 1 in tumors form patients with rectal cancer. Xu L, Duda DG, di Tomaso E, Ancukiewicz M, Chung DC, Lauwers GY, Samuel R, Shellito P, Czito BG, Lin PC, Poleski M, Bentley R, Clark JW, Willett CG, Jain RK.Cancer Res. 2009 Oct 15; 69(20): 7905-10

2. PDGF-D improves drug delivery and efficacy via vascular normalization, but promotes lymphatic metastasis by activating CXCR4 in breast cancer. Liu J, Liao S, Huang Y, Samuel R, Shi T, Nexerova K, Huang P, Kamoun WS, Jain RK, Fukumura D and Xu L. Clin Cancer Res. 2011 Jun 1; 17(11): 3638-48

In review:

Dwaipayan Sen, Rupali A Gadkari, Sudha Govindarajan, Nishanth Gabriel, Yesupatham Sathish Kumar, Rekha Samuel, N Srinivasan, Alok Srivastava, Giridhara R Jayandharan. Targeted modifications in adeno-associated virus serotype (AAV)- 8 capsid improves its hepatic gene transfer efficiency *in vivo*. Hum Gene Ther Methods 2012; in review

Posters:

1. Functionally competent engineered blood vessels from human induced pluripotent stem cells-derived from Type 1 Diabetes patients. *Samuel R*, Daheron L, Kamoun WS, Batista A, Liao S, Buecker C, Schäfer R, Au Patrick, Melton D , Scadden DT, Duda DG,Fukumura D & Jain RK. Massachusetts General Hospital, Boston, USA, Annual Clinical Research Day, October 6th, 2011

Manuscript in progress

2. Pre-gestational Diabetes with Chorangiosis and Nodular chorangioma. A case report. *Samuel R*, Perumal S, Srivastava A, Benjamin S, Mathews J. International Federation of Placenta Association (IFPA). 18-21st September, Hiroshima, Japan.

Abstract: Placenta, 2012; 33 ;(9): A1–A137

ONGOING RESEARCH SUPPORT (3)

Start up fund from CSCR (1)
Christian Medical College Fluid Research Grants (2).

PENDING RESEARCH SUPPORT (1)

Department of Biotechnology, Government of India. (PI: Rekha Samuel, 2012-2014, total award: 42 lakhs) Placental Pericytes and Microvascular Dysfunction in Type 2 Diabetes.

Alok Srivastava, MD, FRACP, FRCPA, FRCP

RESEARCH PROGRAM:

My work in stem cell research involves two areas:

1. Clinical translation of stem cell research: This involves two aspects –

I. Policies and regulations for clinical translation of stem cell research in India – This work is done through the National Apex Committee for Stem Cell Research and Therapy of the Department of Health Research, Ministry of Health. (www.icmr.nic.in/icmrnews/NAC.htm)

II. Stem cell transplantation studies – This involves working with colleagues in different disciplines to test therapeutic strategies in animal models and human stem cell transplantation studies. Apart from long standing and continuing involvement with different aspects of haematopoietic stem cell transplantation, currently these studies include work on cartilage repair (collaboration with Dr. V. Madhuri), wound healing (collaboration with Dr. B. Perakath) and limb ischemia (collaboration with Dr. Indrani Sen).

2. Basic research with stem cells: This work also involves two areas –

I. The first is towards developing gene therapy for hemophilia using adeno associated vectors for gene transfer. (collaboration with Dr. G. Jayandharan in CSCR and Dr. Arun Srivastava, University of Florida, USA and Dr. Amit Nathwani, University College London, UK). The aim is to combine two different strategies for more efficient transduction for transfer of the FIX human clotting factor gene in human studies. Pre-clinical work is ongoing at present.

II. We are also keen to apply the evolving knowledge of the bone marrow stem cell niche to human diseases. Current data suggests that blood diseases such as marrow failure or dysplasia may not always be related to defects in the hematopoietic stem cells only but may actually be the effect of changes in the different components of the niche. Towards this end we are developing a collaborative work involving several scientists to look at the clinical, haematological, cytogenetic, molecular and cellular (HSC, endothelial and mesenchymal) elements of the niche.

Students:

1. Salar Abbas works on the BM niche

2. Sangeeta Hareendran works on AAV vector modifications (in collaboration with Dr. G. Jayandharan)

3. Nishant Gabriel works on AAV vector modifications (in collaboration with Dr. G. Jayandharan)

Selected publications:

1.Scadden D, Srivastava A. Advancing stem cell biology toward stem cell therapeutics. Cell Stem Cell. 2012 Feb 3;10(2):149-50.

2.G.R Jayandharan, A. Srivastava, A. Srivastava. Application of molecular genetics in haemophilia: from diagnoses to therapy. *Sem Thromb Haemost* 2012; 38: 64-78.

(There are several more in collaboration with G. Jayandharan – See his report in this document)

3. Balasubramanian P, Desire S, Panetta JC, Lakshmi KM, Mathews V, George B, Viswabandya A, Chandy M, Krishnamoorthy R, Srivastava A. Population pharmacokinetics of cyclophosphamide in patients with thalassemia major undergoing HSCT. Bone Marrow Transplant. 2012 Sep;47(9):1178-85.

4. Moorthy RK, Sam GA, Kumar SV, Chacko G, Mathews V, Chacko AG, Srivastava A, Rajshekhar V. Intralesional mesenchymal stromal cell transplant in a rodent model of cortical cryoinjury. Neurol India. 2011 Jul-Aug;59(4):573-8.

5. George B, Mathews V, Viswabandya A, Lakshmi KM, Srivastava A, Chandy M. Allogeneic hematopoietic stem cell transplantation is superior to immunosuppressive therapy in Indian children with aplastic anemia—a single-Centreanalysis of 100 patients. Pediatr Hematol Oncol. 2010 Mar;27(2):122-31.

6. Rajasekar R, Lakshmi KM, George B, Viswabandya A, Thirugnanam R, Abraham A, Chandy M, Srivastava A, Mathews V. Dendritic cell count in the graft predicts relapse in patients with hematologic malignancies undergoing an HLA-matched related allogeneic peripheral blood stem cell transplant. Biol Blood Marrow Transplant. 2010 Jun;16(6):854-60.

7. Rajasekar R, Mathews V, Lakshmi KM, George B, Viswabandya A, Chandy M, Srivastava A. Cellular immune reconstitution and its impact on clinical outcome in children with beta thalassemia major undergoing a matched related myeloablative allogeneic bone marrow transplant. Biol Blood Marrow Transplant. 2009 May;15(5):597-609.

