inStem

CSCR

Centre for Stem Cell Research
(a unit of inStem, Bengaluru)
Christian Medical College Campus
Bagayam, Vellore-632002

ANNUAL REPORT
2012-2013

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Introduction

CSCR

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

This Centre for Stem Cell Research stands for bringing stem cell science to patient care. The aim will be develop stem cell research in ways that will either help understand disease biology better or develop therapies where there are major unmet needs. 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. Collaborations will also be sought with industry to bring diagnostic or therapeutic products to the market. 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 institutions and scientists working in this field in the country.

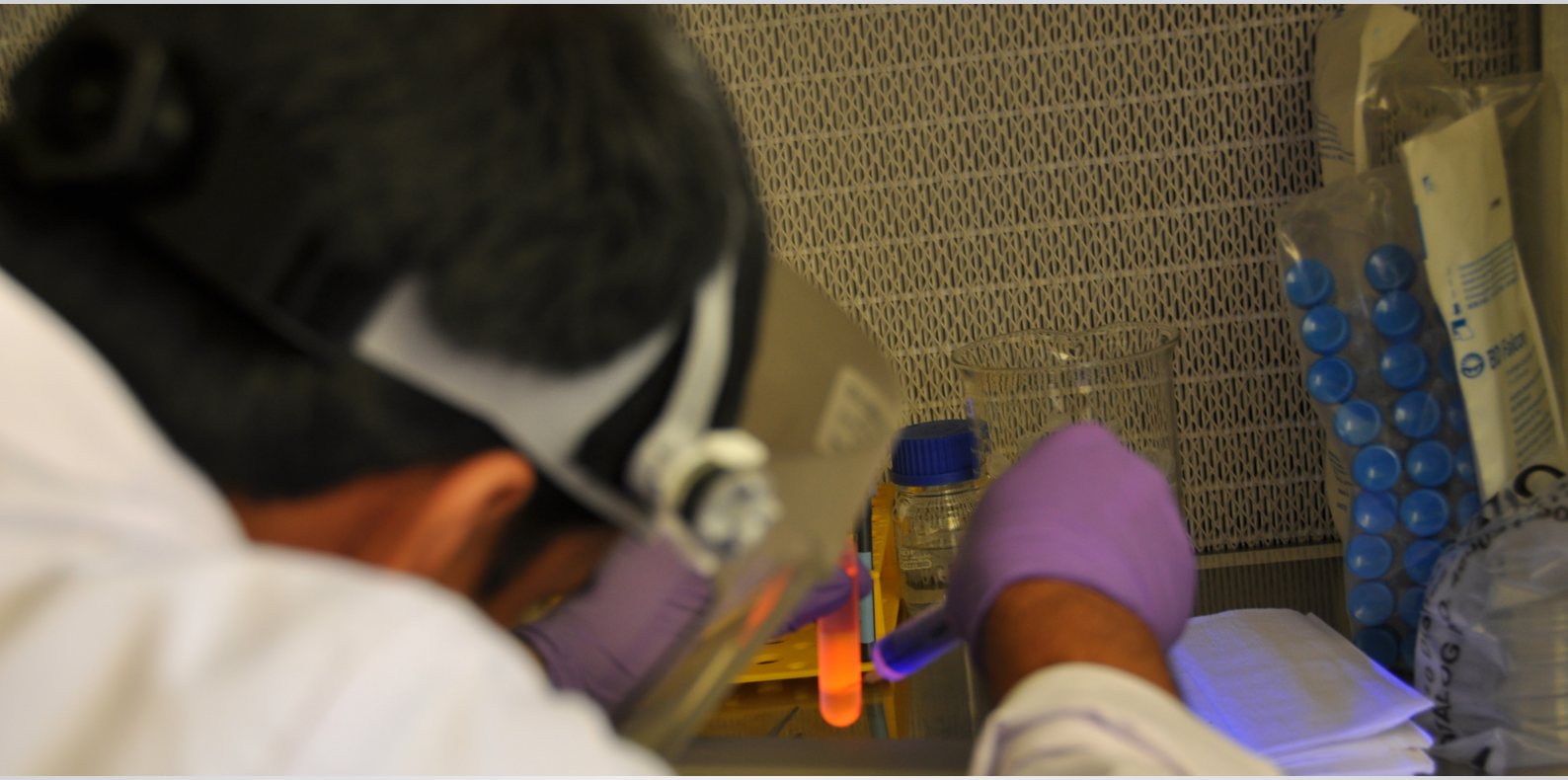
GOVERNANCE – 2005 TO 2010

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.

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. It will continue to function at the Bagayam campus of CMC, Vellore with its emphasis on translation stem cell research and regenerative medicine. Efforts will be directed at developing thematic programs and greater interactions through collaborative work between scientists at Bengaluru and Vellore.

Core Scientific Activities and Initiatives: 2012 -2013



Over the last one to two years, the emphasis has been on developing thematic research programs that are targeted at specific medical problems. The current areas of collaborative research towards specific themes are the following: gene therapy using AAV and lentiviral vectors, a musculoskeletal regenerative medicine program, a stem cell niche biology program and a vascular biology program. Details are in the report that follows. Individual scientific projects do cover a larger range of subjects where new collaborations need to developed – such as induced pluripotent stem cells to create disease models as well as the possibility of developing safe iPS cells for potential clinical applications, evaluation of the heterogeneity of mesenchymal stromal cells of different origins as well its clinical applications. Several clinical problems are also being evaluated in animal models of these diseases by physician scientists from CMC, Vellore, also. A few early clinical trials are also being undertaken utilizing the cell processing facility for such studies.

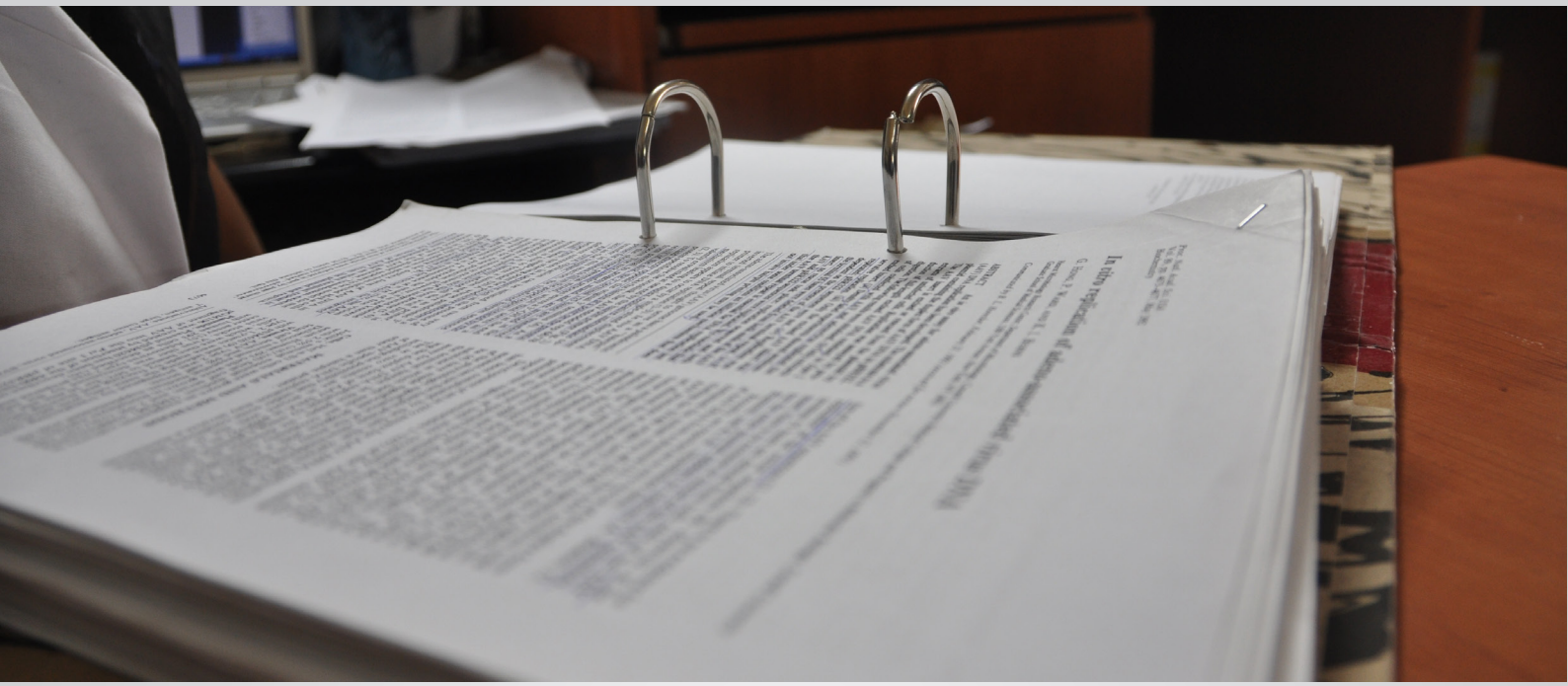
The newer developments in the last year have been the following: the AAV gene therapy program is moving towards a clinical trial in collaboration with industry in India for product development. The collaboration with Emory University, USA has also developed further where work is being initiated with a novel lenti vector on which there is potential to file an IPR after its evaluation. An MOA has been signed with Aptgenetics, a collaboration of Humurine, USA to establish a collaboration for developing humanized mice at CSCR for scientific research in different applications. It is expected that this work will be initiated in the next year.

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

A major challenge in 2012-13 was the extreme paucity of core funds that are usually available to science institutions. This greatly hampered scientific work at a time when several new scientists had joined CSCR. The situation has improved in the second half of 2013 which should lead to better development of the collaborative programs.

Alok Srivastava

TRANSLATIONAL STEM CELL RESEARCH AND REGENERATIVE MEDICINE PROGRAMS:



1. Gene therapy program: (Group: At CSCR - Jayandharan GR, RV Shaji, Alok Srivastava; Other collaborators: listed below under each program)

The importance of gene therapy for treating disorders with specific genetic defects has been recognized for long. Applications have been attempted in both hereditary genetic disorders and certain acquired conditions, particularly cancers. The gene therapy program at CSCR is being developed around application of two viral vectors. The two major sub-themes of this program are:

a. Adeno-associated virus (AAV) vectors

Vector Biology and Pre-clinical studies: In this area, the laboratory led by Dr. G. Jayandharan, has developed various novel AAV based delivery systems by a through understanding of biology of virus and host cellular interactions. For example, an array (n=60) of novel capsid variants based on AAV serotypes 1-10 has been generated by strategic modification of capsid ubiquitination sites. These capsid modified forms with enhanced efficiency and reduced immunogenicity have the ability to target multiple tissues and thus applicable for different diseases. Indeed, translational work done in the hemophilia mouse model with hepatic gene transfer of AAV8 expressing coagulation factor IX has demonstrated their efficacy. Intellectual property protection has also been filed for some of these vectors. Concurrently, work is ongoing on development of other innovative AAV based vector systems. MicroRNA regulated vectors or aptamer/ nanoparticle coated dual AAV vector systems or generation of immune escape phenotypes and their proposed testing in hemophilia and other pre-clinical settings (eg. Leukemia) in collaboration with other investigators are some examples in this direction (see details in Dr. Jayandharan's laboratory report).

Gene Therapy for Hemophilia: *Other Collaborators*: Dr. Amit Nathwani, UCL, UK; Dr. Sanjay Singh, Gennova Biopharmaceuticals - Industry partner in India. More clinical collaborators from CMC to be added when the clinical program starts.

The basic science work done at CSCR combined with the success of clinical trials conducted by Dr. Amit Nathwani from UK reported about 2 years ago using similar vectors, have paved the way for developing a clinical trial with the AAV vector / capsid modified self complementary variants of AAV8. There is a huge unmet need for the clinical management of several thousand patients with hemophilia in India who cannot receive prophylactic clotting factor concentrates lifelong at high doses due to economic reasons. Given the fact that consistent expression of 3-5% at least of FIX expression has been seen in nearly all the patients treated so far with this vector in the UK trial and with almost no significant clinical toxicity in nearly two years of follow-up of the fist patient, there is huge scope for adopting this technology for conducting our own clinical trials of gene therapy in hemophilia. Towards this end, Dr. Alok Srivastava, is working on the following aspects: i. Developing the process for review, approval and monitoring of such research proposals; ii. Production of GMP grade vector in collaboration with industry; iii. The development of the clinical trial protocol (see details in report of Dr. Alok Srivastava)

b.Lenti viral vectors for thalassemia gene therapy – Other collaborators: Trent Spencer, Emory University, USA and Fulvio Mavilio, Genethon, France)

A collaboration has been established with the gene therapy group at Emory University, USA for developing lenti viral vectors that could be used for gene therapy for thalassemia. The origin of this collaboration lies in the fact that this group had got in touch with Dr. Alok Srivastava nearly two years ago to set up a collaboration for a phase I gene therapy trial for hemophilia A using lentiviral modified autologous hematopoietic stem cells for which they have already filed an IND with the USA FDA. During these discussions, the possibility of developing of using similar vector but with a different payload was broached. This has now evolved into a very vigorous collaboration with the following plan for its preclinical development:

- Vector design – This is being done jointly between the three groups involved – Emory, Genethon and CSCR. The construct has been being developed by Emory and is being sent anytime now to CSCR for expansion and evaluation. Based on the initial results, the plan is file for IPR on this vector. It has been designed with that aspect in mind.

- Evaluation of efficiency of the vector – This will be done by Dr.R V Shaji at CSCR using the hematopoietic stem cells (HSC) based assays for assessing hemoglobin production that he has established over the last 3-4 years. This is a unique model to evaluate this vector, including its efficacy in HSCs from patients with thalassemia, before testing them in in-vivo models of transgenic mice with thalassemia. This has also been planned.

Depending on the results of these studies over the next 1-2 years, including the safety profile of these vectors in terms of their random integration which will be evaluated in collaboration with other labs, further development of this work into a clinical program will evolve. Needless to say, thalassemia is a major public health problem in India and there are huge unmet needs as well in this area. There are nearly 3-4 groups in the world now that are in active clinical trials for thalassemia using this approach and it is will be very good for us to be able to work in this area. Success with lentiviral vector based gene therapy using autologous HSCs is also being reported in various other hereditary genetic disorders. If this is successful, there will be scope to expand this into those conditions as well.

2. Musculoskeletal Regeneration Program - (Group: At CSCR – Vrisha Madhuri, Sanjay Kumar, Vikram Mathews, Alok Srivastava; in CMC, Vellore- Noel M Walter, Dr. Sanjay K Chilbule, Mr.Karthikeyan, Dr. Vivek Dutt, Dr Abhay Gahukambale, Dr. Sridhar Gibikote, Dr. Balakumar, Dr. Smitha Elizabeth Mathew, Dr. Albert Abhinay Kota, Dr. Sukria Nayak, Dr. Sathya Subramani Other collaborators – (National) Dr. Jyotsna Dhawan and Dr. Ramaswamy Subramanian, Institute for Stem Cell and Regenerative Medicine, Bangalore, Dr. Prabha D. Nair, Dr. H K Varma and Dr. Annie John, from Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum. Dr. Dhirendra S. Katti and Dr. Amitabha Bhandhopadyay, Indian Institute of Technology, Kanpur, Dr. H. Krishnamurthy, and Dr Dasaradhi Palakodeti, National Centre for Biological Sciences (NCBS), Bangalore (International). Dr.Moustapha Kassem, and Dr.Henrik Daa Schroder, Dr. Lea Bjerre,Dr. Linda Harkness, and Dr. Louise Helskov Jørgensen, Odense University Hospital, Denmark

Musculoskeletal injury and dysfunction results in the more than 20% of all health care encounters. In India, the burden of musculoskeletal disorders accounts for 10% of the disability according to an ICMR study. New treatment and preventive strategies require collaboration between clinicians, engineers and basic scientists. Number of cell therapies, stem cell technology, and cell/ biomaterial based technologies and other regenerative products have been developed in the past decade. At present in the international scenario, there are musculoskeletal regeneration programs in several universities such as University of Pennsylvania (UPENN) and Pittsburgh and University of California, Los Angeles (UCLA).

In Vellore, a group of orthopaedic surgeons, other clinicians and scientists have now been working for several years to develop an active musculoskeletal regeneration program.

This group is actively involved in the research projects related to the regeneration of the musculoskeletal tissues such as articular and physeal cartilage, bone and muscles. This group conducts the in vitro studies of the differentiated and stem cells of musculoskeletal system to translate them in small and large animals. The ultimate goal of the group is to use these cells for the regeneration of cartilage (articular and physeal), bone and muscles in humans. The translational project relating to physeal regeneration is in its final stages while the project related the bone regeneration for long bone defects is about to commence clinical translation.

Congenital pseudarthrosis of tibia occurs in NF1 positive individuals where the bone quality is altered, there is poor bone production and abnormal activity of osteoclasts lead to bone destruction, fracture and non-union in the tibia in affected individuals. The CPT model would allow in-vivo exploration of therapeutic interventions. Focal and diffuse articular cartilage loss in the hip joint occurs in a number of childhood disorders. The sheep model of articular cartilage loss and replacement using scaffolds and autologous chondrocytes is being carried out to look at possible therapeutic interventions.

Simultaneously, this group also works on testing of scaffolds for the above applications and molecular diagnosis of the rare musculoskeletal disorders like osteogenesis imperfecta, fibrodysplasia ossificans progressiva and progressive psuedorheumatoid dysplasia.