Book chapter:

Stem Cell treatments around the world: Boon or bane? A. Srivastava in Mesenchymal Stromal Cells. Eds: P. Hematti and A. Keating. 2012

Patents (applied for):

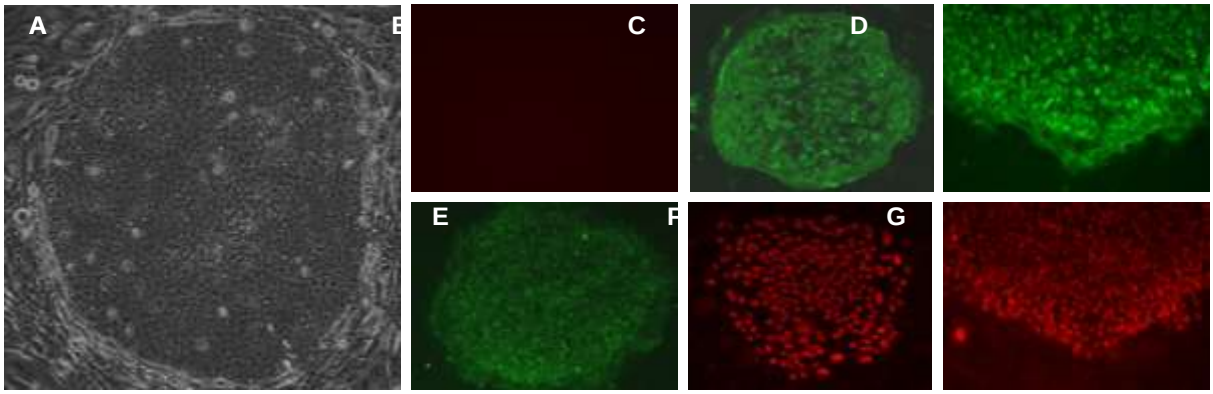
1. Indian Provisional patent-1714/CHE/2012. Nucleotide sequence, recombinant vector, methods and kit there of.
2. Indian Provisional patent-1911/CHE/2012. A vector, recombinant cell, methods and kit there of.
3. Indian Provisional patent-2231/CHE/2012. Nucleotide sequences, recombinant vectors, methods and kit there of.

RV Shaji, PhD. Professor, Adjunct Scientist (Dec 2008 - present)

RESEARCH PROGRAM:

1. **Application of human induced pluripotent stem cells for understanding the mechanisms of human diseases.**

Ectopic expression of four transcription factors *OCT4, SOX2, KLF4* and c*MYC* can reprogram somatic cells to a pluripotent state. These induced pluripotent stem cells (iPSCs) exhibit many of the molecular and functional characteristics of embryonic stem cells (ESCs). Their *in vitro* and/or *in vivo* differentiation permits the study of many cell types and they allow constant access to different cell types including those, which are difficult-to-obtain cell populations like neurons, cardiomyocytes and hepatocytes. Human iPSCs have been derived from several terminally differentiated cells obtained from patients with genetic diseases. We have established the methods to generate human iPSCs using retroviral and episomal expression of transcription factors. The clones have been characterized by gene and protein expression and in-vitro differentiation. We are currently working on generation of iPSCs from patients with hematological diseases and their differentiation to erythroid cells for transcriptional and epigenetic changes towards understanding the disease mechanisms.



Identification of novel genetic regulators of reprogramming: The process of pluripotency induction could be accelerated by alternate reprogramming factors or chemical molecules like epigenetic modifiers and cell cycle regulators etc. However, the process is inefficient resulting in marked heterogeneity in the levels of pluripotency in the generated iPSC clones. Using RNAi and over expression strategies we are currently carrying out projects to identify the new regulators of reprogramming.

Transcriptional regulatory mechanisms in human erythropoiesis: Using a robust ex-vivo erythropoiesis system developed in our laboratory we differentiate haematopoietic stem cells and progenitors to erythroid cells. We are carrying out experiments to understand the transcriptional changes that occur in different stages of human erythropoiesis in normal and disease conditions. Our experimental strategies include ChIP-sequencing. Using globin genes as a candidate for understanding the developmental and differentiation stage specific

transcriptional regulation of mammalian genes our experiments are also focused to study the mechanisms for increased foetal globins in adults. We have identified several genotypes of thalassaemia patients with increased foetal globin production and using the ex-vivo erythropoiesis system we are planning to study the transcriptional mechanisms involved in globin switching.

LABORATORY HIGHLIGHTS OF YEAR 2011-12.

a. Manpower: Four Junior research fellows have been recruited

b. Research projects:

1. Generation of human induced pluripotent stem cells from adult somatic cells (May 2010-October 2011). Funded by Department of Biotechnology. Completed.
2. Understanding the molecular basis of human globin gene regulation (May 2009- May 2014). Funded by Department of Biotechnology. Ongoing.
3. RNAi screen to identify regulators of reprogramming (November 2012-Nov 2015). Funded by Department of Biotechnology. Ongoing.

c. Publications:

1. Kannan VM, Syed Mohammed MA, Chettri P, Sumitha PB, Srivastava A, **Shaji RV**. Retroviral silencing and pluripotency in mouse iPS clones. (Abstract presented in ISSCR meeting 2011, Toronto)

2. Sumitha PB, Syed Mohammed MA, Kannan VM, Srivastava A, **Shaji RV.** Initial culture conditions of human dermal fibroblasts increase efficiency and pace of their reprogramming. (Abstract presented in ISSCR meeting 2011, Toronto)

3. David S, Jayandharan GR, Abraham A, Jacob RR, Devi GS, Patkar N, **Shaji RV**, Nair SC, Viswabandya A, Ahmed R, George B, Mathews V, Chandy M, Srivastava A. Molecular basis of Wiskott-Aldrich syndrome in patients from India. Eur J Haematol. 2012 Jun 9 .

4. Mayuranathan T, Rayabaram J, Edison ES, Srivastava A, **Shaji RV**. A novel deletion of β-globin promoter causing high HbA2 in Indian population. Haematologica. 2012 May 11.

5. Edison ES, Sathya M, Rajkumar SV, Nair SC, Srivastava A, **Shaji RV**. A novel β-globin gene mutation HBB.c.22 G>C produces a hemoglobin variant (Hb Vellore) mimicking HbS in HPLC. nt J Lab Hematol. 2012 Apr 4.

6. Edison ES, **Shaji RV**, Chandy M, Srivastava A. Interaction of hemoglobin E with other abnormal hemoglobins. Acta Haematol. 2011;126(4):246-8.

Aparna Venkatraman, PhD, Associate Professor, July 2012-present

RESEARCH PROGRAM:

ROLE OF NICHE IN MAINTENANCE OF STEM CELL QUIESCENCE

Dr. Aparna returned to CSCR, in July 2012 from a three and half year of Senior Research Associateship in Dr. Linheng Li's laboratory at Stowers institute for Medical research, Kansas City,Missouri,USA. The overseas associate ship in niche areas in biotechnology, Department of Biotechnology, Government of India, Ministry of Science and Technology, funded this. During herassociateship, sheworked on the biology of hematopoietic stem cell and its surrounding niche cells in different animal models elaborated below.

LABORATORY AT STOWERS:

Maintenance of stem cell quiescence by epigenetic regulation

Proper maintenance of a quiescent state is necessary to preserve the adult stem cell, but how this state is regulated is poorly understood, especially at the epigenetic level. Imprinting is a form of epigenetic regulation in which gene expression occurs in a mono-allelic manner. During my overseas associateship we unraveled an unexpected enrichment of imprinted gene *H19* along with other growth-restricting imprinted genes in quiescent hematopoietic stem cells. Conditional deletion of the *H19* imprinting control region(ICR) only from the maternal allele, led to reduced HSC quiescence, impaired function and implicated miR-675(derived from *H19*) as an important effector. By demonstrating a role for the maternal *H19* imprinting locus in maintenance of adult HSC quiescence and long-term function, this body of work established for a role for this unique form of epigenetic regulation in adult stem cells.

(Manuscript under revision)

Molecular profiling of hematopoietic stem cell niche components

Molecular signals emanating from the bone marrow microenvironment are required for hematopoetic stem cell (HSC) maintenance. However the identity of the cell types and mechanisms behind integration of molecular signals emanating from different niche components are still not well understood. During Dr. Aparna's overseas associate ship she provided a comprehensive analysis of the transcriptome of various niche components (stromal and non-stromal components) using a combination of molecular markers and specific reporter mice. Network analysis, clustering and cross talk analysis further revealed how molecular signals from different niche components integrate for the regulation of different states of HSC. In addition, the data provided evidence for an unexpected hierarchical identity of different stromal cells.

(Manuscript under preparation)

LABORATORY AT CSCR:

Adult stem cell niches are known to support both cycling and quiescent stem cells: while the former are responsible for tissue turnover, their quiescent counterparts serves as a reservoir of stem cells to replenish cycling cells upon tissue injury to support tissue regeneration. Tissue injury associated with chronic inflammation is hallmark for inflammatory bowel diseases Ulcerative colitis. Aparna is setting up laboratory at CSCR, to study role of niche cells in aetio-pathogenesis of Ulcerative colitis in various animal models.

LABORATORY HIGHLIGHTS OF YEAR 2011-12.

a. Manpower: (0)

b. Awards: 2012 ISSCR Travel award to attend 10th Annual Meeting in Yokohama, Japan.

c. Publications:

1. Sugimura R, He XC, **Venkatraman A**, Arai F, Box A, Semerad C, Haug JS, Peng L, Zhong X, Suda Tand Li L. Non-canonical Wnt signaling maintains hematopoietic stem cells in the niche. ***Cell***, 2012, Jul 20;150(2):351-65.
2. Zhao M, Ross JT, Itkin T, Perry JM, **Venkatraman A**, Haug JS, Hembree M, Deng C-X, Lapidot Y, He XC, and Li L. FGF signaling facilitates post-injury recovery of mouse hematopoietic system. ***Blood***, 2012, Aug 30; 120(9):1831-42.
3. Sukumaran A, **Venkatraman A**, Jacob M. Inflammation-induced effects on iron-related proteins in splenic macrophages and the liver in mice. ***Blood cells Mol Dis***. 2012 Jun 15;49(1):11-9.
4. Santhanam S, Rajamanickam S, Motamarry A, Ramakrishna BS, Amirthraj JG, Ramachandran A, Pulimood A, * **Venkatrman A.** Mitochondrial electron transport chain complex dysfunction in the colonic mucosa in Ulcerative colitis. ***Inflamm Bowel Dis***., 2012 Feb 28. doi: 10.1002/ibd.22926. (* corresponding author)].
5. Zelickson BR, Benavides GA, Johnson MS, Chacko BK, **Venkatraman A** et al. Nitric oxide and hypoxia exacerbate alcohol-induced mitochondrial dysfunction in hepatocytes**. *BiochimBiophysActa.*,** 2011 Dec;1807(12):1573-82. Epub 2011 Sep 24.
6. Santhanam S, ***Venkatraman A** and Ramakrishna BS. Impaired mitochondrial acetoacetyl CoA thiolase activity in patients with Ulcerative colitis. ***Gut***. 2007 Nov;56(11):1543-9. Epub 2007 May 4. (* Shared first author)

BOOK CHAPTERS

1. **Venkatraman A,** Zhao M and LiL**.** The Hematopoietic Stem Cell Niche. Biomaterials and Regenerative Medicine Book, 2012.

PATENTS:

1. Perry JM, Li L and Venkatraman **A.** Methods, kits and compositions for stem cell self-renewal. Filed on 19th Oct 2011.

B. Adjunct Scientists at CSCR

Poonkuzhali Balasubramanian, PhD. Adjunct scientist, July 2011-present

RESEARCH PROGRAM:

PHARMACOGENETICS OF CYTARABINE AND DAUNORUBICIN IN THE LEUKEMIC STEM CELL COMPARTMENT IN ACUTE MYELOID LEUKEMIA

Acute myeloid leukemia (AML) is a clinically and biologically heterogeneous disease. The success of treatment with chemotherapeutic agents, cytarabine and daunorubicin in AML is hampered by drug resistance and relapse which are major causes of treatment failure, probably due to the drug resistant leukemia initiating cells or leukemic stem cells (LSC). Quantitative differences between LSC and the bulk of the leukemic blasts can explain differences in treatment outcome; for example, specific expression patterns of genes encoding the enzymes/proteins involved in the metabolic pathway of these drugs. We aim to identify the mechanisms involved in the drug resistance phenomenon shown by LSC compartment by evaluating pattern of expression of these genes in the 2 compartments. The objectives are to isolate and characterize putative AML LSC (pLSC) fraction at diagnosis and/or relapse using FACS sorting and methyl cellulose clonogenic assays; to characterize the mechanisms of drug resistance by RNA expression of the candidate genes in the pLSC and other fractions in these patients and to compare with in vitro cytotoxicity/ intracellular drug levels in each fraction; to do gene expression profiling of the pLSC fraction from patients with different prognostically relevant mutations like nucleophosmin1, FLT3- ITD and CEBPA in order to understand the role of these markers in drug resistance and relapse and thereby differences in treatment outcome.

LABORATORY HIGHLIGHTS OF YEAR 2011-12.

a. Manpower: One self-funded junior research fellow (UGC, India) is working in this project.

b. Publications in 2011-12

1. Savitha Varatharajan, Ajay Abraham, Salar Abbas, Wei Zhang, Shaji R.V, Rayaz Ahmed, Aby Abraham, Biju George, Alok Srivastava, Mammen Chandy, Vikram Mathews and ***Poonkuzhali Balasubramanian***. Carbonyl reductase-1 RNA expression influences in-vitro daunorubicin cytotoxicity and intracellular daunorubicinol levels in AML cells. Eur J Clin Pharmacol, 2012 In Press.
2. Ajay Abraham, Savitha Varatharajan, Salar Abbas, Wei Zhang, Shaji R.V, Rayaz Ahmed, Aby Abraham, Biju George, Alok Srivastava, Mammen Chandy, Vikram Mathews and ***Poonkuzhali Balasubramanian.*** Pharmacogenetics of Cytidine Deaminase in Acute myeloid leukemia blasts and normal lymphocytes. Pharmacogenomics, 2012 Feb;13(3):269-82.