3. Stem cell niche program – (Group: At -: CSCR) AparnaVenkatraman, Sanjay Kumar, Murugan Ramalingam; Alok Srivastava; In CMC, Vellore – Biju George and Eunice Sindhuvi (Haematology), Sukesh C Nair (Blood and marrow morphology) Marie Therese (Histopathology), Vivi M Srivastava (Cytogenetics).

There is increasing interest in the understanding of the niche of different adult stem cells that control their fate under physiological conditions – from maintaining them in quiescence to moving them towards division and differentiation. The possible role of the niche in the pathogenesis of diseases of those organs is also a subject of intense evaluation. It is the aim of this group to develop techniques and biological assays to assess the various elements of different stem cell niche components and apply them to the understanding of the pathogenesis of diseases of that organ. This program will currently have two parts:

a. Over the last 10 years or so, the bone marrow niche of the hematopoietic stem cells has been extensively analyzed. These studies have helped dissect the different cellular and molecular elements that contribute to cell fate during hematopoiesis and helped to understand the interactions between them. These studies have shown that there is an endosteal component to the niche where HSCs are generally quiescent while they are much more actively dividing at the endothelial end of the niche. Adrenergic nerve fibres also play a role in this niche as do temperature and oxygen gradients. Extension of these evaluations in transgenic animals has also shown that diseases or phenomenon which may have been presumed to be due to defects or characteristics of the hematopoietic stem cells may actually be related characteristics or defects of the niche elements. .

In the first part of this program, our aim to establish assays for evaluating the different cellular elements of the bone marrow niche in the normal marrow and then use those assays to assess certain diseases. In the beginning we have chosen to evaluate these elements in patients with bone marrow failure syndromes for several reasons. This condition is relatively more common in developing Asian countries than in Western countries with not only the median age being almost two decades lower but also nearly a quarter to third of the patients being children or very young adults. This is a very different profile from what is reported in the Western literature. While both genetic and environmental elements have been implicated very little is known about the cellular changes that may be contributing to these diseases. We see a large number of these patients of all ages and the etiology of their disease remains an enigma.

The evaluation of these patients is planned in the following manner:

i. The clinical documentation of these cases will be done by Drs. Biju George and Alok Srivastava according to a standardized protocol.

ii. Their morphologic diagnosis will be established by Dr. Sukesh C. Nair and histopathological diagnosis by Dr.Marie Therese who will also use various immunohistochemical techniques on the bone marrow trephine biopsy to mark the cellular elements in situ.

iii. We will also evaluate these patients for known mutations that are associated with these conditions using genomic DNA from the marrow cells as well cellular subpopulations, if needed. This work will be done under the supervision of Dr. Eunice Sindhuvi.

iv. The hematopoietic stem and progenitor cells phenotypic, functional and location will be evaluated by Dr. Aparna Venkatraman using FACS, in vivo stem and bone marrow transplantation assays and immuno-fluorescence based on her extensive post-doc work that she has done in this area.

v. The mesenchymal stromal cells (MSC) will be evaluated by Dr. Sanjay Kumar using different culture, co-culture and molecular technologies using his considerable experience in this field. He has substantial experience in this field with having worked with MSCs for over a decade. He will apply these skills to address the biology of subset of progenitor cells involved with cellular defects in bone marrow microenvironment and bone formation.

vi. Dr. Murugan Ramalingam is able to create artificial 3D nanomaterial environments of different chemical gradients to evaluate cellular growth characteristics. This model will also be utilized in these cellular studies.

This can be a difficult subject to study because there are many variables in the niche and our ability to dissect them are not always robust. Also, bone marrow failure syndromes are a heterogenous group of diseases and so we will need to carefully select the cases that we choose to evaluate. Yet with the burgeoning information about the niche and given our advantage with access to the clinical material and trained people particularly in CSCR. The plan is to initially evaluate about 10 each of the low risk cases and very high risk cases (closer to the leukemic spectrum). With those preliminary data, a more elaborate project is to be developed. The start of this project has been delayed because of the lack of funds during the past 12 months but has now been initiated.

b. The second part of this program, is the assessment of the gastrointestinal stem cell niche. This work will be initiated by Dr. Aparna Venkatraman, given her background in gastrointestinal epithelial work in the past and her close collaboration with this group, for human samples, if needed. Beginning with a mouse model of ulcerative coliitis (UC), this work will aim to characterize the colonic stem cell niche and understand key mechanistic indices which influence stem cell quiescence and differentiation and then dissect their role in epithelial cell dysfunction leading to development of UC.

4. Vascular Biology Program – Group: At CSCR -:

Rekha Samuel, Sanjay Kumar; In CMC Vellore: Jiji Elizabeth Mathews and Santhosh Benjamin, (Obstetrics and Gynecology) Nihal Thomas (Endocrinology, Diabetes and Metabolism)and MS Seshadri (retired Professor, Endocrinology, Diabetes and Metabolism), Indrani Sen (Vascular Surgery), Paul MJ and Sukria Nayak (General Surgery), Renu George (Dermatology), Ruchika Agarwal and Debashish Danda (Clinical Immunology and Rheumatology); Other collaborators: Colin Jamora, Institute for Stem Cell Biology and Regenerative Medicine, (inSTEM), Bangalore), H. Krishnamurthy (National Centre for Biological Sciences (NCBS), Bangalore), Niranjan Joshi and Mohanasankar Sivaprakasam (Healthcare Technology Innovation Centre, Chennai).

The broad goal of the vascular biology program at CSCR is to understand the cellular and molecular mechanisms involved with the interaction of human endothelial progenitor and perivascular cells that lead to functional stable vasculature in vivo. A major focus of the lab is examining microvascular dysfunction in Type 2 diabetes utilizing in vivo multiphoton imaging and cranial window models in Severe combined immunodeficient mouse mice. Using placental hyperglycemia as a model to extrapolate vascular defects of Type 2 diabetes, we also examine the blood placental barrier using ultrastructural studies, in vitro and in vivo murine models. We use two approaches to obtain vascular progenitor cells; first, exploiting human induced pluripotent stem cells (hIPS)- derived vascular endothelial cells and pericytes, and second, isolation of vascular progenitor cells from adult somatic (e.g. adipose) tissue. Another area of interest in the lab involves exploring signaling pathways that influence the interaction of vascular and epithelial progenitor cells with immune cells responsible for causing vasculopathy of systemic sclerosis (diffuse scleroderma).

The strengths of the vascular biology lab include the multidisciplinary team and the clinical relevance of our experimental models.

Ongoing experimental models/ projects include:

a. Microvascular defects in Gestational diabetes mellitus (GDM): Upto 10-70% of GDM women and babies develop Type 2 diabetes in India. India ranks second to China in global prevalence of Type 2 Diabetes. Since access to target organs of Type 2 Diabetes e.g. kidney or retina is challenging, we utilize a 9 month old organ, the GDM placenta to examine early changes of the vasculature in the mother and newborn. Involvement of the members: Rekha Samuel (Pathology, vascular biology, human induced pluripotent stem cell technology and in vivo microscopy), Sanjay Kumar (Molecular biology and gene manipulation), Jiji Elizabeth Mathews, Santhosh Benjamin, Nihal Thomas and MS Seshadri (Clinical correlation, and expertise), H. Krishnamurthy (Flow sorting), Niranjan Joshi and Mohanasankar Sivaprakasam (Engineering and Image Analysis).

b. Generating Functional blood vessels: The holy grail of vascular regenerative medicine is creating stable and functional blood vessels in vivo in vascular disease. We have been able to generate durable blood vessels in mice; from human induced pluripotent stem cell derived vascular progenitor cells. We are also keen on isolating vascular progenitor cells from alternate sources such as peripheral blood, adipose tissue or from walls of blood vessels and replacing animal products with substitutes such as human platelet lysate in culture conditions. Involvement of the members: Rekha Samuel (Pathology, vascular biology, and in vivo microscopy), Indrani Sen and Sukria Nayak (Establishing murine ischemic models of disease, and clinical correlation), H. Krishnamurthy (Flow sorting), Niranjan Joshi and Mohanasankar Sivaprakasam (Engineering and Image Analysis)

c. Vasculopathy in Systemic sclerosis (SSc)/ Diffuse scleroderma: Despite 40 years of active research in SSC, the pathogenesis is still unknown and treatment options are limited. Vascular injury in a seminal event in pathogenesis of SSc, that contributes to significant morbidity and multisystem disease involvement. The access to a Snail transgenic murine model that recapitulates human SSc disease, human SSc samples and the potential to manipulate specific proteins in the mouse system, provides an innovative approach to examine vasculopathy in SSc. Involvement of the members: Rekha Samuel (Pathology, vascular biology, human induced pluripotent stem cell technology and in vivo microscopy), Colin Jamora, (Epidermal progenitor and stem cell biology, Snail transgenic mouse model, molecular analysis), H. Krishnamurthy (Flow sorting), Renu George, Ruchika Agarwal, Debashish Danda and Paul MJ (Clinical Correlation and Expertise).

Translational potential of our research: Utilizing vascular progenitor cells in a translational setting remains a significant challenge due to inherent endothelial dysfunction in vascular disease. It is therefore imperative to use meaningful animal models and preclinical studies to examine defects of microvasculature in diseases such as T2D, before one could envisage targeting of specific cytokines, using autologous vascular cell therapy, or vascularization of engineered tissues at the clinic.

Scientific Research Profile

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

RESEARCH ACTIVITIES: Sanjay guides a research group consisting of two graduate students and one laboratory technical staff who study biology of mesenchymal stem cell (MSC) and their roles in disease manifestation, their cellular reprogramming and exploring MSC preclinical therapeutic applications in mice models. His research also encompasses the gene therapy area and use of AAV/lenti vectors to genetically modify MSC, express therapeutic proteins and use of genetically-modified MSC in Nod/Scid mouse models. Currently he has optimized the cellular reprogramming of term-placenta derived mesenchymal stem cells after characterizing their phenotype, cellular growth kinetics and their multilineage differentiation capabilities showing in vitro plasticity. At the same time performing experiments related to how stem cells communicates with other cells using exosomes and genetically-engineered human umbilical cord mesenchymal stem cell-derived exosomes for tumor-specific targeting. Also, how to therapeutically use the placenta-derived MSC in acute radiation sickness. This research group brings into focus

sickness. This research group brings into focus the primary role of mesenchymal stem cells and their involvement in disease pathogenesis; all focused on testing stem cell based therapies into pre-clinical animal model system, one day facilitating the novel approaches to clinical research.

Laboratory highlights

PATENTS:

- Indian Provisional patent application No. 5171/CHE/2012. Dated 12th December 2012. METHOD OF PREPARATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS. Principal Inventor: Sanjay Kumar.
- Indian complete Patent Application No. 57/CHE/2014. Dated 6th January 2014.
  A PROCESS OF LABELLING CELLS AND AMETHOD OF TRACKING THEREOF.
  Principal Inventor: Sanjay Kumar.

MTCC / Gene bank depositions:

- Deposited the reprogramming plasmid vector to Microbial Type Culture and Gene Bank (MTCC) under Budapest Treaty for easy access to every scientific community.

BOOK CHAPTER: Bone Defect Repair in Mice by Mesenchymal Stem Cells- Humana Press, USA part of the Springer Publishing Group.

Publications:

Having impact factor ≥ 5

- Adeno-associated virus (AAV) vectors in gene therapy: Immune challenges and strategies to circumvent them (2013) Hareendran S, Balakrishnan B, Sen D, Kumar S, Srivastava A, Jayandharan GR. Rev Med Virol. 2013 Nov;23(6):399-413

Having impact factor < 5

- Long-term cultured human term placenta-derived mesenchymal stem cells of maternal origin displays plasticity. Sabapathy V, Ravi S, Srivastava V, Srivastava A, Kumar S. Stem Cells Int. 2012:174328.Mar 26.
- Mobilization of bone marrow mesenchymal stem cells in vivo augments bone healing in a mouse model of segmental bone defect. Kumar S, Ponnazhagan S. Bone. 2012 Apr; 50(4):1012-8.
- Comparative Studies to Evaluate Relative in vitro Potency of Luteolin in Inducing Cell Cycle Arrest and Apoptosis in HaCaT and A375 Cells. Vazhapilly Cijo George, Devanga Ragupathi, Naveen Kumar, Palamadai Krishnan Suresh, Sanjay Kumar, Rangasamy Ashok Kumar (Asian Pacific J Cancer Prev, 2013:14 (2), 631-637).
- Bone healing by endogenous stem cell mobilization. Kumar S, Ponnazhagan S. Bone. 2012. Sep;51(3):635.

Manuscript communicated, which are at various stages of peer review process:

- Neurospheres derived from integration-free iPSCs of human placental MSC augments motor and sensory functions in SCID mice following spinal cord injury. (Stem Cell Research)
- Non-invasive in vivo imaging of indocyanin green (ICG) labeled human stem cells in SCID mice injury models. (Cell Transplant)
- Biomechanical characterization of acellular human amniotic membrane scaffolds and comparative study with synthetic scaffolds for potential use in bioengineering applications. (Regenerative Medicine)
- Plasticity of long-term cultured human perinatal tissue-derived Mesenchymal Stem Cells provides a unique therapeutic opportunity. (J Cell Mol Med)
- Human wharton's Jelly Mesenchymal Stem Cells plasticity augments scar-free skin wound healing with hair growth in SCID mice. (Plos One)

Awards:

- Ramalingaswami Fellowship (Sanjay Kumar)
- ICMR Travel Award to attend International Society of Stem Cell Research 2013 conference at Boston USA (Sanjay Kumar)

Human resource development:

- Trained several short-term trainees/students from different background of B.Sc, B.Tech.,M. Sc. and M. Tech. in short-term projects towards partial fulfilment of their respective degrees.
- Conducted Stem cell workshops/courses and demonstrated mouse bone marrow mesenchymal stem cell isolation, in vitro characterization and immunophenotypic characterization.

Managing core facilities as a faculty in charge :

- Flow Cytometry-FACS Core Facility
- In vivo small animal whole body imaging system
- Also, organizing PhD pre-registration course work for Stem Cell Module as a course coordinator.

Invited talks:

- Therapeutic potential of adult mesenchymal stem cells. CME program at Sri Ramachandra Medical College, Porur, Chennai.
- Genetically modified mesenchymal stem cells for enhanced bone regeneration in a mouse model of segmental bone defect at Stem Cell Biology symposium at Lifeline Hospital, Chennai.
- Engineering Mesenchymal Stem Cells for bone targeted homing at International Stem Cell Biology conference held at ARCTREC, Navi Mumbai.
- Therapeutic potential of genetically modified adult stem cells for osteopenia. National Stem Cell Symposium held at Dhanalakshmi Engineering College, Trichy, Tamilnadu.
- Therapeutic potential of adult bone marrow-derived mesenchymal stem cells. Vellore Institute of Technology (VIT), Tamilnadu.

International and national scientific meetings attended:

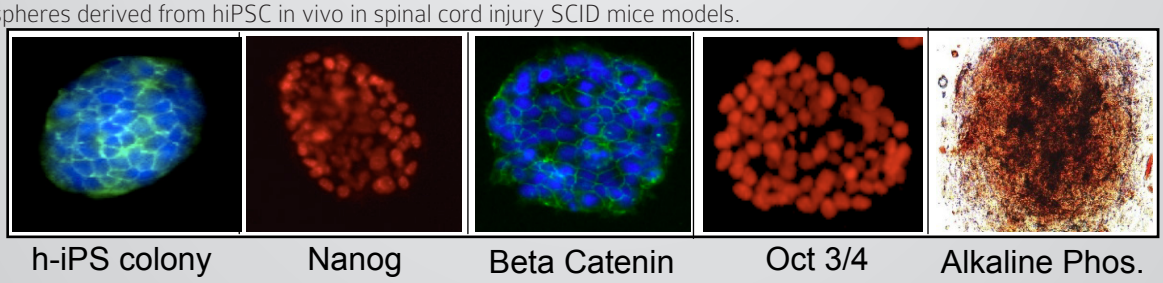
- American Society of Gene and Cell Therapy Conference-2011
- International Stem Cell Biology Conference, Navi Mumbai-2012
- World Stem Cell Summit, Los Angeles, CA-2011
- Cold Spring Harbor Asia Conferences: CSHA/ISSCR Joint Meeting on Stem Cells in Science and medicine, Suzhou, China-2012 (Graduate Student)
- National Stem Cell Symposium 2011
- American Society of Gene and Cell Therapy Conference-2012
- Stem Cell Biology Symposium, Chennai, 2013
- International Society of Stem Cell Research (ISSCR) Annual Meeting, Boston-2013
- American Society of Gene and Cell Therapy Conference-2013

Ongoing research support :

Ongoing Projects:

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

Ongoing work: We have derived iPSC like colonies from human placental MSC and currently characterizing and validating the basic features of induced pluripotent (iPS) cells in vitro differentiation assays, in vivo teratoma assays as well as evaluating therapeutic potential of neurospheres derived from hiPSC in vivo in spinal cord injury SCID mice models.



- Therapeutic applications of human perinatal-tissue derived Mesenchymal Stem Cells (MSC) eg placenta, umbilical cord and amniotic membranes.

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.