ONGOING RESEARCH SUPPORT (RELATED TO STEM CELLS)

Pharmacogenetics of cytarabine and daunorubicin Resistance in the Leukemic Stem Cell Compartment in acute myeloid leukemia- **Principal Investigator**	ICMR-INSERM	40.14	December1, 2010 2 years

Requested for one year extension (till December 2013), awaiting confirmation from ICMR
Pending Research support:

Modulation of drug resistance in acute myelogenous leukemia: role of Nrf2 and ABCB6- submitted pre-proposal to ICMR

PATENTS: None

Dr. Thomas Kuriakose, MS (Opthalmology)

RESEARCH PROGRAM:

Isolation, Cultivation and Characterization of Human Corneal Endothelial Cells (HCEC)

Smith Jasper, Swetha Sara Philip, Zia Sultan Pradhan* , Thomas Kuriakose*, Alok Srivastava†, Augustine Thambaiah P.† , S.Senthilnathan†, Anna Pulimood‡, Geeta Chacko°*

*- Department of Ophthalmology, Christian Medical College, Vellore
†- Centre for Stem Cell Research, CMC, Vellore.
‡- Wellcome Lab, CMC, Vellore
° - Department of Pathology, CMC, Vellore.

The corneal endothelium responsible for corneal transparency has limited in-vivo proliferative capacity. Therefore, an irreversible loss of endothelial cells leads to loss of transparency and is treated by corneal transplantation. In vitro proliferation of HCECs, made possible by the development of defined culture conditions with growth factors, is probably due to the presence of dormant adult stem cells.

This project was developed to isolate HCECs and culture them in media supplemented with growth factors. We also proposed to perform sphere forming assays to help isolate adult stem cells to ensure long term culture. In future, we also intend to expand these cells on amniotic membrane which will act as a carrier for transplantation.
So far we have not been able to grow these cells to confluence or in large numbers. Assuming it is cell damage during harvesting or due to the enzymes used to separate the cells from the decemet membrane, different methods of cell harvesting was tried but of no avail.

At this point the experiments have come to a standstill as the researcher who was involved in bulk of the bench work had to leave for higher studies. We are now in the process of identifying a new researcher for the bench work.

Dr. I. Sen, MS (Vascular surgery)

RESEARCH PROGRAM:

USE OF AUTOLOGOUS BONE MARROW MONONUCLEAR CELL THERAPY AS A LIMB SALVAGE PROCEDURE IN NON RECONSTRUCTABLE CRITICAL LIMB ISCHAEMIA

Indrani Sen[1], Edwin Stephen[1], Dheepak S[1], Sunil Agarwal[1], Alok Srivastava[2] Department of Vascular Surgery[1], Haematology[2] at the Christian Medical College, Vellore 632004, India .

PROJECT REPORT

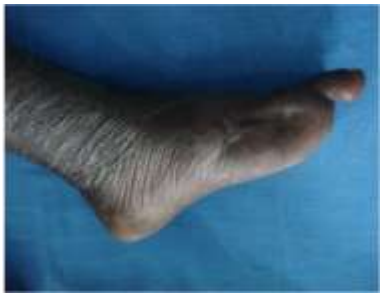
Background

Non-recontructable peripheral arterial disease (PAD) has a high rate of limb loss secondary to ischemic pain and gangrene.

Methods

This is a prospective study using multiple-site intramuscular (calf) injection of autologous bone- marrow derived mononuclear cell (BM-MNC) in patients with non-reconstructable PAD who would have otherwise required amputation. Baseline, assessment was followed by two-week and six-month review. Primary outcome was defined as prevention of major limb amputation. Secondary outcomes were relief of rest pain, ulcer healing and improvement in tissue indices of perfusion.

Results



Fifteen patients (16 limbs) were included. There were 15 males. All patients had rest pain/ulcers at presentation. Non-healing ulcers were present in 13 cases. After the procedure, non-significant improvements in ABI were observed. Three below-knee amputations were subsequently performed 6 weeks after the BM-MNC implantation. The patients who did not have major amputations demonstrated improvement in symptom severity three months after the procedure, as evidenced by alleviation of rest pain and improvements by at least one level in Rutherford and Fontaine classifications, and have not required amputations at a mean follow-up of 12 months. This specific BM-MNC implantation technique was fully

successful in eight patients, as major amputation was avoided and the other applicable criteria were met.

Conclusions

Short-term results indicate the use of BM-MNC implantation as a means of limb salvage therapy for patients with severe PAD shows promise in postponing or avoiding amputation in a patient population currently presented with few alternatives to amputation.

LABORATORY HIGHLIGHTS

a. Man Power: not applicable

b. Awards: not applicable

c. Publications in 2011-12: not applicable

ONGOING RESEARCH SUPPORT : not applicable

PATENTS : not applicable

Eunice Sindhuvi, PhD (Haematology)

RESEARCH PROGRAM:

Haemoglobin E (HbE) ($\alpha_2\beta_2$ $^{26\ Glu\rightarrow Lys}$) is one of the most common variant haemoglobins in the world and is also seen in a higher frequency in the North-eastern India. HbE is caused by a GAG→AAG mutation in the codon 26 of the β globin chain which results in the substitution of lysine for glutamate. The erythroid cells of individuals with the variant haemoglobin E, exhibit a quantitative deficiency in their content of β^E globin and its messenger RNA. The mechanism of the defective production of β^E chains is a reduction of β^E mRNA. The mutation producing HbE, affects RNA processing in two ways. It results in the slow excision of the intervening sequence-1(IVS I) and also activates an alternative splicing in exon-1. Since this new splice site competes with the normal site, the amount of normally spliced β^E mRNA is reduced. The abnormally spliced transcript is non-functional because a part of exon 1 is missing and it synthesizes a truncated protein. There are no further publications proving these hypotheses. In a group of patients with HbE-β thalassaemia, we identified that patients who produce more HbE had a milder phenotype. The abnormal splicing or β-globin transcript levels could not explain the difference in production of HbE. Hence this study is aimed to identify the mechanisms involved in the regulation of βE gene expression during early stages of erythropoiesis. An exvivo erythropoiesis system using progenitor cells obtained from peripheral blood will be established. Factors regulating β globin transcription and translation will be analyzed and correlated with the amount of β^E globin produced. The results obtained in different subsets of HbE syndromes will be correlated with the clinical and haematological parameters.

The results of this project will aid in understanding the molecular pathology of HbE at the transcriptional and the translational level. This also will help to explain the phenotypic heterogeneity of Haemoglobin E diseases.

Significant findings

- Abnormally spliced β^E transcripts are not found/below the detectable limit in early, intermediate and late stages of exvivo erythropoiesis system.
- The antisense transcripts were found to be more prominent in reticulocytes when compared to the early stages of erythropoiesis. The antisense transcripts were confirmed by DNA sequencing.
- FLVCR (cytoplasm heme exporter) was found to be expressed higher in early precursors than later erythroid cells.
- There was a correlation between the heme levels and the FLVCR expression. HRI balances heme and globin synthesis by sensing intracellular heme concentrations.
- HRI expression was found to be higher in controls when compared to patients.

a. Publication

Divya.J, Shaji R.V, Chandy M, Srivastava A, Eunice S.Edison. Splicing patterns of βE mRNA in HbE Syndromes.ISHTM 2011

ONGOING RESEARCH SUPPORT

Transcriptional and Translational processes of βE gene (DBT) 2009-2012; Extension of two years requested and sanction order is awaited.

Dr. George Tharion, MS (Physical Medicine and Rehabilitation)

RESEARCH PROGRAM:

Spinal cord regeneration using stem cell transplantation- Phase II.

(BT/PR12619/MED/31/70/2009)

Overview of program

Despite aggressive research to find a cure for paralysis following spinal cord injury is been conducted around the world, no successful treatment is available so far. The investigators, in an earlier project supported by the DBT explored the role of olfactory ensheathing cells for spinal cord regeneration in rat models. (*Motor recovery following olfactory ensheathing cell transplantation in rats of spinal cord injury. Neurology India 2011: 59; 566-572*). Further the role bone marrow stromal cells in cord recovery as well as its potential for neuronal transdifferentiation was studied. It has been suggested that olfactory ensheathing cells provide guidance and structural conduit for axonal growth as it is normally designed to do in the olfactory pathway. The mesenchymal stem cells provide matrix and local growth factors as well as myelinate the injured fibers thereby facilitate regeneration. With back ground work a phase-II project was initiated. Since the different cell types facilitate spinal cord recovery through different mechanism, the main objectives in this study is to explore the efficacy of transplantation of different cell combinations in rat models of spinal cord injury. Initially cells cultured from rat olfactory mucosa and the cells from the bone marrow stromal cells will be characterized before transplantation. Further the role of chondroitinase enzyme in breaking the glial scar to support the axonal regeneration is also being explored. Painless magnetic cortical stimulator to study the regeneration of corticospinal tract is also being developed. Outcome of the transplantation were evaluated by BBB score, motor evoked potential studies and by histological methods.

c. Manpower:

Durai Murugan, Senior Research Fellow, M.Phil/Bharathidasan University, Trichy.
Janani, Junior Research Fellow. M.Sc. Vellore Institute of Technology, Vellore.

d. Awards: Not applicable.

e. Publications in 2011-12: No stem cell publication.

ONGOING RESEARCH SUPPORT:

Spinal cord regeneration using stem cell transplantation- Phase II – DBT, 43.72 Lakhs, 12.7.2010 to 11.1.2013.

PENDING RESEARCH SUPPORT: Not applicable.

PATENTS: Not applicable.

New projects initiated in 2011

1. Dr Sumita Danda, Clinical Genetic Unit, CMC- Isolation and characterization of pericytes from muscles of DMD (Duchenne muscular dystrophy) patients

2. Establishing methods for evaluating the bone marrow hematopoietic stem cell niche in humans. Dr. Alok Srivastava, Centre for Stem Cell Research.

Programmes :

A. Scientific programmes

Stem Cell Research – Policy and Regulation- Winter symposium January 2012

A workshop on "Policies for the regulation of clinical trials with stem cells in India" was organized with support from the ICMR under the mandate of the NAC SCRT on the 5th of January 2012. This was acollaboration with the Princes Margaret Hospital, Toronto (Dr. Armand Keating and Dr. Sowmya Viswanathan) as an extension of a similar program that is done in Canada as well the NIH Centrefor Regenerative Medicine (Dr. Mahendra Rao).

Two discussions were arranged on the 6th of January 2012. The first of these was on induced pluripotent stem cells, taking advantage of the visit of Dr. Konrad Hochedlinger from Centrefor Regenerative Medicine, Massachusetts General Hospital, Boston, USA to attend the CMC Winter Symposium on Cellular and Molecular Medicine. The second discussion forum was on the understanding of the hematopoietic stem cell niche and its applications in the assessment of human disease taking advantage of the presence of Dr. David Scadden of the Harvard Stem Cell Institute, Boston, USA. Both these scientists visited Vellore as part of the CMC 10th Winter Symposium.

Dr. Alok Srivastava continues to chair the National Apex Committee for Stem Cell Research and Therapy, which will be reviewing the revised guidelines for stem cell research apart from its other activities.

Training:

I. Ph.D Program

CSCR has an active PhD programme and the students can register for PhD under Sree Chitra Thirunal Medical Science and Technology (SCTIMST), Thiruvananthapuram, CSCR or Thiruvalluvar University. Fourteen students registered for PhD in 2011-2012.

II. Other training programs

The Centre for Stem Cell Research held a workshop on 'Culturing and characterization of Mesenchymal stromal and hematopoietic stem cells'' from April 11-21st, 2011. There were 11 participants from all over India.

Short term student projects (Bi-annual)

SHORT TERM STUDENTS

S. No	Name	Duration	Qualification	University	Project title	PI /Lab
1	Mr. Mansoor Hussain	Dec 09 – May10	M.Sc Biotechnology	Jamal Mohammed College, Madurai Kamarajar University	Developing Adeno-associated virus capsid surface exposed Serine/Threonine mutants and tests their efficacy in vitro in Hela and HepG2 cell lines.	Dr. Jayandharan / Lab -4
2	Ms. Ramya	Dec 09 – May10	M.Sc Animal Biotechnology	Bharathidasan University	Developing an AAV helper virus encoding a NOTCH transgene to augment transgene expression from single stranded AAV vectors	Dr. Jayandharan / Lab -4
3	Mr. Sagar Gupta	Nov 09 – Apr 10	B. Tech (GN)	Bharathidasan University	Strategies to Improve Somatic cell Reprogramming	Dr. Shaji / Lab -2
4	Ms. Shreemoyee Dutta Majumder	Nov 09 – Apr 10	M. Sc Bio technology	KIIT University Bhubaneshwar	Molecular Regulation of Human Globin Genes.	Dr. Shaji / Lab -2
5	Mr. Abhiyan Viplav	Apr – Jun 11	B. Tech	Amity university	Generation of AAV vectors for gene therapy	Dr. Sanjay / Lab -3
6	Ms. Heizline Blessie. D	May - Oct 11	B. Tech	Allahabad Agricultural University	Analysis of globin Transcripts in Erythroid Cells	Dr. Shaji / Lab -2
7	Ms. Karthika. P	Jun – Jul 11	M.Sc Microbial Gene technology	Madurai Kamaraj University	Retroviral cloning miRNA369 and miRNA200c and their functional characterization in HEK 293 cells	Dr. Shaji / Lab -2
8	Mr. Sathish Kumar. Y	Jun – Dec 11	M.Sc Molecular Biology	MGR Medical University	Generation of Bioengineered AAV Threonine Mutant Vectors).	Dr. Jayandharan / Lab -4
10	Ms. Ankita Mitra	Dec 11– May 12	M. Sc Bio medical Genetics	VIT University, Vellore	Differentiation of Stem Cells	Dr. Shaji / Lab -2