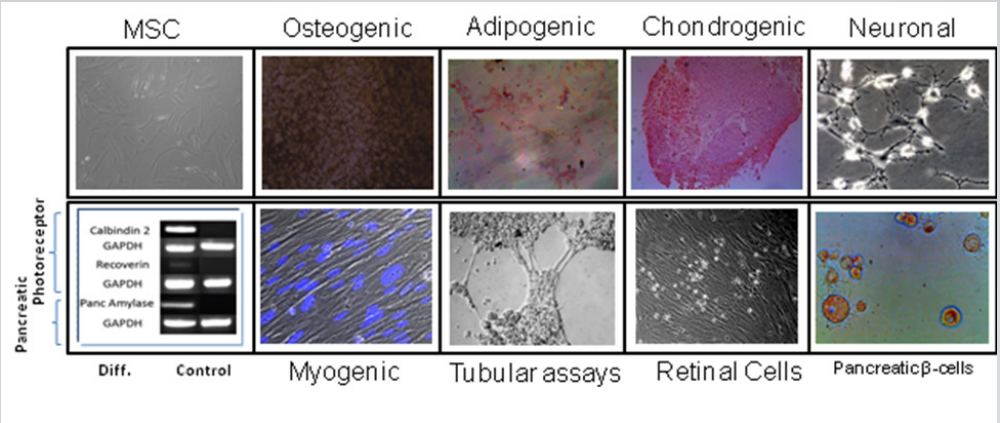


Figure: Pluripotency of the cultured MSCs was evidenced by induced differentiation towards mesenchymal as well as trans-lineage differentiation towards ectodermal and endodermal lineages displaying plasticity in their differentiation potential.

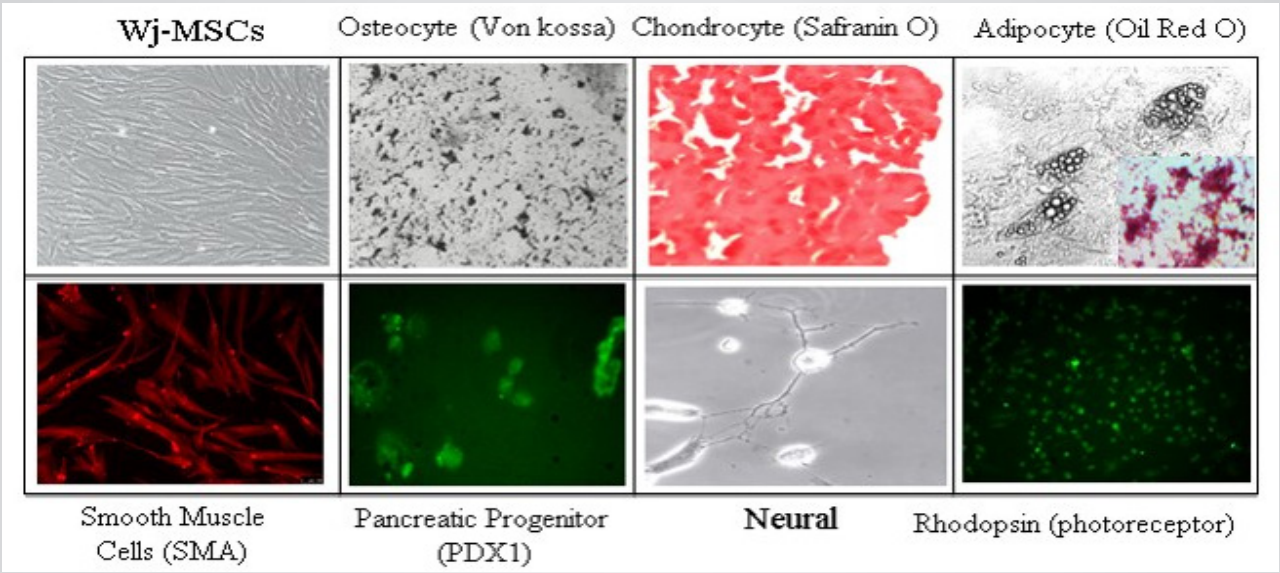
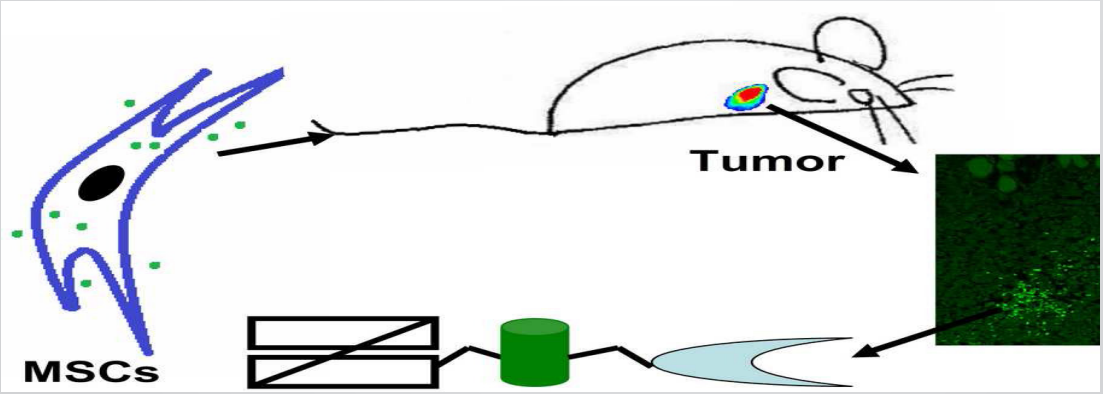


Figure: In vitro multilineage differentiation plasticity of wharton jelly of human umbilical cord derived MSC.

- A novel multifaceted approach to widen the therapeutic window of spinal cord injury in SCID mice model using hPD-MSC/neuro-progenitors and/or PTEN modulation in axons by AAV-shRNA.
- Therapeutic applications of genetically manipulated human term-placenta-derived mesenchymal stem Cells (PD-MSCs) as drug cells for treating acute radiation sickness (ARS) and/or radiation-induced cutaneous damages.
- Genetically-engineered human wharton jelly of umbilical cord mesenchymal stem cell (WJ-MSC)-derived exosomes for tumor-specific targeting.



MSC loaded with Endostatin and HSV-tk

Ongoing / submitted grants to funding agencies :

- Ramalingaswami fellowship contingency support Grant (2011-2015) Ramalingaswami fellowhip project, DBT.
- A novel multifaceted approach to widen the therapeutic window of spinal cord injury in SCID mice model using hPD-MSC/neuro-progenitors and/or PTEN modulation in axons by inducible shRNA (Grant-DBT).
- Therapeutic applications of genetically manipulated human term-placenta-derived mesenchymal stem Cells (PD-MSCs) as drug cells for treating acute radiation sickness (ARS) and/or radiation-induced cutaneous damages (Grant BRNS).
- Creating a preclinical non-human primate (marmoset) facility to carry out the spinal cord regeneration experiments at the Centre for Stem Cell Research (CSCR), Vellore. Infra Structure Grant: (Grant DBT).
- Generation of an epigenetic factor shRNA library for studying the mechanisms of stem cell differentiation, disease pathogenesis and drug resistance. (Grant DBT; Co-PI: Shaji RV).

Completed grants:

- Site-specific excisable AAV-based vector technology for consistent and reliable generation of virus-free pluripotent stem (iPS) cells. DBT Grant # BT/PR15420/MED/31/122/2011

Collaborators:

International- Selvarangan Ponnazhagan, Professor, Dept. of Pathology, UAB.
CSCR/CMC-Dr. RV Shaji, Dr. Jayandharan GR, Dr. Rekha Samuel, Dr. Vrisha Madhuri, Dr. Alok Srivastava, Dr. BS Ramakrishna, Dr. George Tharion, Dr. Suresh Devasahayam, Dr. Paul MJ, Dr. Antony Devasia.

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 Prgramme :

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 was 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 were injected every 2 weeks in the right knee joints of the 20 SD rats following the surgical intervention. Another group of 20 rats acted as control that underwent only surgical procedure. The osteoarthritis in the rat knee joints is being evaluated using Mankin score for histopathology or radiologically using micro CT.
- In second project, the discarded cartilage from the hip joint of the children with the Perthe's disease (6 patients), Idiopathic chondrolysis (5 patients) and slipped capital femoral epiphysis (3 patient) have been procured. The cartilage tissue is undergoing characterization using the markers like autotoxin, CD44, collagen II and collagen X using immunohistochemistry and routine hisotpathological evaluation for pathological and regeneration changes.
- 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 MSCs from CPT are being transfected with luciferase and GFP to use them for bioilluminence and immunohistochemistry respectively.
- The study is awaiting the approval from animal ethics committee in which thepolycaprolactone 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.
- Fifth project involves studying the effect of shock wave on growth plate in rat metatarsal during organ culture. In this study the rat metatarsal will be subjected to shock wave and then the effect of shock wave on metatarsal growth plate will be assessed by Histology and Micro CT.
- A study is proposed by collaboration with Dr. Henrik Schroeder, Odense University, Denmark. Objective of this study is to treat Human sphincter muscle injury using Good manufacturing protocol (GMP) grade Human Autologous Muscle satellite cells.

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

This pilot study is 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. Recruitment in the study is over after 5 children with 8 physeal bars were transplanted autologous cultured chondrocytes. GMP (good manufacturing practices) protocol was standardized as a first step as this study involved the human intervention. Five children with mean age of 3 years (range 2-10 years) underwent mean 266.2 million (range 30 to 460 million cells) autologous cultured chondrocytes transplant in the eight physeal bar (5 distal femurs and 3 proximal tibias) Average duration of follow up following the index surgery is 8.8 months (range 5 to 14.5 months). Till date none of the children has faced any complication related to either cartilage harvest or the index surgery. First child transplanted has undergone revision bar excision. Deformity is recurring in 4rth child as deformity correction is planned. Other children needs further follow up to comment on the outcome.

2. In vitro and in vivo testing of a layered 3-D composite scaffold for articular cartilage tissue engineering

This is DST funded project of which a part is being conducted at our unit. The part of the project is being conducted at IIT Kanpur under Dr Dhiren Katti. Objectives of this project to understand the in vitro influence of the 3D layered scaffold on MSCs behavior and in vivo study to test the clinical efficacy of the scaffold with MSCs in rabbits for articular cartilage regeneration. Chitosan–Gelatin (CG) cross-linked with clay scaffolds are received from Dr. Katti. Chondrocytes isolated from the rabbit articular cartilage were loaded over the CG scaffold and cultured. Simultaneously chondrocyte were cultured as a monolayer to compare the efficacy of scaffold. Viability of the chondrocytes cultured on the CG scaffold was evaluated by LIVE/DEAD assay using confocal microscopy. Expression of the chondrocyte specific gene such as Collagen 2 and SOX 9 were assessed by using real time PCR.Bone marrow and periosteum MSCs are isolated from rabbits and are being characterized for markers such as CD29, CD 90, CD73 and CD 105. These cells are differentiated into three different lineages such as chondrocyte, osteoblast and adipocytes. Biochemical properties like Proteoglycan production (GAG), Collagen secretion and DNA content are assessed.

Laboratory highlights of year 2011-12

- **MANPOWER:** Recruitment and training adjunct scientist (4), Research associates (1), senior Research Investigator (1), Senior research fellows and PhD student(1), Junior research fellow (1) Project assistant (2), Research nurses and technical staff (4)

Name	Designation	Qualification/University
Dr Vrisha Madhuri	Professor and Head, Paediatric orthopaedics Unit Adjunct scientist, Centre for stem cell Research	MS Orth, MCh Orth (L'Pool)
Dr Abhay Gahukambale	Asst Prof and Adjunct scientist (on Leave)	MS Ortho, Dr MGR Tamilnadu Medical university
Dr Vivek Dutt	Asst Prof and Adjunct scientist	MS Ortho, Dr MGR Tamilnadu Medical university
Dr B Balakumar	Asst Prof and Adjunct scientist	MS Ortho, Dr MGR Tamilnadu Medical university. Postdoctoral fellowship paed ortho CMC Vellore
Dr Sanjay Chilbule	Research associate	MS Ortho, Mumbai university. Postdoctoral fellowship paed ortho CMC Vellore
Dr Vishak Manoj Bhaskar	Senior research investigator	MBBS
Mr Karthikeyan	Senior Research Fellow & PhD student	Bharthiyar University Coimbatore Registered at SriChitra Tirunal Institute of medical Sciences, Trivandrum
Ms Sowmya	Junior Research Fellow	B-Tech, Bioengineering, Shastra University
Ms Ruth Rosy	Statistician/social worker	MSc Biostatistics Madras University
Ms. Mona santhanam	Junior Research Fellow	M.Tech, Anna University, Chennai
Mr.David Livingston	Project Assistant	M.Tech Anna Univeristy,Trichy

Award

- Dr Vrisha Madhuri was invited speaker for Annual conference of Paeditraic Orthopaedics society of North America (POSNA) conducted at Toronto on May 1-4, 2013 for "Chondrolysis: Pathophysiology And Treatment"
- Dr Vrisha Madhuri was invited speaker for the Annual meeting of British Limb Reconstruction Society 2013, held at Leicester, United Kingdom on 21-22 March 2013 for the i) Physeal defects and management and ii) Paediatric Clubfoot Deformity.
- Dr Vrisha Madhuri appointed as a PhD guide for SriChitra Tirunal Institute of medical Sciences, Trivandrum.
- Dr.Vrisha Madhuri appointed as a PhD guide for Tamil Nadu Dr. M G R Medical University, Chennai.
- Best Poster award at Annual conference of Paediatric Orthopaedics Society of India (POSICON) 2013, Ahmedabad, Author- Dr Sangeet Gangadharan, Dr Vrisha Madhuri
- Papers presented at Annual meeting of British Limb Reconstruction Society 2013 held at Leicester, United Kingdom on 21-22 March 2013
  1. Dr Balakumar- Limitations of Guided Growth in Sick Physis
  2. Dr Sanjay Chilbule- Postseptic Gap Non-Unions in Paediatric Upper Limb Long Bone Diaphyseal Defect: Role of Fibular Grafting.
- Dr Balakumar received the fellowship for International travel support (ITS) by Department of Science and technology for travel to UK for BLRS meeting 2013.
- Mr Karthikeyan registered as PhD student under Sri Chitra Tirunal Institute of Medical Sciences, Trivandrum with Dr Vrisha Madhuri as a guide.

Publications in 2012-13 (published/ in review/in revision.*corresponding author):

Published-

- Sowmya R, Rajagopal K, Dhanesh Kumar, Nair PD, Madhuri V. Enhanced encapsulation of chondrocytes within chitosan hyaluronic acid hydrogel: A new technique, Biotechnology Letters (Provisionally accepted)
- Balakumar B, Babu S, Varma HK, Madhuri V. Triphasic ceramic scaffold in paediatric and adolescent bone defects. J Pediatr Orthop B. 2014 Mar;23(2):187-95.
- Mathew SE, Madhuri V*. Clinical tibiofemoral angles in South Indian children. Bone Joint Res. 2013 Aug 14;2(8):155-61.
- Arora S, Dutt V, Palocaren T, Madhuri V Slipped upper femoral epiphysis: Outcome after in situ fixation and capital realignment technique.Indian J Orthop. 2013 May;47(3):264-71.
- Rajagopal K, Chilbule SK, Madhuri V*. Viability, proliferation and phenotype maintenance in cryopreserved human iliac apophyseal chondrocytes. Cell Tissue Bank. 2013 Aug 11 (epub ahead of print).
- Madhuri V*, Arora SK, Dutt V. Slipped capital femoral epiphysis associated with vitamin D deficiency: A series of 15 cases. J Bone Joint J. 2013;95-B(6):851-4.
- Madhuri V, Dutt V, Gahukamble AD, Tharyan P. Conservative interventions for treating diaphyseal fractures of the forearm bones in children. Cochrane Database Syst Rev. 2013 30;4:CD008775.
- James D, Madhuri V*, Gahukamble AD, Choudhrie L, Pancharatnam P. Burkholderia pseudomallei Osteomyelitis of the Metatarsal in an Infant.J Foot Ankle Surg. 2013;52(3):370-3.
- Balakumar B, Madhuri V*. A retrospective analysis of loss of reduction in operated supracondylar humerus fractures. Indian J Orthop. 2012;46(6):690-7.
- Ekbote AV, Danda D, Kumar S, Danda S, Madhuri V, Gibikote S. A descriptive analysis of 14 cases of progressive-psuedorheumatoid-arthropathy of childhood from south India: Review of literature in comparison with Juvenile Idiopathic Arthritis. Semin Arthritis Rheum. 2013;42(6):582-9.
- Madhuri V*, Gangadharan S, Gibikote S. Bipolar physeal injuries of the clavicle in a child. Indian J Orthop. 2012;46(5):593-5.
- Madhuri V, Balakumar B, Walter NM, Prakash H, Dutt V, Chowdhurie L. Function after total calcanectomy for malignant tumor in a child: is complex reconstruction necessary? J Foot Ankle Surg. 2012;51(1):71-5

Completed project

Externally funded -

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

CMC Vellore funded-

- 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
- 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
- Culture characteristics of cryopreserved human growth plate chondrocyte- PI- Mr.Karthikeyan R , CO-PI- Dr Vrisha Madhuri

Outside student project – Student Name- Sowmya.R Institute- SASTRA University, Chennai Course- B.Tech (Bioengineering); B.Tech thesis title: Chitosan based scaffolds for cartilage tissue engineering ; Guide- Dr. Vrisha Madhuri ; Carried out at Lab 5 at CSCR, CMC Vellore
Result: 'S' grade for Project thesis and viva-voce ('S' Grade interpretation: 10/10)

Ongoing research support:

Internally funded projects-(fluid research grant)

- Autologous cultured chondrocytes from Iliac crest in the treatment of Physeal bars in children-PI Dr Vrisha Madhuri(Recruitment is over. Operated children are being -followed up)
- Evaluation of culture characteristics of human growth plate chondrocytes. PI- Dr B Balakumar CO-I – Dr Vrisha Madhri
- Role of pamidronate in modulating the osteogenic potential of the mesenchymal stem cells derived from the human lipofibromatous tissue derived from three children with the congenital pseudarthrosis tibia with neurofibromatosis type 1. PI- Dr Smitha Elizabeth Matthew, department of accident and emergency medicine.CMC Co-I- Dr Vrisha Madhuri; Mr Karthikeyan

Externally funded projects:

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

    Budget - Rs 49.02 Lakhs; Principal Investigator – Dr Vrisha Madhuri.; In collaboration with IIT Kanpur with Dr Dhiren Katti as the PI for the IIT Kanpur part of the project.