11	Ms. Kanchan Kumari	Dec 11 – Jun 12	M.Sc Bio technology	VIT University, Vellore	Cellular factors modulating AAV replication and their inhibition of gene therapy of Haemophilia -B	Dr. Jayandharan / Lab -4
12	Ms. Prachi Agrawal	Jan – Jun 12	M.Sc Biotechnology	Jaipur National University, Rajasthan	Gene transfer using Ser/Thr mutant AAV in murine models of Haemophilia - A	Dr. Jayandharan / Lab -4
13	Ms. Roselyn Jebamalar Newton	Sep 11– Feb 12	M.tech	VIT University, Vellore	Differentiation of Stem Cells	Dr. Shaji / Lab -2
14	Ms. Sandhyaa. V	Jan – Jun 12	M.Sc Regenervative medicine	Manipal University, Bangalore	Trans lineage differentiation and molecular characterization of human placenta-derived MSC	Dr. Sanjay / Lab -3
15	Ms. Sarita Das	Jan - Jun 12	M.Sc Bio tech	KIIT University Bhubaneshwar	Study gene expression profile changed in human BM MSC, umbilical cord derived MSC, placenta-derived-MSC and cord blood derived MSC	Dr. Sanjay / Lab -3
16	Ms. Shantoshini Dash	Jun –Dec 12	M.Sc Biotech (Integrated)	KIIT University Bhubaneswar Odisha	Gene Transfer in Murine Model of Hemophilia-B	Dr. Jayandharan / Lab-4
17	Ms. Monika Kumari	Jun–Dec 12	M.Tech Biotech (Integrated)	Shoolini University, Solan	Blocking adaptive immune response against AAV vectors in-vivo	Dr. Jayandharan / Lab-4
18	Mr. Manjunath	Sep12 – Mar 13	M.Tech Bio medical Engineering	VIT University, Vellore.	Design development and mechanical testing of a bioengineering construct composition for mesenchymal stem cell transplantation studies for spinal cord injury model in scid mice	Dr. Sanjay/ Lab-3
19	Ms. Sarangi. T.K	Jan- Jun 2013	M.Sc Biotech	VIT University, Vellore.	Cloning, expression & purification of basic fibroblast growth factor (bFGF) & testing recombinant protein in maintenance of iPS cells.	Dr. Sanjay/ Lab-3 (Yet to Join)

20	Ms. Sreelakshmi V.M	Jan- Jun 2013	M.Sc Biotech	VIT University, Vellore.	Cloning, expression & purification of Leukemia Inhibitory Factor (LIF) & test functionality in human iPS cells.	Dr. Sanjay/ Lab- 3 (Yet to Join)
21	Mr.Ijaz Mohammed Abdulla	Dec 12 – May 13	M.Sc Biotech (Integrated)	VIT University, Vellore.	Gene therapy for hemophilia A	Dr. Jayandharan / Lab-4 (Yet to Join)
22	Ms. Rohini K Murthy	Dec 12 – May 13	B.Tech Bioengineering	SASTRA University, Thanjavur	Gene therapy for hemophilia B	Dr. Jayandharan / Lab-4 (Yet to Join)

Meetings /Events

2011

- 11- 13th January 2011
  Visit of Haukeland University Team, Norway to discuss collaborative opportunities.

  25th January 2011
  Dr. Taslimarif Saiyed Director and COO, Centre for Cellular and Molecular Platforms (A DBT initiative), NCBS-TIFR, Bangalore, visited CSCR to present C-Camp's facilities .

- 9th February 2011
  Dr. Mahendra Rao, Vice President, Stem Cell Research and Regnerative Medicine, Invitrogen Inc, USA (former Head, Stem Cell Research, National Institute of Aging, NIH, USA) "MSC and HSC cell therapy trends"

- 12th March 2011.
  Dr Murali Gururajan, Research Scientist (Instructor) at Cedars-Sinai Medical Centre(affiliated to University of California at Los Angeles), Los Angeles, USA "MicroRNAs: Role in B cell differentiation, B cell derived diseases and solid tumors"

- 6th August 2011
  Dr Rao V.L. Papineni, Senior Principal Investigator, Research and Development Carestream Health INC USA. "Advances in Multimodal Molecular Imaging: Sights and Sound in Probe Targeting"

- 6th December 2011
  Professor Mackay-Sim, Director National Adult Stem Cell Research Centre, Griffith University, Brisbane, Australia has extensively investigated the regeneration and repair of the nervous system. He has conducted clinical trials of cell transplantation on patients with spinal cord injury using elegant methods which has proved the safety and potential use of these methods for clinical application.

- 12thDecember 11.
  Dr. Pawanbir Singh, MBBS, MS, PhD, from Stem Cell Technologies Inc. Canada "Feeder independent culture and in vitro differentiation of pluripotent stem cells"

2012

- 16th January 12

  Professor S Homer-Vanniasinkam IBSc MD FRCSED FRCS Consultant Vascular Surgeon, The General Infirmary at Leeds Clinical Sub-Dean, University of Leeds Medical School Chair, Translational Vascular Medicine, University of Bradford Director, Northwick Park Institute for Medical Research, London Honorary Professor, Division of Surgical & Interventional Sciences, UCL "Potential application in characterizing stem cells applied for a patent for early detection of MRSA bacterial infection"

- 1st February 2012

  Dr. Gerold Feuer, Professor, Department of Microbiology and Immunology, SUNY Upstate Medical University,Founder and Director, Humurine Technologies "Humanized" Severe Combined Immunodeficient Mice for Investigations of Viral Pathogenesis and Stem Cell Biology"

Governance of CSCR

a. CSCR Committees

Dr. Sunil Chandy, Director , CMC	Chairperson
Dr VijayRaghavan, Acting Director inStem	Member
Dr Ramaswamy S, Dean inStem	Member
Dr Jyotsna Dhawan, Dean inStem	Member
Dr.Alfred Job Daniel	Principal
Dr Alok Srivastava, Head CSCR	Member Secretary

b. CSCR Sub Committee (Finance):

J.S.& F.A, DBT	Chairperson -
Director inStem	Member
Dr. Jyotsna / Dr. Ramaswamy, Dean inStem	Member
Dr. Alka Sharma / Dr. T.S. Rao, DBT	Member
Director CMC	Member
Associate Director (Fin) CMC	Member
Head CSCR	Member Secretary

In addition, CMC, Vellore has established two further committees to assist in the management of CSCR:

1. The Core Committee which has been appointed by the Principal, CMC,Vellore and consists of 4 senior faculty of CMC, Vellore to work on a regular basis in an advisory capacity with the Head, CSCR particularly for scientific and personnel related issues. The Head, CSCR is the convener.

2. The Steering Committee which is chaired by the Director, CMC, Vellore and consists of relevant administrative officers of CMC, Vellore as well as the Core Committee members to provide an administrative interface between CMC, Vellore and CSCR. The Head, CSCR is the member secretary."

Administration:

Purchase process at CSCR CMC Vellore

The integration of inStem Bengaluru, the Department of Biotechnology and Christian Medical College, Vellore in July 2011 paved the way for CSCR to function as the translational wing of inStem Bengaluru.

As stated in the MOA, the administrative and financial powers lies with CMC, keeping in view the physical location of CSCR in the CMC Campus at Bagayam, Thus keeping in view of the above and for the efficient running of the centre in relation to procurement of materials, the Material department of CMC facilitates the purchase process for CSCR.

The procurement of material in any organization involves arranging for the supply of a required material on time, at a suitable price from the right vendor to meet production programme, sales plans or operational needs. Purchase Unit of Materials Department is the centralized procurement unit, which deals with the purchase of materials required by Christian Medical College, except Drugs and Pharmaceuticals. Purchase Department is located outside the main CMC Hospital building, on the Ida Scudder Road at Vellore.