  Project under this DST project
    - Differentiation of human periosteal derived Mesenchymal stem cells into chondrocyte using Parathyroid hormone related peptide. PI- Dr.Vrisha Madhuri
    - A study is planned in collaboration with Dr. Jørgen Kjems, Aarhus University, Denmark. Aim of this study is to profile the miRNA expression during chondrogenesis of periosteum MSCs. Pi- Dr.Vrisha Madhuri
    - Chitosan based scaffolds for cartilage tissue engineering. PI-Dr.Virsha Madhuri
    - Role of Parathyroid hormone related peptides in retaining and reverting chondrocyte phenotype. Pi- Dr.Vrisha Madhuri
- Treatment of large segmental bone defects with custom made triphasic hydroxyappetite scaffolds loaded with mesenchymal stem cells in children.

  Funding agency- Dept of Biotechnology Govt of India; Budget – 56 lakhs; PI- Dr Vrisha Madhuri , Status – Approved, Awaiting for DCGI approval.

Poonkuzhali Balasubramanian, PhD. Adjunct scientist,

RESEARCH PROGRAM: RESISTANCE IN THE LEUKEMIC STEM CELL COMPARTMENT IN MYELOID LEUKEMIA

LABORATORY HIGHLIGHTS OF YEAR 2012-13.

Mr. Ajay Abraham

- Abstract Achievement Award Recipient 2012- American Society of Haematology (ASH) annual meeting-2012
- Best poster Award in "Annual Research Day"- Christian Medical College, Vellore. Oct' 12
- 2012- DBT travel award to attend American Society for Haematology meeting.

Ms. Sreeja Karathedath

- Travel grant for poster – Indian society for Haematology and Transfusion medicine (ISHBT – 2012)

Ms. Savitha Varatharajan

- Charpak Fellowship to work in France for 6 months 2012

PUBLICATIONS IN 2012-13

- Novel NPM1 mutation in the 3'-untranslated region identified in two patients with acute myeloid leukemia. Abraham A, Karathedath S, Kumaraswamy V, Jayavelu AK, M S, Srivastava VM, Zhang W, Zhou T, George B, Srivastava A, Mathews V, Balasubramanian P. Leuk Lymphoma. 2013 Sep 2. [Epub ahead of print]
- Ajay Abraham, Sreeja Karathedath, Savitha Varatharajan, Preetha Markose, Ezhilarasi Chendamarai, Ashok Kumar J, Biju George, Alok Srivastava, Vikram Mathews & Poonkuzhali Balasubramanian. ABCB6 RNA expression in leukemias—expression is low in acute promyelocytic leukemia and FLT3-ITD-positive acute myeloid leukemia. Ann Hematol. 2013 Jun 22. [Epub ahead of print]
- Balasubramanian P, Chendamarai E, Markose P, Fletcher L, Branford S, George B, Mathews V, Chandy M, Srivastava A. International reporting scale of BCR-ABL1 fusion transcript in chronic myeloid leukemia: first report from India. Acta Haematol. 2012;127(3):135-42.
- 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.
- Abraham A, Varatharajan S, Abbas S, Zhang W, Shaji RV, Ahmed R, Abraham A, George B, Srivastava A, Chandy M, Mathews V, Balasubramanian P. Cytidine deaminase genetic variants influence RNA expression and cytarabine cytotoxicity in acute myeloid leukemia. Pharmacogenomics. 2012 Feb;13(3):269-82.
- Chendamarai E, Balasubramanian P, George B, Viswabandya A, Abraham A, Ahmed R, Alex AA, Ganesan S, Lakshmi KM, Sitaram U, Nair SC, Chandy M, Janet NB, Srivastava VM, Srivastava A, Mathews V. Role of minimal residual disease monitoring in acute promyelocytic leukemia treated with arsenic trioxide in frontline therapy. Blood. 2012 Apr 12;119(15):3413-9.
- Varatharajan S, Abraham A, Zhang W, Shaji RV, Ahmed R, Abraham A, George B, Srivastava A, Chandy M, Mathews V, Balasubramanian P. Carbonyl reductase 1 expression influences daunorubicin metabolism in acute myeloid leukemia. Eur J Clin Pharmacol. 2012 Dec;68(12):1577-86.

ONGOING RESEARCH SUPPORT

S. No.	Title of Project	Funding Agency	Amount (Lakhs)	Date of sanction and uration
1.	Pharmacogenetics of cytarabine and daunorubicin in acute myeloid leukemia- Principal Investigator	DBT	67.77	Feb 28, 2009 5 years
2.	Mechanisms of imatinib resistance in chronic myeloid leukemia- Principal Investigator	DBT	64.77	Feb 28, 2009 5 years
3.	Pharmacogenetics of cytarabine and daunorubicin Resistance in the Leukemic Stem Cell Compartment in acute myeloid leukemia- Principal Investigator	ICMR-INSERM	20.07	December1, 2010 3 years
4.	Pharmacogenetic and pharmacodynamic analysis of fludarabine based conditioning regimen for HSCT – Principal Investigator	DBT	61.87	March 2013 3 years

PATENTS: None

PENDING RESEARCH SUPPORT

- Modulation of drug resistance in acute myelogenous leukemia: role of Nrf2 and ABCB6- Submitted to ICMR, 2013 (PI: Poonkuzhali B; Co-I: Vikram Mathews, Shaji R.V, Biju George, Alok Srivastava) Rs. 76.56 Lakhs
- Pharmacogenomics in Hematopoietic Stem cell Transplantation- Submitted to ICMR, 2012 (PI: Poonkuzhali B; Co-I: Biju George, Vikram Mathews, Shaji R.V, Alok Srivastava)
- "Identification of novel nuclear receptor drug targets using short hairpin RNA (ShRNA) screen in myeloid leukemias" Submitted to Lady Tata Trust, 2013 (PI: Poonkuzhali B; Co-I: Shaji R.V, Biju George, Alok Srivastava) Rs. 55.44 Lakhs

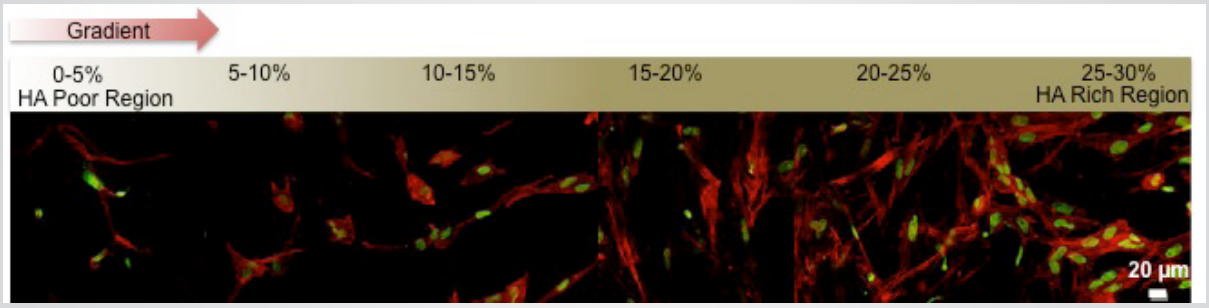
Murugan Ramalingam, PhD., FIoN., FRSC., Associate Professor (Scientist G), June 2012- present

RESEARCH PROGRAM: Area: Stem Cell Nanotechnology

Research Highlights:

Combinatorial nanofiber libraries for high-throughput screening of stem cell behavior and their interface tissue regenerative capacity and underlying mechanisms (from start-up funds):

This project involves the development of combinatorial nanofiber libraries suitable for high-throughput screening of stem cell-matrix interaction in terms of adhesion, migration, orientation, proliferation, differentiation and tissue organization. Scaffolds play a key role in tissue engineering and regenerative medicine wherein they provide structural support for cells to adhere, grow and guide them to synthesize tissues and organs. Scaffolds made of multiple biomaterials are typically required to mimic the structural and compositional features of native cellular microenvironment (niche) in order to regulate cellular and biological functions. Screening the effect of scaffold compositions and properties toward stem cell behavior that optimize tissue organization is the key for the selection of scaffolding system for use in tissue regenerative medicine. 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 microenvironment. Therefore, this project aims to develop a 3D combinatorial gradient nanofiber scaffold libraries suitable for high-throughput screening of cellular responses such as cell adhesion, proliferation, migration, orientation and differentiation under defined 3D microenvironment that maximize tissue organization and growth and to study their interface tissue regenerative capacity and underlying mechanisms in the process of soft and hard tissue development.



A representative confocal image of human bone marrow-derived stromal cell responses to gradient microenvironment. HA denotes hydroxyapatite, a major bone mineral substance.

- Images
- Publications (2012-2013)
- Honors and awards
- Invited talks
- List of team members (postdocs, students, JRFs, short-term trainees, others)
  Dr. Murugan Ramalingam, Associate Professor
- Collaborators

Prof. Ali Khademhosseini, Hardvard University and MIT, USA

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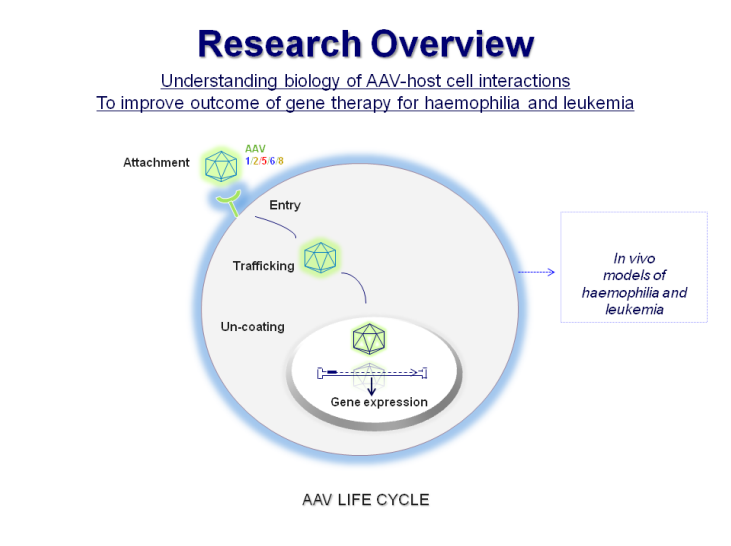
Jayandharan. G . Rao, PhD. Associate Professor, Dept of Haematology/ Adjunct Scientist, CSCR Jan 2010-present

RESEARCH PROGRAM: Adeno-associated virus mediated 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. Thus the research in this sub-theme is 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. These strategies are being simultaneously tested for their efficacy and safety in therapeutic models (Fig. 1). My individual grants were conceived to achieve this, albeit in parts with the expertise of a multitude of collaborators, for an efficacious and safe gene therapy approach for various disease states.



Over the last 4 years, my laboratory has developed an array novel AAV serotype vectors (>60) with an ability to target multiple tissues efficiently. This was achieved by in-depth structural analysis of AAV capsids. These studies identified highly conserved phosphodegrons, which are sites, marked for ubiquitination and degradation by the cellular proteasomal machinery (Collaborator (C): N. Srinivasan, IISc). Based on this finding, we strategically modified phosphodegrons or equivalent residues in all the ten serotypes of AAV (1-10) and generated vectors with enhanced transduction and reduced immunogenicity. Indeed, we have shown that targeting one such phosphodegron in a hepatotropic AAV serotype- 8, can improve coagulation factor levels in pre-clinical models of haemophilia (C: Alok Srivastava, SC Nair, CMC). Similar vectors based on AAV1 serotype are being developed for targeted suicide gene transfer approach to improve therapeutic outcome in a murine model of acute myeloid leukemia (C: Vikram Mathews, CMC). This initiative to treat AML has other innovative components such as the identification of novel AAV strains that have naturally evolved to infect leukemic cells, the generation of a highly specific vector system that can infect leukemic cells by aptamer targeting (C: Ramanuj Dasgupta, inStem) or the development of dual AAV-nanoparticle carrier systems to achieve bimodal killing of leukemic cells (C: Uma Maheswari, SASTRA university, Praveen Vemula, inStem). In another collaborative initiative we are generating an AAV optogenetic switches to study developmental process in Zebrafish models (C: Satish Kitambi, Karolinska Institute).

The focus of these collaborative efforts has been to maximize the reach and translational impact of the novel vector systems we have developed so far in the laboratory. However, we also see scope for further refinement with AAV vectors. The on-going work on miRNA regulatable vector systems or ZFN- DNA editing modular AAV vectors or immune escape phenotypes of AAV vectors and their testing in pre-clinical settings are some examples in this direction. Such initiatives are likely to further the repertoire of AAV vectors that can be applied for potential gene therapy in humans

A.PROJECTS

ONGOING RESEARCH SUPPORT

- Swarnajayanti Fellow (PI: Jayandharan) Jan 2012 - Dec 2016 - 330 Lakhs
  Department of Science and Technology, India-Optimized AAV serotype 2 vectors by bioengineering surface exposed motifs to improve the efficacy of therapeutic gene transfer
- Innovative Young Biotechnologist Award (PI: Jayandharan) April 2011- March 2014 -45 lakhs, 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
- Research grant (PI: Jayandharan, Co-I: Alok Srivastava, Sukesh C Nair) June 2011 June 2014 DBT, India-45 lakh - Efficacy of bio-engineered adeno-associated virus serotype 8 vectors for the potential gene therapy of hemophilia A.
- Research grant (PI: Jayandharan, Co-I: Noel Walter, Alok Srivastava, Viju Daniel) Sep 2013- Aug 2016. Total Award: 33 lakhs. DBT, India - Dissecting the molecular regulators of blood induced joint damage to develop targeted gene transfer strategies for haemophilia

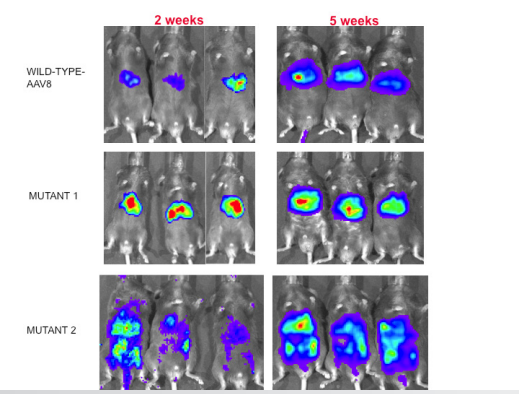
- Research grant (PI: Jayandharan)June 2010 onwards Total Award: -95 lakhs
  Bayer Inc, USA-AAV vectors for the potential gene therapy of hemophilia B: Modulation of the host immune response via the NF-kB pathway

PENDING RESEARCH SUPPORT (PI: Jayandharan)

- 2014-2017. DBT, India. -105 lakhs
  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.
- 2014-2017.DBT, India-92 lakhs
  Exploiting adeno-associated virus modulated host cellular microRNAome to improve its therapeutic gene transfer
- 2014-2019. DBT, India. -312 lakhs

Development of a targeted suicide gene transfer approach using Adeno-Associated Virus (AAV) vectors to improve therapeutic outcome in a murine model of Acute Myeloid Leukemia

B. IMAGE – Novel AAV vectors demonstrating enhanced systemic transduction efficiency.



C.RECENT SELECTED PUBLICATIONS

- Ramesh B. Batchu, Okasana V. Grundyn, Amberly M. Moreno-Bost, Susann Szman, Giridhara R. Jayandharan, Arun Srivastava , Bala K Kolli, Donald W Weaver, Frits van Rhee, Schott A Gruber. Efficient lysis of epithelial ovariancancer cells by MAGE-A3-induced cytotoxic T lymphocytes using rAAV-6 capsid mutant vector. Vaccine 2014; in press.
- Dwaipayan Sen, Balaji Balakrishnan, Nishanth Gabriel, Prachi Agrawal, Vaani Roshini, Rekha Samuel, Alok Srivastava, Giridhara R. Jayandharan*. Improved adeno-associated virus (AAV) serotype 1 and 5 vectors for gene therapy. Sci Rep; 2013, 3: 1832. DOI: 10.1038/srep01832.
- Sangeetha Hareendran, Balaji Balakrishnan, Dwaipayan Sen, Sanjay Kumar, Alok Srivastava, Giridhara R. Jayandharan*. Adeno-associated virus (AAV) vectors in gene therapy: Immune challenges and strategies to circumvent them. Rev Med Virol; 23:399-413.
- Nishanth Gabriel, Sangeetha Hareendran, Dwaipayan Sen, Rupali A. Gadkari, Govindarajan Sudha, Mansoor Hussain, Ramya Dhaksnamoorthy, Rekha Samuel, Srinivasan Narayanaswamy, Alok Srivastava, Giridhara R. Jayandharan*. Bio-Engineering of Adeno-Associated Virus Serotype (AAV)-2 Capsid at Serine/Threonine/Lysine Residues Improves Its Transduction Efficiency Both In Vitro and In Vivo. Hum Gene Ther Methods 2013;24:80-93.
- Dwaipayan Sen, Rupali A. Gadkari, Govindarajan Sudha, Nishanth Gabriel, Yesupatham Sathish Kumar, Ruchita Selot, Rekha Samuel, Sumathi Rajalingam, V. Ramya, Sukesh C. Nair, Narayanaswamy Srinivasan, Alok Srivastava, Giridhara R. Jayandharan*. Targeted modifications in adeno-associated virus (AAV) serotype -8 capsid improves its hepatic gene transfer efficiency in vivo. Hum Gene Ther Methods 2013; 24(2):104-16.
- Balaji Balakrishnan, Dwaipayan Sen, Sangeetha Hareendran, Vaani Roshini, Sachin David, Alok Srivastava, Giridhara R. Jayandharan*. Activation of the Cellular Unfolded Protein Response by Recombinant Adeno-Associated Virus Vectors. PLoS ONE 2013; 8(1): e53845. doi:10.1371/journal.pone.0053845.
- Dwaipayan Sen, Aaron Chapla, Walter N, Daniel V, Alok Srivastava, Giridhara R. Jayandharan*. Nuclear Factor (NF)-kB is a major regulator of blood induced joint damage in a murine model of hemophilia. J Thromb Haemost 2013, 11: 293-306
- Liujiang Song, Xiaomiao Li, Giridhara R. Jayandharan, Yuan Wang, George V. AshlanidiV, Chen Ling, Li Zhong, Guangping Gao, Mervin C. Yoder, Changquan Ling, Mengquan Tan, Arun Srivastava. High-efficiency transduction of primary human hematopoietic stem cells and erythroid lineage-restricted expression by optimized AAV6 serotype vectors in vitro and in a murine xenograft model in vivo. PLoS ONE 2013; 8(3):e58757.
- Liujiang Song, M. Ariel Kauss, Etana Kopin, Manasa Chandra, Taihra Ul-Hasan, Eren Miller, Giridhara R. Jayandharan, Angela E.Rivers, George V. AshlanidiV, Chen Ling, Baozheng Li, Wenquin Ma, Xiaomiao Li, Lourdes M. Andino, Li Zhong, Alice F. Tarantal, Mervin C. Yoder, Kamehameha K. Wong Jr, Mengqun Tan, Saswati Chatterjee, Arun Srivastava. Optimizing the transduction efficiency of human hematopoietic stem cells using capsid-modified AAV6 vectors in vitro and in a xenograft mouse model in vivo. Cytotherapy 2013,15(8):986-998.

- Giridhara R. Jayandharan*, Arun Srivastava, Alok Srivastava. Application of molecular genetics in haemophilia: from diagnoses to therapy. Sem Thromb Haemost 2012; 38: 64-78.
- Giridhara R. Jayandharan*, Alok Srivastava. Hemophilia: disease, diagnosis and management. J Genet Syndr Gene Ther; 2012. doi: 10.4172/2157-7412.S1-005.
- Li Zhong, Giridhara R. Jayandharan, George V. Ashlanidi, Sergi 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.
- George V. Aslanidi, Angela E.Rivers, Luis Oritz, Lakshmanan Govindasamy, Chen Ling, Giridhara R. Jayandharan, Sergi Zolotukhin, Mavis Agbandje-McKenna, Arun Srivastava. High-efficiency transduction ofhuman monocyte-derived dendritic cells by capsid-modified recombinant AAV2 vectors. Vaccine. 2012; 30: 3908-17.
- Giridhara R. Jayandharan, George V. Ashlanidi, Ashley T Martino, Stephen C. Jhan, 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.
- Geoffrey L. Rogers, Ashley T. Martino, George V. Ashlanidi, Giridhara R. Jayandharan, Arun Srivastava, Roland W Herzog. Innate immune responses to AAV vectors. Front Microbiol, 2011; 2: e194.
- Wenquin 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.
- 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, Mavis 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.
- Giridhara R. Jayandharan, Li Zhong, Brandon K. Sack, Angela E. Rivers, Mengxin Li, Baozheng Li, Roland W Herzog, Arun Srivastava. Optimized AAV-protein phosphatase 5 helper-viruses for efficient transduction by single-stranded AAV vectors: Therapeutic expression of Factor IX at reduced vector doses. Hum Gene Ther 2010; 21: 271-83.
- David M Markusic, Roland W Herzog, George V Aslanidi, Brad E Hoffman, Baozheng Li, Mengxin Li, Giridhara R. Jayandharan, Chen Ling, Irene Zolotukhin, Wenquin Ma, Sergei Zolotukhin, Arun Srivastava and Li Zhong. High-efficiency transduction and correction of murine hemophilia B using AAV2 vectors devoid of multiple surface-exposed tyrosines. Mol Ther 2010; 18: 2048-56.
- Chen Ling, Yuan Lu, Jasmine K. Kalsi, Giridhara R. Jayandharan, Baozheng Li, Wenquin Ma, Binbin Cheng, Samantha W.Y. Gee, Katherine E. McGoogan, Lakshmanan Govindasam, Li Zhong, Mavis Agbhandje-McKenna and Arun Srivastava. Human Hepatocyte Growth Factor Receptor is a cellular Co-Receptor for AAV3. Hum Gene Ther 2010; 21:1741-7.
- Chunping Qiao, Wei Zhang, Zhenhua Yuan, Jin-Hong Shin, Jianbin Li, Giridhara R. Jayandharan, Li Zhong, Arun Srivastava, Xiao Xiao, Dongsheng Duan. AAV6 capsid tyrosine to phenylalanine mutations improve gene transfer to skeletal muscle. Hum Gene Ther 2010; 21: 1343-8.
- Mengxin Li, Giridhara R. Jayandharan, Baozheng Li, Chen Ling, Wenquin Ma, Arun Srivastava, Li Zhong. High-Efficiency transduction of fibroblasts and mesenchymal Stem Cells by tyrosine-Mutant AAV2 vectors for their potential use in cellular therapy. Hum Gene Ther 2010; 21: 1527-43.

D.PATENTS [PCT application completed]

- Novel AAV vectors- IN 1714/CHE/2012,
- US 13/886,241,
- EUROPEAN UNION EP13166332.0
  PRINCIPAL INVENTOR: Jayandharan GR
  CO-INVENTORS: Dwaipayan Sen, Sangeetha Hareendran, Nishanth Gabriel, Ruchita Selot, Akshaya K, Balaji B, Alok Srivastava [CMC, Vellore], Sudha Govindarajan, Rupali G, N Srinivasan [IISc, Bengaluru]

- NF-kB in joint disease- INDIA E-2/10024-2013-CHE
  PRINCIPAL INVENTOR: Jayandharan GR
  CO-INVENTORS: Dwaipayan Sen, Aaron Chapla, Viju Daniel, Alok Srivastava, Noel Walter [CMC, Vellore].

E. HONORS AND AWARDS

Jayandharan GR

- 2012-17 Swarnajayanti Award, Department of Science and Technology,Government of India
- 2012 Travel award, Department of Science and Technology, Government of India, 54th American Society for Hematology meeting, Atlanta, USA
- 2011-14 Innovative Young Biotechnologist award, Department of Biotechnology, Government of India
- 2011 Eberhard Mammen Young Investigator award, Thieme Publishers
- 2010-12 Bayer Hemophilia Early Career Investigator Award, Bayer Inc, USA

F. MENTORSHIP

AWARDS

- 2013 American Society for gene and cell therapy society Abstract award, Salt Lake city. (Dwaipayan Sen, Post-doctoral Fellow)
- 2012 Young Investigator Award, SSC meeting of the International Society on Thrombosis and Haemostasis, Liverpool, United Kingdom (Dwaipayan Sen, Post Doctoral Fellow)
- 2012 CSIR Senior Research fellowship (Sangeetha H, PhD student)

- 2012 UGC Junior Research fellowship (Balaji B, PhD student)
- 2011 Best Poster Award, CMC Annual Research Day (Sangeetha H, PhD student)

LAB MEMBERS-CURRENT

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	PhD student	Msc/ Cochin University of Science and Technology, Kochi
Mrs. Sangeetha Hareendran	PhD student	Msc/ Cochin University of Science and Technology, Kochi
Mr. Balaji B	PhD student	M.Phil/ Madras veterinary college, Chennai
Ms. Akshaya Krishnagopal	Junior Research Fellow	M.Phil/ Madras University, Chennai
Ms. KalaiVani	Technician	BSc/Thiruvalluvar University, Vellore

TRAINEES FOR MASTER'S THESIS WORK (6-12 MONTHS)

Monika Kumari, M.Tech	Shoolini University of Management, Solan
Shantoshini Dash, MSc	KIIT University, Bhubaneshwar, Odisha
Ijaz Mohammed Abdull, MSc	Vellore Institute of Technology, Vellore
Rohini K Murthy, B.Tech	SASTRA University, Thanjavur
Kayal Vizhi, MSc	Thiruvalluvar University, Vellore.
Apurv Jain, M.Pharm	JSS College of Pharmacy, Ooty.
Pratheppa, M.Tech	SRM University, Chennai.
Santhosh, M.Tech	SRM University, Chennai.
Anjana Chandrasekhar, MSc	Vellore Institute of Technology, Vellore
Mr Rajnikanth, MSc	Thiruvalluvar University, Vellore.
Prachi Agarwal, MSc	Jaipur National University, Jaipur.
Kanchan Kumari, MSc	Vellore Institute of Technology, Vellore.
Mansoor Hussain, MSc	Jamal Mohamed College, Trichy.
Ramya Moorthy, MSc	Bharthidasan Univeristy, Trichy.
Sathish Kumar, MSc	Madras University, Chennai.
Deeksha Varma, M.Tech	SRM university, Chennai

G. INVITED TALKS [2012 onwards]

- January 2014: Indian Institute of Technology, Kanpur. Adeno-associated virus vectors-host virus interactions, bioengineering and potential in gene therapy.
- December 2013: All India Cell Biology conference, Indian Institute of Science, Bengaluru, Biology of Adeno-associated virus vectors.
- Sep, 2013: KSR college, Tiruchengode, Biology of viral vectors.
- July, 2013: International Society of thrombosis and hemostasis, Amsterdam, Improved AAV8 vectors for gene therapy of hemophilia.
- June, 2013: School of Biotechnology, VIT University, Vellore. Gene Therapy using AAV vectors.
- May, 2013: Madurai Kamaraj University, Madurai. National Seminar on Modern Biotechnology: concepts and practice.
- May 2013: JSS college of Pharmacy, Ooty. Gene therapy: principles and practice.
- April, 2013: Musculoskeletal Congress, World Federation of Hemophilia- Chicago- State of the Art, talk- "NF-kB genes in Blood induced Joint disease"
- March 2013: Advanced School in Biomedical Nanotechnology-SASTRA University- Tanjore- Invited talk-"Gene Therapy"
- Sep 2012: Emerging trends in Cancer and Stem Cell biology-Bharathidasan University- Trichy-Invited talk- Stem Cell Gene Therapy.
- July 2012: World Federation of Hemophilia Congress- Paris-Invited chair for session on "Genetics of hemostasis".

H. COLLABORATORS

- N. Srinivasan, IISc, Bengaluru
- Alok Srivastava, CMC, Vellore
- Vikram Mathews, CMC Vellore
- Sanjay Kumar, CSCR, Vellore
- Rekha Samuel, CSCR Vellore
- Sukesh Nair, CMC Vellore
- Viju Daniel, CMC Vellore
- Noel Walter, CMC Vellore
- George Tharion, CMC Vellore
- Deepa Bhartiya, NIRRH, Mumbai
- Ramanuj Dasgupta, inStem, Bangalore
- Praveen Vemula, inStem, Bangalore
- Ramkumar Sambasivan, inStem, Bangalore
- S. Ramasamy, inStem, Bangalore
- Shravanti Rampalli, inStem, Bangalore
- Dasaradhi Palakodeti, inStem, Bangalore
- Uma Maheswari, SASTRA University, Tanjore.
- Ramesh Batchu, Wayne State University, Detriot.
- Ashok Kumar, MKU, Madurai.
- Arun Kumar Dhayalan, Pondicherry University, Pondicherry.
- Roop Malik, TIFR, Mumbai.
- Rose Ann Padua, INSERM, Paris
- Satish Srinivas Kitambi, Karolinska Institute, Stockholm

Dr. Rekha Samuel, MD, Professor of Pathology.
July 13th 2011-present. (Annual Report, 2013).

RESEARCH PROGRAM: Vascular Progenitor/ Stem Cell Biology

The goal of the vascular progenitor/ stem cell biology research program is to understand the cellular and molecular cues involving the interaction of human endothelial progenitor and perivascular cells that leads to functional stable vasculature in vivo. We examine functional parameters of human tissue engineered vascular constructs in immune deficient mouse models. The major focus of the laboratory is dissecting pathophysiological and molecular mechanisms of microvascular dysfunction in Type 2 Diabetes. We are involved with projects that lead to generation of stable functional vasculature using human vascular endothelial progenitor cells and pericytes from Gestational Diabetic placenta, and vascular progenitor cells from adult somatic tissue, such as adipose tissue. We recognize the contribution of pivotal non-vascular cells including epithelial and immune cells participating in vasculopathy in diseases such as diffuse scleroderma. It is hoped that our preclinical studies would help our understanding of inherent endothelial dysfunction in vascular disease; ultimately translating in targeting specific cytokines, autologous vascular cell therapy, or vascularization of engineered tissues in the clinic.

Other core responsibilities at CSCR: Faculty in charge of the Imaging and Histopathology Core Facilities.

Funding:

I. From CMC: Principal Investigator (PI)

A. Fluid Research Grants from CMC:

1. Isolation of placental perivascular cells and endothelial progenitor cells from Gestational Diabetes to explore early microvascular functional abnormalities. Fluid Research Grant, Christian Medical College, IRB Min 7737, 2012-2014. Rs.80, 000.
2. Isolation and expansion of human endothelial progenitor cells (epcs) from peripheral blood using human platelet lysate (hPL) as a substitute for fetal bovine serum. Fluid Research Grant, Christian Medical College, IRB Min 7846, 2012-2014. Rs.80, 000.

II Extramural funding: Principal Investigator (PI)

A. Accepted

1.Placental Pericytes and Microvascular Dysfunction in Type 2 Diabetes. Department of Biotechnology (Stem Cell Research and Regenerative Medicine). 2012-2014. BT/PR5915/MED/31/172: Rs.42, 22,200.

2.Blood placental barrier in Hyperglycemia of pregnancy. Fast track Scheme for young scientists, Department of Science and Technology (DST), Science and Engineering Research Council SB/FT/LS-196/2012. 2012-2015: Rs. 24,79,000.

3. Generating functional blood vessels using adult vascular stem cells. Indian Council of Medical Research 2012-0803. 2012-2014:Rs. 29,55,080.

4. Non-invasive long-term in vivo vascular imaging using Multi photon Laser Scanning Microscopy. Department of Biotechnology (Bioengineering). BT/PR7990/MED/32/282/2013. 2013-2016: Rs. 50,00,000.

B. Submitted Concept proposal; requested submission of full proposal

1. (As PI):
Back To The Future: Placental Vascular Progenitor Cells Predict Adult Type 2 Diabetic Microvascular Complications. Indian Council of Medical Research. ID. 2013-2825

2. As Co-investigator:
DBT Call for concept notes or ideas under RNAi Science and Technology: 'Elucidating the role of PAI-1 mediated signaling in cutaneous fibrosis'. PI: Colin Jamora, inStem, Bangalore.

Publications 2013

- Chen Y, Huang Y, Reiberger T, Duyverman AM, Huang P, Samuel R, Hiddingh L, Roberge S, Koppel C, Lauwers GY, Zhu AX, Jain RK, Duda DG. Differential effects of sorafenib on liver versus tumor fibrosis mediated by SDF1a/CXCR4 axis and Gr-1+ myeloid cell infiltration in mice. Hepatology. 2013 Nov 14. [Epub ahead of print
- Goel S, Gupta N, Walcott BP, Snuderl M, Kesler CT, Kirkpatrick ND, Heishi T, Huang Y, Martin JD, Ager E, Samuel R, Wang S, Yazbek J, Vakoc BJ, Peterson RT, Padera TP, Duda DG, Fukumura D, Jain RK. J Natl Cancer Inst. 2013 Jul 30. [Epub ahead of print]
- Generation of functionally competent and durable engineered blood vessels from human induced pluripotent stem cells. Samuel R, Daheron L, Liao S, Vardam T, Kamoun WS, Batista A, Buecker C, Schäfer R, Han X, Au P, Scadden DT, Duda DG, Fukumura D, Jain RK. Proc Natl Acad Sci U S A. 2013 Jul 30; 110(31): 12774-9
- Improved adeno-associated virus (AAV) serotype 1 and 5 vectors for gene therapy. Sen D, Balakrishnan B, Gabriel N, Agarwal P, Roshini V, Samuel R, Srivastava A, Jayandharan GR. Sci Rep. 2013 May 13; 6:1832.