Purchase Unit receives the Purchase Requests from the User Departments with necessary approvals. Purchase Orders are processed to procure the materials (based on the approval by Administrative Committee / Purchase Committee or by other special Committee, UPS Committee and Computer Committee) from the right source within the stipulated period through a systematic procedure. The materials are purchased from abroad as well as from domestic vendors. The unit is responsible for the processing of purchase orders as well as the follow up of orders and works in collaboration with the Stores unit and Central Receiving Section (CRS). The purchase committee plays a major role in the purchase decisions of the institution.

The General Superintendent enters into a contract with the approved vendor for the material required by placing a Purchase Order (PO). The PO is then signed by Deputy General Superintendent (Materials) on behalf of the GS.

The User department raises Purchase Requests with complete specification of materials needed in the appropriate Purchase Request forms (Table I). There are three different types of Purchase Requests used in CMC based on the nature of material and the type of funds.

Table 1 – Different Kinds of Purchase Requests

Sl.No	Purchase Requests	Materials ordered	Fund
1.	Greed Colour PR	Chemicals, Reagents & Kits	Maintenance Fund
1	Yellow Colour PR	Equipments	Any Fund
2	White Colour PR	All Items & Funds other than 1& 2	

The user department needs to obtain necessary approvals (Table II & III) for accepting the PR in Purchase Unit for processing orders.

Table 2 – Approvals needed for Purchase of Materials – Based on value & fund

Material Value Rs	Accounts	HOD	Treasurer	Director
Below 1000	**Maintenance Fund**	✓		
Above 1000	**Maintenance Fund**	✓	✓	
Up to 5000	**Special Fund**	✓	✓	
Above 5000	**Special Fund**	✓	✓	✓

Material Value Rs	Accounts	HOD	Treasurer	Director
Above 1000 & already purchased	Special Fund	✓	✓	✓
Below 50000 without AC Approval	Development/ Project Fund	✓	✓	✓
With AC Approval	Any Fund	✓	✓	

Table 3 – Special Approvals – Based on Type of Materials

Materials	Approval From	PR Send to Purchase with
Computers & Printers	Computer Committee	Quotation from the approved vendor & Computer Committee approval letter
UPS 1 KVA & above	UPS Committee	Quotation & UPS Committee approval letter
Equipments above Rs.50000	GAAT & AC	Quotation & Administrative Committee Minute Ref. Details and Condemnation Certificate in case of materials purchased against condemnation.
Any materials needed urgently for emergency use	Director	Quotation and Urgent Purchase approval column duly signed by Director

1. Members of GAAT – A Committee for MEDICAL EQUIPMENT:

Chairman -	Associate Director (Finance)
Convener -	Medical Superintendent
Member	Associate Director (Medical)
Member	Treasurer
Member	Associate Medical Superintendent
Member	Deputy General Superintendent (Purchase)
Member	HOD, Engineering Electronics
Member	Member- (Additional member from AC)
Dr. Mathew Joseph,	Professor of Neurosurgery
Dr. Sukesh Chandran,	Professor of Clinical Pathology
Member	Assoc. Dy. Nursing Superintendent

2. GAAT – B Committee for NON – MEDICAL EQUIPMENT:

Chairman	Associate Director (Finance) –
Convener –	General Superintendent
Member	Associate Director (Administration) –
Member	Treasurer -
Member	Deputy General Superintendent (Purchase
Member	HOD, Engineering Electronics
Dr. Vathsala Sadan	Additional member from AC
Dr. Simon Rajaratnam,	Professor of Endocrinology

3. Members of the Administrative Committee

Dr.Sunil Chandy	Director
Dr.Thomas Kuriakose	Assoc.Director

Dr.John C.Muthusami	Assoc.Director
Dr.George Joseph	Assoc.Director
Dr. Raju Titus Chacko	Assoc.Director
Dr.D.J.Christopher	Assoc.Director
Dr.Kenny David	Assoc.Director
Dr.M.J.Paul	Council Secretary
Dr. C.E.Eapen	Medical Superintendent
Dr.Alfred Job Daniel	Principal
Mr.Denzil Ranjitsingh	Treasurer
Mrs. Rosaline Jayakaran	Dean, College of Nursing
Dr(Mrs)Jayarani Premkumar	Nursing Superintendent
Dr.Anna Pulimood	Member
Mrs.Rajeswari Siva	Member
Dr.Nylla Shanthly	Member
Dr.Sukria Nayak	Member
Mr.E.Josam Titus	Assoc.General Supdt.
Mr.J.P.Peter	General Superintendent & Secretary

Standard Operating Procedures :

Ordering Procedures

Purchase requests are accepted in Ordering Section after initial screening for approvals from the concerned authorities. The accepted PRs are registered and ordering process starts. POs are placed immediately for materials, which satisfies criteria for purchase.

Enquiry Procedures

Materials which do not fit the criteria, an enquiry to be floated to the suppliers listed in the Vendor list. A specific due date (approximately 7-15 days) is given for the enquiries which

falls on Mondays. The quotations are collected in a locked tender box which is opened on the next day of the tender due date by the authorized clerk in the presence of supervisors of ordering section and the Dy.General Superintendent (DGS). The quotations obtained are registered in the tender register duly signed by the supervisor and DGS. The quotes are then tabulated and POs are placed immediately with L1 (lowest quote) for the materials spelt out with details of proper brand and description without any confusion (the same will be ratified in Purchase Committee). The PRs for quotations received with high value purchase, policy related issues, etc are taken to Purchase Committee for decision making & placement of POS.

Purchase Committee:

The Purchase Committee comprises of the following

Chairman	Purchase Committee
Convener	from Purchase unit (DGS or his representative)
Director	Representatives of Director
Treasurer	-
Medical Superintendent	-
Nursing Superintendent	-
Mechanical Engineering Department	-
BME Department	-
Stores Unit and CSSD.	

The purchase committee meets on every Thurdays with agenda related to the purchase transactions from Friday to Wednesdays. The purchase committee agenda comprises of -

Matters related to the previous PC minutes, price revisions for the POs placed, any changes to the previous approvals and tabulations due to price revisions are dealt in this section
Orders placed as per the lowest, urgent purchase approval from director for spare parts, from authorized dealers/manufacturers are ratified here. Purchase requisitions- The PRs received with quotations for proprietary items monopoly items, tabulations for the materials requested in a PR.

Confirmatory section-This section particularly deals with confirmatory purchase requests for the materials which do not have any previous approvals and ratification of confirmatory PO's.
Regular Purchase Orders for ratification -placed based on the previous price and approved price lists, as per the AC approval, placed for kits, chemicals and reagents.

Annual Tender Procedures

1. Annual Tender Notice is published during the month of February every year in the newspapers for the selected consumables in A-Class category of items needed by the institution.
2. The tender with the details of materials required, description, quantity, specific instructions along with the terms and conditions of the institution is printed and issued to the suppliers on request.
3. The suppliers have to pay a sum of Rs 150/- for the cost of the tender application form. They submit the tender forms duly filled with necessary documents, information, samples etc, within the specified due date which normally falls on the mid of April.
4. The tenders are collected in a sealed tender box, which are opened in the presence of the representatives of the Internal Audit and Accounts department after the specified tender due date.
5. The tenders are accepted for tabulation, which received with tender registration fee of Rs.300/- and counter signed by the representatives of Internal Audit and Accounts department.
6. The tenders are tabulated and presented to the special purchase committee for annual tender.
7. The Standing Orders are placed based on the Annual Tender Purchase Committee decision with details of schedule of supply.