- Targeted modifications in adeno-associated virus (AAV) serotype -8 capsid improves its hepatic gene transfer efficiency in vivo. Sen D, Gadkari RA, Sudha G, Gabriel N, Sathish Kumar Y, Selot R, Samuel R, Rajalingam S, Ramya V, Nair SC, Srinivasan N, Srivastava A, Jayandharan GR. Hum Gene Ther Methods. 2013 Feb 26. [Epub ahead of print]
- Bio-engineering of AAV-2 capsid at specific serine, threonine or lysine residues improves its transduction efficiency in vitro and in vivo. Gabriel N, Hareendran S, Sen D, Gadkari RA, Sudha G, Selot R, Hussain M, Dhaksnamoorthy R, Samuel R, Srinivasan N, Srivastava A, Jayandharan GR. Hum Gene Ther Methods. 2013 Feb 4. [Epub ahead of print]

Posters:

- 1. Does the intrauterine hyperglycemic milieu impact Type 2 Diabetic microvascular dysfunction? Rekha Samuel, Jiji Elizabeth Mathews and Mandalam Subramanian Seshadri. EM: Frontiers in Bioimaging Conference, National Centre for Biological Sciences, Bangalore Feb 3rd-5th 2014.
- Perivascular triton tumor: EM aiding diagnosis in a rare tumor. Indrani Sen, Rekha Samuel, Albert Kota, Jennifer Prabhu, Sunil Agarwal. EM: Frontiers in Bioimaging Conference, National Centre for Biological Sciences, Bangalore Feb 3rd-5th 2014.
- Back to the Future: Examining Type 2 Diabetic vasculature using the Gestational Diabetic Placenta. Rekha Samuel , Jiji Elizabeth Mathews and Mandalam Subramanian Seshadri. Diabetes in Pregnancy Study Group of India Conference, Feb 15th -16th 2014.
- Generation of functional durable engineered blood vessels from human induced pluripotent stem cells, ISSCR, Boston. June 2013. Rekha Samuel, Laurence Daheron, Shan Liao, Trupti Vardam, Walid S. Kamoun, Ana Batista, Christa Buecker, Xiaoxing Han, Patrick Au, David T. Scadden, Dan G. Duda, Dai Fukumura , Rakesh K Jain.

Awards

- Best poster award, Stem Cell Society of Singapore, Annual Symposium, November 18-19th 2013, "Engineered functional durable blood vessels from human iPS cells: implications for modeling vascular disease".

Invited talks

1. "Stem Cells and vascular tissue engineering" Vellore Institute of Technology, Tamil Nadu. 25th February, 2014.
2. "Creating functional blood vessels from adult stem cells". Post Graduate Department of Biotechnology, Chinmaya Womens' College of Arts & Sciences, Science Academies Workshop, Kannur, Kerala. 8th October 2013.
3. "An Introduction to Stem Cell Biology". Post Graduate Department of Biotechnology, Chinmaya Womens' College of Arts & Sciences, Science Academies Workshop Kannur, Kerala. 8th October 2013.
4."Stem Cell Imaging-Current perspectives and future challenges". Vellore Institute of Technology, Tamil Nadu. 4th October, 2012.

Training

1.Practical Course, Mouse Embryology Course, inStem, Bangalore, India: March 10th-23rd, 2013.
2.iPS Reprogramming course- June 9-11th, 2013. Boston.

Lab members:

1.Elizabeth Jayex Panakkal, Junior Research Fellow. MSc, Biotechnology, Vellore Institute of Technology.
Research Interest: Microvascular dysfunction in Type 2 Diabetes.
2.Chitra Premkumar, Graduate Technician. BSc Zoology, Auxilium College, Vellore. Research Interest: Vascular progenitor cell characterization.
3.Saranya Rajendran, Graduate Technician. BSc Microbiology, D.K.M College, Vellore. Research Interest: Vascular progenitor cell characterization.

Collaborators

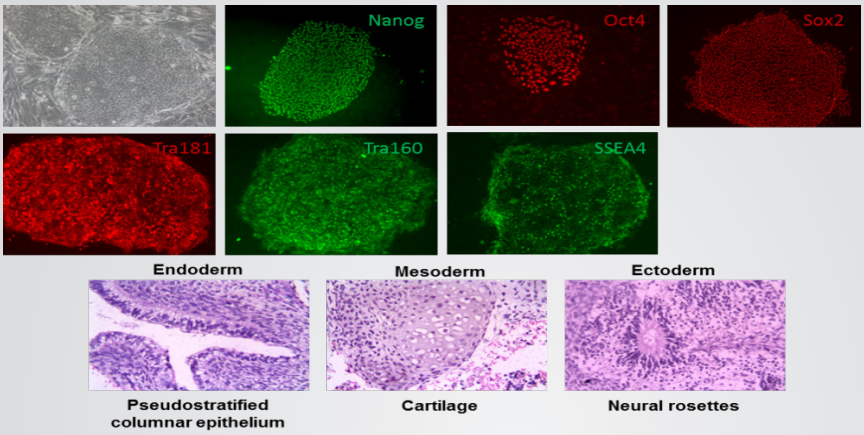
1. Jiji Elizabeth Mathews, DGO, MD. Professor and Head, Obstetrics and Gynecology, Unit V, CMC, Vellore.
2. MS Seshadri, MD, PhD, FRCP. Professor and Retired Head of Endocrinology, Diabetes and Metabolism, CMC, Vellore.
3. Niranjan Joshi, Ph.D. Researcher, Healthcare Technology Innovation Centre, Madras.
4. Mohanasankar Sivaprakasam, Ph.D. Assistant Professor, Indian Institute of Technology, Madras, & Director, Healthcare Technology Innovation Centre, Madras.
5. Colin Jamora, PhD. Associate Professor and Laboratory Director, IFOM-inStem Joint Research Laboratory, Bangalore.
6. H. Krishnamurthy, PhD. Director of Flow Cytometry Facility, Centre for Cellular and Molecular Platforms, NCBS, Bangalore.
7. Sukria Nayak, MS, FRCS. Professor and Head, Surgery, Division, IV, CMC, Vellore.
8. Indrani Sen, MS. MCh Registrar, Division of Vascular Surgery, CMC, Vellore.
9. Ashish Kumar Gupta, MS, M.Ch. Professor and Head, Plastic Surgery, Division II, CMC, Vellore.
10.Renu George, MD. Professor and Head of Dermatology, Unit 1, CMC, Vellore.
11.Debashish Danda, MD, DM, FRCP. Professor and Head, Clinical Immunology and Rheumatology, CMC, Vellore.

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

RESEARCH PROGRAM:
Research Interests:

1.Induced pluripotent stem cells-Mechanism of reprogramming and disease models

Our lab has made significant progress in generation of mouse and human induced pluripotent stem cells (iPSCs). The characterized colonies could be differentiated in-vitro and in-vivo. We have two major interests in this field; one, understanding the transcriptional and epigenetic regulation in the reprogramming process and two, use of iPSCs to study human disease mechanisms. In a model system using retroviral silencing and expression of pluripotency genes we have identified several epigenetic factors that play crucial roles in the late stage of reprogramming. We have initiated a project on generating an inducible shRNA library using which we can study the role of these epigenetic factors in the different stages of reprogramming. In the second project we are working on generation of human iPSCs and their differentiation to haematopoietic stem cells and erythroid cells to study the molecular basis of red cell diseases.



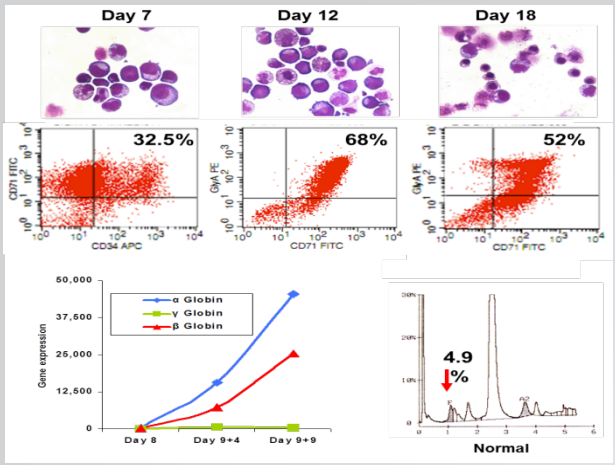
Human iPSCs generated in our laboratory

2. Human erythropoiesis- Transcriptional and epigenetic regulation

We have established the methods for ex-vivo erythropoiesis by differentiation of purified CD34+ haematopoietic stem and progenitor cells (HSPCs) to erythroid cells. We could use this method to obtain large number of erythroid cells from patients with red cell diseases. Using this method we are currently investigating the role of non-coding transcripts in human erythropoiesis by RNA-sequencing of cultured erythroid cells obtained at different stages of erythropoiesis. We identified several miRNAs that are differentially regulated during erythropoiesis. Current experiments are to understand the role of these miRNAs in developmental regulation of erythroid genes using cultured erythroid cells obtained from adult and cord blood HSPCs. Using the ex-vivo erythropoiesis model we are also carrying out ChIP-seq experiments to understand the role of transcriptional cofactors in developmental regulation of human erythropoiesis. From the cultured erythroid cells obtained from HSPCs we studied globin genes in the patients with haemoglobinopathies and identified novel transcriptional mechanisms that cause these diseases.

3.Gene therapy for haemoglobinopathies (In collaboration with Dr.Alok Srivastava, CSCR and Dr.Trent Spencer, Emory University)

In this project we will evaluate the new generation lentiviral vectors for the application of gene therapy for haemoglobinopathies. Based on the outcomes of the previous lentiviral vectors used in the pre-clinical studies in haemoglobinopathies we decided several modifications in the vector design for efficient expression of beta and gamma globin genes in human erythroid cells. Several vectors with various regulatory sequences will be constructed and the titers will be determined by Spencer lab. These modifications include use of different combinations of globin cluster hypersensitive sites, UTR sequences and introns and promoters. The efficiency of these vectors for the high level expression of globin genes will be tested and pre-clinical experiments will be carried out at CSCR. We have established ex-vivo erythropoiesis of adult human CD34+ haematopoietic stem and progenitor cells (HSPCs) from normal individuals and the patients with beta-thalassaemia. Transduced HSPCs will be differentiated to erythroid cells and we will measure the globin gene expression in the late stages of erythropoiesis and the efficiency of the vectors will be estimated. Experiments will be carried out in the cells with different types of beta globin mutations as it is known that the therapeutical benefits may vary depending on the genotypes. In CSCR we will also perform experiments to identify the aberrantly spliced and polyadenylated transcripts from the transgene in human erythroid cells and the data obtained will be used by Spencer Lab to modify the sequences in the vector to improve the efficiency of the globin gene expression. These modified vectors will be used in animal models in our pre-clinical studies.



Ex-vivo erythropoiesis using human adult CD34+ HSPCs

Collaborators:

- Alok Srivastava, CSCR
- Sanjay Kumar, CSCR
- Eunice Sindhuvi Edison, Department of Haematology, CMC
- François Moreau-Gaudry, INSERM U1035, University Bordeaux Segalen

Research Fellows:

- Kannan VM: Senior Research Fellow and PhD student under Thiruvalluvar University
- Musheer Aaalam Syed: Senior Research Fellow and PhD student under Thiruvalluvar University
- Sumitha PB: Senior Research Fellow and PhD student under STIMST
- Janakiram Rayabaram: Senior Research Fellow and PhD student under Thiruvalluvar University
- Thiyagaraj Mayuranathan: Senior Research Fellow and PhD student under Thiruvalluvar University

Research Projects as Principal Investigator:

- Generation of human induced pluripotent stem cells. Funded by Department of Biotechnology 2010-2012 (Completed)
- Molecular Basis of Human globin gene regulation. Funded by Department of Biotechnology 2009-2014
- Molecular Basis of Fanconi Anaemia in Indian population. Funded by Department of Biotechnology 2009-2014
- RNAi screen to identify the novel regulators of somatic cell reprogramming. Funded by Department of Biotechnology 2012-2015.
- Generation of human induced pluripotent stem cells for studying the mechanisms of haematological diseases. Funded by Indian Council for Medical Research (Sanctioned).

Recent Publications:

- Mayuranathan T, Rayabaram J, Das R, Arora N, Edison ES, Chandy M, Srivastava A, Velayudhan SR. Identification of rare and novel deletions that cause (Óβ)0 - thalassaemia and HPFH in Indian population. Eur J Haematol. 2014 Jan 29. [Epub ahead of print].
- Human Grainy head like factor regulates reprogramming. Sumitha P Bharathan, Syed Musheer Mohammed Aaalam, Kannan V. Manian, Alok Srivastava, Shaji R.Velayudhan (Abstract accepted for ISSCR conference 2013, Boston) (Manuscript under preparation).
- Variegated expression of iprinted genes in mouse induced pluripotent stem cells. Kannan V. Manian, Syed Mohammed Musheer Aalam, Sumitha P. Bharathan, Alok Srivastava, Shaji R. Velayudhan. (Abstract accepted for ISSCR conference 2013, Boston) (Manuscript under preparation).
- Mayuranathan T, Rayabaram J, Edison ES, Srivastava A, Shaji RV. A novel deletion of β-globin promoter causing high HbA2 in an Indian population. Haematologica. 2012 Sep;97(9):1445 -7.
- Jain S, Edison ES, Mathews V, Shaji RV. Int J Hematol. 2012 May;95(5):570-2. A novel β-globin gene mutation (HBD: c.323G>A) masking the diagnosis of β-thalassemia: a first report from India. Int J Hematol. 2012 May;95(5):570-2.
- Edison ES, Sathya M, Rajkumar SV, Nair SC, Srivastava A, Shaji RV. A novel 26 bp deletion [HBB: c.20_45del26bp] in exon 1 of the β-globin gene causing β-thalassemia major. Int J Lab Hematol. 2012 Oct;34(5):556-8.
- Varatharajan S, Abraham A, Zhang W, Shaji RV, Ahmed R, Abraham A, George B, Srivastava A, Chandy M, Mathews V, Balasubramanian P Carbonyl reductase 1 expression influences daunorubicin metabolism in acute myeloid leukemia. Eur J Clin Pharmacol. 2012 Dec;68(12):1577-86
- Cytidine deaminase genetic variants influence RNA expression and cytarabine cytotoxicity in acute myeloid leukemia. Abraham A, Varatharajan S, Abbas S, Zhang W, Shaji RV, Ahmed R, Abraham A, George B, Srivastava A, Chandy M, Mathews V, Balasubramanian P. Pharmacogenomics. 2012 Feb;13(3):269-82.

Eunice Sindhuvi.E, PhD. Adjunct scientist, July 2011-present

RESEARCH PROGRAM: Our aim is to establish an in vitro erythroid system from progenitor/ stem cells which we can use to study the transcriptional and translational processes of β^E globin gene.

RESEARCH FINDINGS:
The major aim of this project is to identify molecular mechanisms at the transcriptional and translational level that govern the expression of β^E gene and whether they explain the heterogeneity of HbE syndromes.

Towards this we have established an erythroid culture system. The cells produced by this protocol clearly emulated the in vivo situation. This system gives us an opportunity to get cells from different stages of erythroid development. Using this system, we are studying the following parameters

Quantitation of alternatively spliced β^E mRNA in different stages of erythropoiesis
HbE (Cod 26 G→A) creates a cryptic splice site in exon 1. This cryptic splice site is thought to compete with the normal splice site and result in the production of abnormally spliced transcript. The amount of abnormally spliced transcript is thought to influence the production of haemoglobin E levels.

Identify and characterize antisense RNA transcripts: Existence of globin antisense transcripts has been observed in the erythroid lineage in both patients and controls. 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.

Measurement of proteins regulating β globin translation: Translation of globin mRNA is intricately regulated. Heme regulated eIF-2a kinase (HRI) balances heme and globin synthesis by sensing intracellular heme concentrations. When heme concentration is high, heme binds to HRI and keeps HRI in inactive state, thereby permitting globin protein synthesis. In case of heme deficiency, HRI is activated by autophosphorylation.Even though Gadd34 was found to be a modifier of translation initiation with respect to globin translation and heme abundance, erythroid cells of E-BETA are more prone to apoptosis by induction of CHOP when compared with controls and homozygous E.

Significant Observations
- An exvivo erythropoiesis system has been established, through which we are able to get the erythroid precursors from patients with HbE syndromes.
- Abnormal splicing (due to β^E mutation) does not occur even in early stages of erythropoiesis.(ex-vivo)
- Presence of antisense transcript of the human HBB gene suggesting the regulatory role of sense-antisense pairing.
- Significant role of HRI in regulating the β globin translation has been observed.
- Confirms an equilibrium state exists between Gadd34 and HRI during the synthesis of haemoglobin

Future work will be to study the role of miRNA in splicing regulation and understand how different kinds of stress contribute to aberrant splicing

Publications / abstract presentations, if any.
- Role Of Stress Responsive Genes Responsible In Molecular Switch From Adaptive Phase To Apoptosis In HbE-β Thalassemia. Submitted to ASH 2013
- Divya.J, Eunice S. Edison, Shaji R.V, Aby Abraham, Biju George, Vikram Mathews, Chandy M, Alok Srivastava. Role of genetic variants influencing Hemoglobin F production in HbE-β thalassaemia. Indian J Hematol Blood Transfus,2010:26:145
- Divya.J, Shaji R. V, Vikram Mathews, Alok Srivastava, Eunice S.Edison. Splicing patterns of β^E mRNA in HbE Syndromes. Indian J Hematol Blood Transfus,2011:27:241
- Codon 26(G →A) (β^E) mutation does not result in aberrant splicing (Manuscript in preparation)

Alok Srivastava, MD, FRACP, FRCPA, FRCP

RESEARCH PROGRAM:

My work in stem cell transplantation and research involves two areas:

a. Clinical stem cell transplantation – This is the clinical stem cell transplantation using hematopietic stem cells for blood diseases. Apart from offering a regular service of this treatment we are also evaluating several novel conditioning regimens to improve outcome of such therapies. Currently the following studies are ongoing /planned:

- A phase I study of using single agent post transplantation cyclophosphamide as graft versus host disease (GVHD) prophylaxis in patients undergoing allogeneic stem cell transplantation for aplastic anemia (AA) (Co-investigator)
- A phase II trial to study if the addition of meloxicam to the standard regimen of mobilization with colony stimulating factor (G-CSF) will improve stem cell mobilization rates in patients undergoing mobilization for autologous stem cell transplantation. (Co-investigator)

b.Stem cell research –

This involves three aspects –

- 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. This has taken very considerable time this year with the new national guidelines being developed and finalized. (www.icmr.nic.in/icmrnews/NAC.htm) In addition, the process of registration and review of all clinics / institutions carrying out stem cell research in the country is also ongoing. More than 60 such clinics / institutions have applied for registration from different states in the country.
- Stem cell transplantation clinical trials – This involves working with colleagues in different disciplines to test therapeutic strategies in animal models and human stem cell transplantation studies. Currently these studies include work on cartilage repair (collaboration with Dr. V. Madhuri) and non healing anal fistulas (collaboration with Dr. Sukriya Nayak).
- Translational research with stem cells / regenerative medicine:

  This work also involves two areas –

  - A major thrust in the last year has been on developing gene therapy towards clinical possible trials. Two areas are being developed:
    - The first is towards developing gene therapy for hemophilia using adeno associated vectors for gene transfer. (Collaboration with Dr. G. Jayandharan in CSCR). We have developed our own engineered AAV vectors with surface modified residues that improve their transduction efficiency. Preclinical studies have been completed. Discussions have been initiated with ICMR and DBT (meeting with Dr. V.M. Katoch and Dr. Vijay Raghavan) to start the process of defining the path to clinical gene therapy studies in India. Collaborations with industry in India is also being explored to look at potential AAV vector production on a large scale. A collaboration has been established with Dr. Sanjay Singh at Gennova Biopharmaceuticals for clinical vector production. A final decision on the choice of vector to be taken to the clinical stage needs to be taken.

      In the meantime, I am working of developing three aspects of this program:

      i.Developing the process for review, approval and monitoring of such research proposals: As this is will be a completely new area of clinical research in India, there are no established systems for review, approval and monitoring of such clinical trial proposals. Given the fact that this has two major aspects – the therapeutic product and the clinical trial protocol, he has initiated discussions with both Department of Biotechnology, GOI and the Indian Council for Medical Research, over the last 6 months to define the path for it in India.

      ii. Production of GMP grade vector: This is the major challenge in the world to get cost-effective production of good quality vectors. After a lot of discussions with many people in the field and given the ability of our pharmaceutical industry in process innovation, links have been established with one industrial biotech group in India to see if this can be taken further.

      iii. The development of the clinical protocol: There is string expertise already available in the team with doing clinical trials in therapeutics of hemophilia. I have also been associated with previous international clinical trials of gene therapy and have maintained discussions with both the leading researchers in this field – Dr. Amit Nathwani from UCL, UK and Dr. Kathy High, from University of Pennsylvania, USA. This protocol will be developed by the time the GMP grade vector issues are resolved. Several colleagues from the medical team and the clinical trials unit in the department of Haematology, CMC, Vellore will be involved.

    - The second of these efforts is towards gene therapy for thalassemia. This is being done in collaboration with Dr. R. V. Shaji in CSCR and with Dr. Trent Spencer at Emory University and Dr. Fulvio Mavilio of Genethon, France. A novel vector has been designed (see report from Dr. R V Shaji) and will be evaluated in an ex-vivo hematopoietic stem cell system developed by Dr. Shaji for hemoglobin production. This work started in CSCR with vectors sent by Dr Spencer and the initial results are very encouraging (see report of RV Shaji).

If successful, this will be taken to in-vivo evaluation in mouse models and its safety as well efficacy further assessed.

PhD Students:

- Salar Abbas works on the BM niche (in collaboration with Dr. Aparna Venkatraman, Dr. Sanjay Kumar and Dr. Eunice Sindhuvi)
- Sangeeta Hareendran works on AAV vector modifications (in collaboration with Dr. G. Jayandharan)
- Nishant Gabriel works on AAV vector modifications (in collaboration with Dr. G. Jayandharan)
- Nancy Berel Janet works on the genetics of Fanconi anemia (in collaboration with Dr. R.V. Shaji)

Selected publications:

- Mathews V, George B, Viswabandya A, Abraham A, Ahmed R, Ganapule A, Sindhuvi E, Lakshmi KM, Srivastava A. Improved Clinical Outcomes of High Risk β Thalassemia Major Patients Undergoing a HLA Matched Related Allogeneic Stem Cell Transplant with a Treosulfan Based Conditioning Regimen and Peripheral Blood Stem Cell Grafts. PLoS One. 2013 Apr 26;8(4):e61637.
- Desire S, Mohanan EP, George B, Mathews V, Chandy M, Srivastava A, Balasubramanian P. A rapid & sensitive liquid chromatography-tandem mass spectrometry method for the quantitation of busulfan levels in plasma & application for routine therapeutic monitoring in haematopoietic stem cell transplantation. Indian J Med Res. 2013 Apr;137(4):777-84.
- Sen D, Balakrishnan B, Gabriel N, Agrawal P, Roshini V, Samuel R, Srivastava A, Jayandharan GR. Improved adeno-associated virus (AAV) serotype 1 and 5 vectors for gene therapy. Sci Rep. 2013 May 13;6:1832.
- Sen D, Gadkari RA, Sudha G, Gabriel N, Kumar YS, Selot R, Samuel R, Rajalingam S, Ramya V, Nair SC, Srinivasan N, Srivastava A, Jayandharan GR. Targeted modifications in adeno-associated virus serotype 8 capsid improves its hepatic gene transfer efficiency in vivo. Hum Gene Ther Methods. 2013 Apr;24(2):104-16.doi: 10.1089/hgtb.2012.195
- Gabriel N, Hareendran S, Sen D, Gadkari RA, Sudha G, Selot R, Hussain M, Dhaksnamoorthy R, Samuel R, Srinivasan N, Srivastava A, Jayandharan GR. Bioengineering of AAV2 capsid at specific serine, threonine, or lysine residues improves its transduction efficiency in vitro and in vivo. Hum Gene Ther Methods. 2013 Apr;24(2):80-93.
- Balakrishnan B, Sen D, Hareendran S, Roshini V, David S, Srivastava A, Jayandharan GR. Activation of the cellular unfolded protein response by recombinant adeno-associated virus vectors. PLoS One. 2013;8(1):e53845. doi:10.1371/journal.pone.0053845.
- Sabapathy V, Ravi S, Srivastava V, Srivastava A, Kumar S. Long-term cultured human term placenta-derived mesenchymal stem cells of maternal origin displays plasticity. Stem Cells Int. 2012;2012:174328. Epub 2012 Mar 26.
- Sellathamby S, Lakshmi KM, Busson M, Viswabandya A, George B, Mathews V, Chandy M, Charron D, Krishnamoorthy R, Tamouza R, Srivastava A. Polymorphisms in the Immunoregulatory Genes are Associated with Hematopoietic Recovery and Increased Susceptibility to Bacterial Infections in Patients with Thalassaemia Major Undergoing Matched Related Hematopoietic Stem Cell Transplantation. Biol Blood Marrow Transplant. 2012 Jan 16.
- Scadden D, Srivastava A. Advancing stem cell biology toward stem cell therapeutics. Cell Stem Cell. 2012 Feb 3;10(2):149-50
- 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.

Book chapter:

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

Invited talks:

- Hematopietic stem cell transplantation – A paradigm for the development of cell therapies. 5th Annual Champalimuad Research Symposium, LV Prasad Eye Institute, Hyderabad, January 2102.
- Dr.V. Ramalingaswamy Oration – Biology and clinical applications of human stem cells. Institute of Liver and Biliary Sciences, New Delhi, August, 2012
- Stem cell transplantation in thalassemia major. Asean Federation of Hematology, Singapore. September, 2012
- Therapeutic drug monitoring in allogeneic stem cell transplantation. Asia Pacific Blood and Marrow Transplantation meeting, October, 2012.
- Challenges to growth of stem cell transplantation in the Asia-pacific countries. BMT Tandem meeting February, 2013. Salt lake city, USA.

Patents (applied for):

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

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

A cure for paralysis following spinal cord injury is not yet available. 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 of bone marrow stromal cells in cord recovery as well as its potential for neuronal transdifferentiation was studied. Olfactory ensheathing cells provide guidance and structural conduit for axonal growth. The mesenchymal stem cells provide matrix and local growth factors as well as myelinate the injured fibers thereby facilitate regeneration. On the basis of these data, a phase-II project was initiated. Since the different cell types facilitate spinal cord recovery through different mechanisms, the main objectives in this study is to explore the efficacy of transplantation of different cell combinations in rat models of spinal cord injury. Cells cultured from rat olfactory mucosa and the cells from the bone marrow stromal cells will be characterized. Further the role of chondroitinase enzyme inhibiting the glial scar to support the axonal regeneration is also being explored. Painless magnetic cortical stimulator to study the regeneration of corticospinal tract is being developed. Outcome of the transplantation were evaluated by BBB score, motor evoked potential studies and by histological methods.

In dose response relationship, 5 lakh transplantation appear to be promising in comparison to 2 lakh and more than 10 lakh cells. The mean BBB score injected with Chondroitinase, OEC, and MSC was 7.1, 5.9, and 4.1 respectively. In summary chondroitinase, OEC, and MSC has moderate therapeutic effects following rat spinal cord injury.

Manpower:

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

Awards: Not applicable.

Publications in 2011-12:

- Motor recovery following olfactory ensheathing cell transplantation in rats of spinal cord injury. Neurology India 2011: 59; 566-57.

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.

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

RESEARCH PROGRAM: ROLE OF THE STEM CELL NICHE IN DEVELOPMENT OF DISEASE

Stem cells receive signals from the surrounding environment to self- renew or to differentiate for tissue regeneration during homeostasis and tissue injury. Dysregulation of these signals can lead to uncontrolled activation of stem cells leading to various disease conditions including cancer. The focus of our lab is to study these processes in two biological systems relevant to human disease. The first is the gastrointestinal system, where stem cell location and lineage tracing can be performed and the second is the blood system, where functional analysis of stem cells can be carried out.

- **Role of the stem cell niche in development of Ulcerative Colitis (IBD)**

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 serve as a reservoir of stem cells to replenish cycling cells upon tissue injury. Tissue injury associated with chronic inflammation is a hallmark for inflammatory bowel diseases like Ulcerative colitis (UC). Compelling evidence suggests that epithelial abnormalities such as depletion of goblet cells are among the central defects underlying development of UC. Identification of the local or niche factors responsible for such abnormalities in the epithelium may contribute to understanding of the aetio- pathogenesis of this disease. Our lab is interested in dissecting the role of niche signals emanating from the surrounding stroma in the aetio-pathogenesis of Ulcerative colitis. For this study, tissue from both an animal model of colitis and human biopsy/surgical tissues from ulcerative colitis (UC) patients will be obtained.

- Identification of different compartments of colonic epithelium and their surrounding niche using cell surface markers. In the animal model different compartment of surrounding niche and colonic epithelium will be identified by immune-histochemistry (IHC) and quantitated by flow cytometry. Various niche components will also be identified by IHC in human biopsy specimens.
- Isolation of different cellular components by flow cytometry and its confirmation by gene expression analysis.

Standardization of the assays for epithelial components and flow analysis is currently ongoing.

- **Role of the stem celllniche in development of myelodysplastic syndrome (MDS)**

Myelodysplastic syndromes are a heterogeneous group of diseases characterized by ineffective hematopoiesis with the propensity of leukemic transformation. Though the mechanisms behind the role of the niche cells in disease evolution are poorly defined, delineation of the hematopoietic stem cell niche and the ability to interrogate its role in hematopoietic disease in animal models have advanced our insights into these processes in recent years. Our lab is interested in dissecting the role of niche signals emanating from the surrounding stroma and characterizing the hematopoietic stem cell phenotype and function in MDS. Experiments are planned to be carried out in bone marrow aspirates and trephine biopsies.

- Phenotypic characterization of hematopoietic stem and progenitor cells by flow cytometry in bone marrow (BM) aspirates from MDS patients. Initial studies involved standardization of the detection of hematopoietic stem and progenitor cells (HSPCs) from BM aspirates.
- Localization of hematopoietic stem and progenitor cells in relation to their niche components. Initial studies involved stand ardization of the detection of niche and HSPCs from BM trephines.

LABORATORY HIGHLIGHTS OF YEAR 2012-13.

Publications

Venkatraman A, He XC, Thorvaldsen JL, Sugimura R, Perry JM et al. Maternal imprinting at the H19-Igf2 locus maintains adult haematopoietic stem cell quiescence. Nature. 2013;500:345-349. Featured in: Goodell MA. Parental Permissions: H19 and Keeping the Stem Cell Progeny under Control. Cell Stem Cell.2013;13:137-138.

HONORS AND AWARDS FOR ALL TEAM MEMBERS INCLUDING STUDENTS AND POSTDOCS-

2013 ISSCR Travel award to attend 11th Annual Meeting in Boston, USA.

INVITED TALKS BY MEMBERS OF THE THEME- (0)

LIST OF TEAM MEMBERS (POSTDOCS, STUDENTS, JRFS, SHORT-TERM TRAINEES, OTHERS)

JRF-1

Short term trainee 1

COLLABORATORS

MDS project

Alok Srivastava, MD, Department of Hematology and CSCR, CMC, Vellore

Marie Theresa, MD, Department of Pathology, CMC, Vellore

Core Facilities and Instrumentation:

CSCR Core Facilities:

The Core Facilities at CSCR host state-of-the art instrumentation to aid researchers both within and outside CSCR. The Core Facilities provide expertise in sample processing and analysis and also help in experiment design. All facilities are accessible to not only scientists working full time at CSCR but also to all other scientists in CMC, Vellore who require these technologies / platforms for their work.

Molecular Biology Core Facility:

- Faculty In-Charge: **Dr. R.V. Shaji, PhD.**
- Technical Officer: **Mr. Vaidyanathan. S.**
- Technical Staff: **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). The facility currently has a 3130 4-capillary DNA sequencer from Applied Biosystems, an ABI 7500 Real-time PCR machine and an Applied Biosystems QuantStudio 12K Flex Real-time PCR for high throughput analysis.

Radioactivity Core Facility

The Radioactivity Core Facility provides researchers a secure access to radiolabelled isotopes and instrumentation for detecting radioactivity. The facility currently has Greiger counters, GE Storm 365 Phosphor imager and a Perkin Elmer Tricarb Liquid Scintillation Counter.

Many departments from CMC, Vellore and outside use this core facility extensively. The molecular biology core also aims to collaborate with people outside CSCR to share expertise and knowledge on platform development and augmentation.

Flow Cytometry Core Facility:

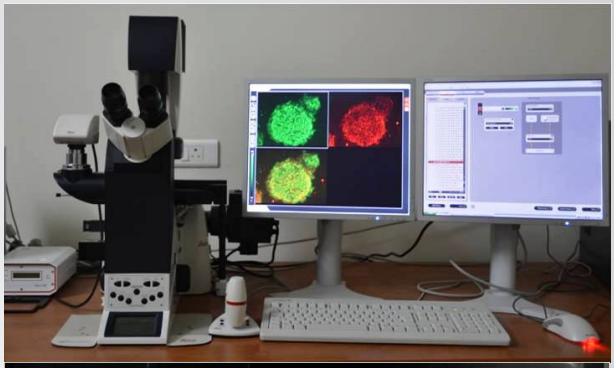
- Faculty In-Charge : **Dr. Sanjay Kumar, PhD.**
- Technical Officer : **Mr. Vaidyanathan. S.**
- Technical staff : **Ms. J. Saranya.**

Flow cytometry is a pivotal tool in cell biology. Many intra and extra-cellular parameters can be analyzed and statistically evaluated with high speed and precision. The Flow Cytometry Core Facility currently houses a BD FACSAria cell sorter for sorting applications and BD FACSCalibur cell analyzer for analysis. The BD FACSAria has a throughput of 70,000 events per second and can do 4-way sorting using a 3 laser 9 colour configuration. The BD FACSCalibur has a 2 laser 4 colour system and is routinely used for cell analysis by scientists both within CSCR and CMC, Vellore.

The Flow Cytometry core aims to conduct regular workshops in flow sorting and cell analysis for human resource development in flow cytometry and provides support to various departments in selecting antibody panels, experiment design and post-acquisition data analysis.

Imaging Core Facility:

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



I. Leica DMI6000B Inverted Fluorescence Microscope

The Leica DMI6000B is an inverted fluorescence microscope comprising of 6 interchangeable filters for detecting various fluorochromes and independent cameras for phase contrast and fluorescence imaging. It is also equipped with a fluorescence intensity manager and programmable function keys for easy access to functions.

II. Leica Light Microscopes

Leica DMIL (upright) and Leica DMI1000 (inverted) are available for users to perform routine light microscopy imaging.

III. Zeiss Inverted Fluoresence Microscope

A Carl Ziess Axiovert 40 CFL equipped with 3 filters for routine flouresence imaging is avaiable along with a ProgRes C3 camera module for acquisition.

IV. Laser scanning confocal microscope system (Olympus FV1000).

The Olympus FV1000 confocal system comprises a motorized microscope with z focus drift compensation facility for bright field, differential interference contrast and fluorescence imaging with motorized XY scanning stage and CO2 incubation facility. It is equipped with the following lasers - 405nm, Multi-Argon (458nm, 488nm and 515nm), 559nm and 635nm. Apart from regular confocal imaging, this microscope can perform Multi-Area Time Lapse, FRET, FRAP and FLIM experiments.

V. Laser scanning multi photon microscope (Olympus FV1000MPE).

The FV1000MPE is an upright multiphoton laser-scanning microscope coupled with Mai Tai HP-Deep See –OL laser with automated broadband wavelengths tuning from 690 to 1040nm.