Purchase of Proprietary Items

The PRs received with quotations of Proprietary items or monopoly suppliers placed in Purchase Committee for approval and placement of Purchase orders.

There are 4 different types of Purchase Orders

1. REGULAR ORDERS, placed in anticipation of supply of material.
2. CONFIRMATORY ORDERS placed for items already purchased directly by user departments.
3. STANDING ORDERS are placed for a specific period of time with instruction to supply periodically.
4. IMPORT ORDERS are placed with overseas vendors in foreign currency.

Clarifications/discrepancies, which needed departmental advice, are communicated to the user departments through e- mail/ local letters. Amendments to the descriptions, quantities, price, terms of PO, etc., are done with the departmental approval and purchase committee approval.

Vendor List

Vendor introduction letters and details and brochures received from new vendors are registered and filed systematically for future reference and use. The potential vendors are selected and added in to the vendor list. The vendor list is based on the type of material.

1. Category A contains the vendors in Vellore
2. Category B contains vendors in South India other than Vellore.
3. Category C contains vendors other than in South India including International vendors.

Follow Up

The Purchase Orders placed are forwarded to suppliers by post/courier/e-mail/fax after verification and filed for future reference. Orders are followed up, if supply is not received within the due date assigned. Reminder Letters are send after 2 days of due date and due date extended based on the type of material. Further reminders are sent or over phone if supplies are not received. User Departments are intimated if there is a delay in supply. In case of confirmation of non supply of material by the supplier, Ordering Section is intimated to source alternate vendors. In such cases a fresh PO is released on the L2 (second lowest) or the next approved alternate after canceling the defaulted PO or a fresh enquiry will be sent. Supply is received in Central Receiving Section. Outstation suppliers normally supply the materials through courier and through Lorry service. Lorry Way bills are received registered and forwarded to CRS for clearance.

Settlement of Payments

Bills received from suppliers are registered, scrutinized against the Goods Received Note forwarded by CRS. Bills & GRNs are forwarded to Accounts Department, if the Invoice complies with the PO. Accounts Department prepares the payment and sends to the suppliers within 30 days or on an "urgent basis" as per the order terms and conditions. A statement of bills forwarded to accounts is prepared and filed for future reference. Cash is given to the local suppliers against preparation of voucher duly signed by the vendor, within 7-10 days from the date of supply, on receipt of Cash Goods Received Note forwarded by CRS. The statement of Cash Payments made to local suppliers is forwarded to Accounts Department for settlement and reimbursement. Expense for the clearance of consignments, labor charges, lorry receipts, etc. paid by CRS is reimbursed by the accountant and petty cash / miscellaneous statement is forwarded to accounts department. Other types of payments, like Full advance payment, Partial advance payments, Payment on delivery, Cash on delivery, Payment through Bank, Inland letter of Credits are obtained and settlement done with Accounts department on completion of order.

Import Section.

Import Orders are placed with CIP (Carriage Insurance Prepaid) pricing with Insurance coverage based on quotations from overseas suppliers or their Indian dealers. Payment is made by Foreign Demand Draft/ Wire Transfer/ Sight Draft basis or by Letter of Credit basis. Customs Clearance is done within 2-3 days on an average and within 1-2 days incase of frozen shipment through an authorized Customs House Clearing Agent from Chennai Airport on arrival of shipments.

Custom Clearance Formalities The necessary documents are forwarded to the custom house clearing agent for arranging clearance from customs authorities. The cleared consignments are sent to CRS by the custom house clearing agent. The service bill along with other import documents is received and service charges are paid. Other Post-import formalities like submitting Bill of Entries to bankers, redeeming bonds submitted to Customs are done within a period of 10-15 days.

Personnel:

Scientist &Technical Staff

S. No.	Name	Designation	Position
1.	Dr. B.S. Ramakrishna	Professor, Department of GI Sciences	Adjunct Scientist
2.	Dr. Sanjay Kumar	Ramalingaswamy Fellow	Scientist
3.	Dr. Vrisha Madhuri	Professor, Department of Paediatric Orthopaedics	Adjunct Scientist
4.	Dr. Murugan Ramalingam	Associate Professor, Centre for Stem Cell Research	Scientist
5.	Dr. Jayandharan. G. Rao	Lecturer, Department of Haematology	Adjunct Scientist
6.	Dr. Rekha Samuel	Professor, Centre for Stem Cell Research	
7.	Dr. Alok Srivastava	Professor, Centre for Stem Cell Research	Adjunct Scientist
8.	Dr. R.V. Shaji	Professor, Department of Haematology	Adjunct Scientist
9	Dr. Aparna Venkatraman	Associate Professor, Centre for Stem Cell Research	
10	Dr. B. Poonkuzhali	Professor, Department of Haematology	Adjunct Scientist
11	Dr. Ari Chacko	Professor, Department of Neurosurgery	Adjunct Scientist
13	Dr. Thomas Kuriakose	Professor, Department of Opthalmology	Adjunct Scientist
14	Dr. Vikram Mathews	Professor, Department of Haematology	Adjunct Scientist
15	Dr. Indrani Sen	Assistant Prof, Department of Vascular Surgery	Adjunct Scientist
16	Dr. Eunice Sindhuvi	Lecturer, Department of Haematology	Adjunct Scientist
17	Dr. George Tharion	Professor, Department of Physical Medicine & Rehabilitation	Adjunct Scientist
18	Dr. Dwaipayan Sen	Post Doctoral Fellow	-
19	Dr. Ruchita Selot	Post Doctoral Fellow	-
20	Dr. R. Sumathy	Scientific Officer	-

21	Mr. Augustine Thambiah	Technical Officer	-
22	Mr. S. Senthilnathan	Technical Officer	-
23	Ms. Sangeetha Hareendran	SRF	-
24	Mr. Salar Abbas	SRF	-
25	Mr. Syed Mohammad Musheer Aalam	SRF	-
26	Mr. Vikram Sabapathy	SRF	-
27	Mr. Nishanth Gabriel	SRF	-
28	Ms. E. Sumitha	SRF	-
29	Mr. Balaji	JRF	-
30	Ms. P.B. Sumitha	SRF	-
31	Mr. Kannan Thoopil	JRF	-
32	Mr. Thyagarajan	JRF	-
33	Mr. Janakiraman	JRF	-
34	Ms. Nancy	ARO	-
35	Mr. S. Balasubramanian	JRF	-
36	Ms. Akshaya Krishnagopal	JRF	-
37	Ms. Aleya Tabasum	Graduate Tech.	-
38	Mr. P. Sathish	Technician	-
39	Ms. J. Esther Rani	Technician	-
30	Ms. Dhavapriya	Graduate Tech	-
41	Ms. G. Kalaivani	Graduate Tech	-
42	Ms. R. Saranya	Graduate Tech trainee	-
43	Ms. J. Saranya	Graduate Tech trainee	-
44	Ms. R. Pavithra	Graduate Tech trainee	-