Training Sessions

The Imaging Core Facility conducts training sessions regularly for both first time and experienced users. The sessions are conducted by application specialists from Leica and Olympus along with the imaging core personnel. This year, 2 sessions for the Olympus FV1000 confocal microscope and 3 sessions for the Leica DMI6000B were conducted.

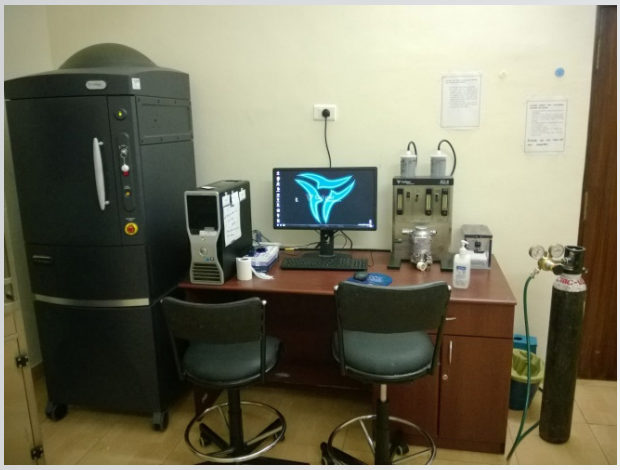
III. Histopathology Core Facility:

- Faculty In-Charge: **Dr. Rekha Samuel, MD.**
- TechnicalStaff : **Mrs. Esther Rani, DMLT and Mr. Satish Perumal , DMLT**

Special stains standardized in 2012-2013:

I. Histology Special Stains:
Alcian Blue, Perl's Prussian Blue, Periodic Acid Schiff, Masson Trichrome, Gordon Sweet Reticulin, Acid Fast Bacillus stain for Mycobacterium Tuberculosis, Toluidine Blue, Masson Fontana, and Verhoeff's elastic stain.

II. Cytology:
Cell block preparation.



In vivo Small Animal Imaging System (PerkinElmer Ivis Spectrum CT)

- Faculty In-Charge : **Dr. Sanjay Kumar, Ph.D.**
- Scientific Officer : **Dr. Prateesh M.D., M.VSc, Ph.D**

The Ivis Spectrum CT supports low dose microCT for longitudinal imaging. It features 3D optical tomography for fluorescence and bioluminescence and has sensitive detection for real time distribution studies for both fluorochromes and PET tracers.

CSCR Laboratory Animal Facility:

- Faculty In-Charge: **Dr. Aparna Venkatraman, PhD**
- Scientific Officer: **Dr. M.D Pratheesh. M.V.Sc ,PhD**
- Technical Staff: **Esther Rani , P. Sathish and 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.

Objective

The main objective of the CSCR-Laboratory Animal Facility is to breed, maintain and supply quality laboratory animals to the scientific community of the centre, as per the sanction from the Institutional Animal Ethics Committee (IAEC). The laboratory animal facility is registered with the 'Committee for the Purpose of Control and Supervision of Experiments on Animals' (CPCSEA) for breeding and conducting experiment on small laboratory animals vide registration no. (Reg. No.88/1999/CPCSEA) dt.April 28, 1999. All activities and protocols of the CSCR-LAF were carried out as per standard operating procedures (SOPs) approved by institutional animal care and use committee (IACUC).

Infrastructure

The CSCR Laboratory Animal Facility (CSCR-LAF) is located in the basement of the CSCR 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. Animals are maintained within individually ventilated microisolator caging system for breeding, holding and experimentation. Temperature and relative humidity of the animal rooms were maintained between 20 to 25 °C and 30 to 70% respectively throughout the year. All the environmental factors were monitored round the clock through individual room sensors. Photoperiod of 12 hrs light and 12 hrs dark maintained with automatic timers. Light intensity (300 lux) and noise level (< 85db) maintained as per CPCSEA regulations. All animal house records were updated and maintained everyday. Ad libitum supply of UV treated autoclaved R.O water and irradiated commercial diets were given to animals.

Strains

The CSCR-LAF maintains nine different strains of mice - including knock out and SCID strains and 1 strain of rats. The majority of rodent strains are bred under strictly inbred conditions.

	Strain	Description	Disease Model	Source	User frequency
1	C57BL/6J	Inbred strain	Multi- Purpose model	Jax Lab, US	Very High
2	BALB c/J mice	Inbred strain	Inbred strain	Jax Lab, US	Low
3	FVB/NCrl mice	Inbred strain	Mouse leukemia model	Charles River, UK	High
4	CD-1	Out bred strain	Sentinel animals, Pseudopregnancy	Charles River, UK	Low
5	B6.129S4-F8tm-1Kaz/J	Mutant Stock; Targeted Mutation	Hemophilia A	Jax Lab, US	Very High
6	B6.129P2-F9tm-1Dws/J	Congenic; Mutant Strain	Hemophilia B	Jax Lab, US	Very High
7	B6;129S4 Pou5f1tm1Jae/J.	Mutant Stock; Targeted Mutation	OCT-GFP model	Jax Lab, US	Low
8	B6.CB17-Prkdcs-cid/SzJ	SCID	Transplantation studies	Jax Lab, US	Very High
9	C.B-17/Icr-Prkd-c<Scid>IcrIcoCrl	SCID	Xeno Graft Research	Charles River, UK	Very High
10	Sprague Dawley	Rat- Outbred strain	Orthopedic surgery	Jax Lab, US	High

Quality control

Routine sentinel animal sampling was done in every three months to ensure the health status of breeding and experimental animals stock. Skin and hair were checked for ectoparasites. Fecal samples were checked for the endoparasites by sedimentation method. Microbiological examination of animal room air, animal feed, water, bedding material, fecal samples and throat swabs were also carried out periodically. Blood samples of sentinel animals were checked for mycoplasma pulmonis by PCR method.

Collaboration
CSCR-LAF signed MOU with Apt Genetics (Hu-murine) for the generation and supply of humanized mouse models on commercial basis.

Protocols established -2012-13
SOP's for mouse liver perfusion and Retro orbital injection were established.

Training-2012-13
Dr. Pratheesh attended Mouse Embryology Symposium, conducted at InStem, Bangalore, 10-12 March 2013.

CSCR current Good Manufacturing Practice (cGMP) Facility

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

The CSCR cGMP facility is committed to provide high quality service to all the cell therapy developers across the country seeking assistance in the manufacture or the supply of clinical grade cells (currently Mesenchymal Stromal Cells (MSC)) for various clinical trials. We have experienced personnel who can help cell therapy developers at all stages, from tissue sourcing, improving culture processes & test procedures to implementing cost effective product manufacture for both autologous and allogeneic cellular therapies.

The Cell Processing Unit (cGMP Facility), under the supervision of Dr. Vikram Mathews, is currently involved in the large scale production of Clinical Grade Human Bone Marrow and Placenta derived MSC, 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 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 55 bone marrow samples (total yield of ~ 5.8 billion MSC) and 7 placenta samples (total yield of ~1.7 billion MSC) have been processed for clinical grade MSC expansion in vitro. During this period 23 patients with steroid refractory aGvHD have been treated with MSC infusion as part of a clinical trial.

Since June 2012 around 5 Bone Marrow samples were processed with an average yield of 100 million MSC per sample. Further 3 placenta samples were processed, with an average yield of 400 million MSC 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.

We are also involved in an ongoing work headed by Dr. Sukria Nayak (Department of Surgery, CMC, Vellore) titled "A prospective, interventional, Phase 2 trial to evaluate the use of mesenchymal stromal cells (MSC) in the treatment of recurrent, complex fistula-in-ano".
In addition to MSC expansion, the cGMP facility was also involved in the culture and expansion of autologous chondrocytes for a 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)

Milestones and Achievements 2008- 2012

2008

- Scientific work was initiated at CSCR

2009

- 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.

2010

- Work initiated towards developing engineered AAV vectors for gene transfer based on the previous work done at University of Florida, USA.
    - 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)

2011

- Infrastructure Development
    - Establishment of Vector Core facility
    - In vivo imaging facility
    - Small Animal Imaging

2012

- Infrastructure Development
    - Nano Technology Facility (Basic)

2013

- MOA with AptGenetics Pvt. Ltd (India) and HuMurine Technologies (USA) to establish a humanized mice facility at CSCR.
- Development of a clinical gene therapy program

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. Two students registered for PhD in 2013-2014

II. Other training programs:

- **Short term student projects (Bi-annual)**

SHORT TERM STUDENTS

S. No	Name	Duration	Qualification	University	Project title	PI /Lab
1.	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
2.	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
3.	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
4.	Mr. Ijaz Mohammed Abdulla	Dec 12 – May 13	M.Sc Biotech (Integrated)	VIT University, Vellore.	Gene therapy for hemophilia A	Dr. Jayandharan / Lab-4
5.	Ms. Rohini K Murthy	Dec 12 – May 13	B.Tech Bioengineering	SASTRA University, Thanjavur	Gene therapy for hemophilia B	Dr. Jayandharan / Lab-4
6.	Ms. Kayal Vizhi	Feb 13 -Apr 13	M.Sc - Biotech	Thiruvalluvar University	Cloning of HSV-TK Gene in AAV Vector	Dr. Jayandharan / Lab-4
7.	Ms. Uma. S	Feb 13 -Apr 13	M.Sc - Biotech	Thiruvalluvar University	Gene Expression Analysis in Human Erythroid Cells	Dr. Shaji / Lab -2
8.	Ms. Sarala. V	Feb 13 -Apr 13	M.Sc - Biotech	Thiruvalluvar University	Gene Extraction Analysis of Human Umbilical Cord derived Mesenchymal Stem Cell	Dr. Sanjay/ Lab- 3
9.	Ms. Deepa. P	Feb 13 -Apr 13	M.Sc - Biotech	Thiruvalluvar University	Gene Extraction Analysis of Human Placenta Derived Mesenchymal Stem Cell	Dr. Sanjay/ Lab- 3
10.	Ms. Shivangi Srivastava	May 13 - Jun 13	M.Sc - Marine Biotech	Goa University	Generation of fluorescently labeled human primary cells for in-vitro and in-vivo applications	Dr. Shaji/ Lab-2
11.	Ms. Pratheppa	Aug 13 - Apr 13	M.Tech - Genetic Engine.	SRM University	AAV Vectors with Immune escape phenotype	Dr. Jayandharan / Lab-4
12.	Mr. Apurv Jain	Jun 13 - Nov13	M.Pharm - Pharmacology	JSS College of Pharmacy.	Gene Therapy for Haemaophilia	Dr. Jayandharan / Lab-4
13.	Mr. Santhosh. A.S	Aug 13 - Apr 13	M.Tech - Genetic Engine.	SRM University	Immune response to AAV Vectors	Dr. Jayandharan / Lab-4

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

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. A DBT designated Scientific Advisory Committee reviews 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.

Governing Council of inStem

Dr. Dr. K. Vijay Raghavan	Secretary, DBT, New Delhi	Chairman
Dr. Satyajit Mayor	Director, inStem Bengaluru	Member
Dr. S. Ramaswamy	Dean, inStem, Bengaluru	Member
Dr. Jyotsna Dhawan	Dean, inStem, Bengaluru	Member
Dr. Satyajith Rath	NII, New Delhi	Member
Dr. Chandrima Shaha	Director, NII, New Delhi	Member
Dr. K. Muniyappa	IISc, Bengaluru	Member
Dr. Chittaranjan Yajnik	Director, KEM Hospital, Pune	Member
Dr. Sunil T Chandy	Director, CMC, Vellore	Member
Dr. Alok Srivastava	Head, CSCR, CMC, Vellore	Member
Mrs. Anuradha Mitra	JS & FA, DBT, New Delhi	Member
Dr. T.S. Rao	Adviser, DBT, New Delhi	Member
Dr. Alka Sharma	Joint Director, DBT, New Delhi	Member
Mr. T.M. Sahadevan	Head, A & F, inStem, Bengaluru	Non Member Secretary

The following are the committee at CSCR Committees

Dr. Sunil Thomas Chandy	Director, Christian Medical College	Chairperson
Dr. Satyajit Mayor	Director inStem, Bengaluru	Member
Dr. S. Ramaswamy	Dean, inStem, Bengaluru	Member
Dr Jyotsna Dhawan	Dean inStem, Bengaluru	Member
Dr.Alfred Job Daniel	Principal, Christian Medical College	Member
Dr Alok Srivastava	Head CSCR	Member Secretary

CSCR Sub Committee (Finance)

Prof. VijayRaghavan,	Director inStem	Chairperson
Ms. Anuradha Mitra,	J.S. & F.A, DBT	Member
Prof. S. Ramaswamy /Prof Jyotsna Dhawan,	Deans inStem	Member
Dr. Sunil Thomas Chandy,	Director, Christian Medical College	Member
Dr. Thomas Kuriakose,	Associate Director (Finance), Christian Medical College	Member
Dr. T.S Rao, Advisor,	DBT	Member
Dr. Alka Sharma,	Director DBT	Member
Dr. Alok Srivastava	Head CSCR	Member Secretary

In addition, CMC, Vellore has established two further committees to assist in the management of CSCR and provide an interface with CMC administration:

Core Committee:

The Core Committee which has been appointed by the Principal, CMC, Vellore and consists of upto 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. This currently consists of the following:

Dr Molly Jacob	Member
Dr Vikram Mathews	Member
Head CSCR	Convener

Steering committee:

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."

Dr. Sunil Thomas Chandy	Director CMC	Chairperson
Dr. Alfred Job Daniel	Principal, CMC Vellore	Member
Dr. Thomas Kuriakose	Associate Dir – Finance	Member
Dr. Anil K Kuruvilla	Associate Dir-General Admin	Member

Dr. D.J. Christopher	Associate Dir.HR	Member
Dr. C.E. Eapen	Medical Superintendent	Member
Mr. Denzil Ranjith Singh	Treasurer	Member
Dr. R Selvakumar	General Superintendent	Member
Dr. Nihal Thomas Addi.	Vice Principal (Research)	Member
Dr. Molly Jacob	Member Core committee	Member
Dr. Vikram Mathews	Member Core committee	Member
Dr Alok Srivastava	Head CSCR	Member Secretary

Personnel:

Scientist:

Dr. Sanjay Kumar	Ramalingaswami Fellow	Scientist
Dr. Murugan Ramalingam	Associate Professor, Centre for Stem Cell Research	Scientist
Dr. Vrisha Madhuri	Professor, Department of Paediatric Orthopaedics	Adjunct Scientist
Dr. Jayandharan. G. Rao	Associate Professor, Department of Haematology	Adjunct Scientist
Dr. Rekha Samuel	Professor, Centre for Stem Cell Research	Adjunct Scientist
Dr. R.V. Shaji	Professor, Department of Haematology	Adjunct Scientist
Dr. Alok Srivastava	Professor, Centre for Stem Cell Research	Adjunct Scientist
Dr. Aparna Venkatraman	Associate Professor, Centre for Stem Cell Research	Adjunct Scientist
Dr. Vikram Mathews	Professor, Department of Haematology	Adjunct Scientist
Dr. Eunice Sindhuvi	Lecturer, Department of Haematology	Adjunct Scientist
Dr. B. Poonkuzhali	Professor, Department of Haematology	Adjunct Scientist
Dr. Ari Chacko	Professor, Department of Neurosurgery	Adjunct Scientist
Dr. Thomas Kuriakose	Professor, Department of Opthalmology	Adjunct Scientist
Dr. Indrani Sen	Assistant Prof, Department of Vascular Surgery	Adjunct Scientist
Dr. George Tharion	Professor, Department of Physical Medicine & Rehabilitation	Adjunct Scientist

Post Doctoral fellows

Dr. Dwaipayan Sen	Post Doctoral Fellow
Dr. Ruchita Selot	Post Doctoral Fellow
Dr. Sabna. C	Post Doctoral Fellow

Scientific and Technical Officers

Dr. Pratheesh. M.D.	Scientific Officer
Mr. Augustine Thambiah	Technical Officer
Mr. Vaidyanathan Subramaniam	Technical Officer

Research Fellows

Ms. Sangeetha Hareendran	SRF
Mr. Salar Abbas	SRF
Mr. Syed Mohammad Musheer Aalam	SRF
Mr. Vikram Sabapathy	SRF
Mr. Nishanth Gabriel	SRF
Ms. E. Sumitha	SRF
Mr. Balaji	JRF
Ms. P.B. Sumitha	SRF
Mr. Kannan Thoopil	JRF
Mr. Thyagarajan	JRF
Mr. Janakiraman	JRF
Ms. Nancy	ARO
Mr. S. Balasubramanian	JRF
Ms. Akshaya Krishnagopal	JRF
Ms. Archana Kini	JRF
Ms. Elizabeth Jayex Panakkal	JRF
Mr. Karthikeyan. R	SRF
Ms. Swomya	JRF
Ms. Mona. S	JRF
Mr. David Livingston	Project Assistant

Technical staff

Ms. Aleya Tabasum	Graduate Tech.	-
Mr. P. Sathish	Technician	-
Ms. J. Esther Rani	Technician	-
Ms. Dhavapriya	Graduate Tech	On Project
Ms. G. Kalaivani	Graduate Tech	On Project
Ms. R. Saranya	Graduate Tech	On Project
Ms. J. Saranya	Graduate Tech	-
Ms. R. Pavithra	Graduate Tech	-
Ms. Chitra Premkumar	Graduate Tech	On Project
Ms. Saranya Rajendran,	Graduate Tech	On Project

Annual Report
2012 - 2013

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