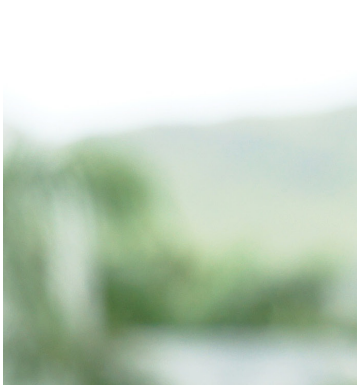
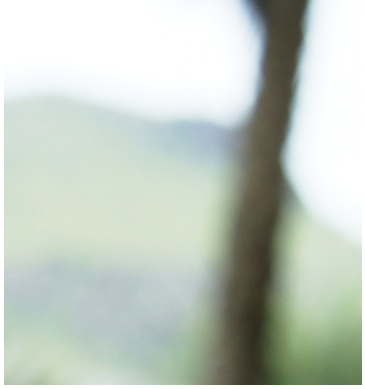
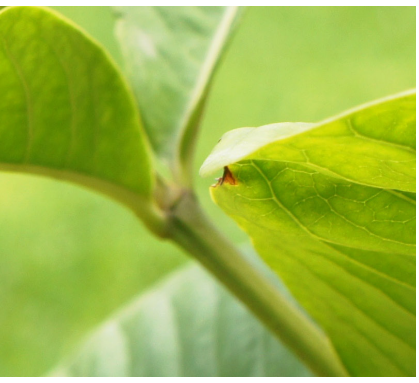
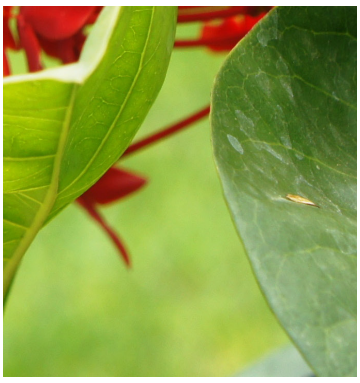







ANNUAL REPORT

2013 - 2014

inStem

CENTRE FOR STEM CELL RESEARCH

(A unit of inStem,Bengaluru)

Christian Medical College Campus,

Bagayam, Vellore-632002

Table of content

INTRODUCTION

Centre for Stem Cell Research (CSCR), (a unit of inStem, Bengaluru)
Christian Medical College Campus, Bagayam, Vellore
The Beginnings …….2005-2010

The establishment of the Center for Stem Cell Research (CSCR) in Vellore was sanctioned 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 later 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 July, 2011, CSCR is integrated with inStem with focus on translation research and clinical studies with stem cells and other novel therapies.

MANDATE

The mandate of the Centre for Stem Cell Research is to bring stem cell science and other innovative approaches to management of patients with unmet needs. This is to be done by developing science which will help enhance understanding of disease biology or develop innovative therapies. This is to 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 doctoral programs as well as 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 FROM 2011

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 by 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 1st July, 2011. It continues to function at the Bagayam campus of CMC, Vellore with its emphasis on translation stem cell research and regenerative medicine. Efforts are directed at developing thematic programs and greater interactions through collaborative work between scientists at Bengaluru and Vellore as well as other institutions and scientists in India and overseas. It is now governed by a CSCR committee chaired by the Director, CMC and includes the Director and Deans of inStem. It also has a finance subcommittee which is part of finance committee of inStem both of which report to the inStem Governing Body, chaired by the Secretary, DBT. Given the predominantly translational nature of the research at CSCR, it also has a separate Scientific Advisory Committee.

CORE SCIENTIFIC ACTIVITIES AND INITIATIVES: 2013 -2014

As mentioned in the report last year, emphasis continues on developing thematic research programs that are targeted at specific medical problems. These include the gene therapy program, using viral vectors - AAV and lentiviral for hemophilia and thalassemia, a musculoskeletal regenerative medicine program addressing different aspects of repair of correction of bone and cartilage defects in children, a stem cell niche biology program applied to the bone marrow and gastro-intestinal niches and a vascular biology program directed primarily at models to evaluate the vascular changes in type II diabetes mellitus. Details are in the reports that follow.

A truly innovative program that is starting clinical trial is the use of long scaffolds loaded with mesenchymal stromal cells for bone grafting in children. This will be one of the first such studies in the world for long bone defects. This is a collaboration between CMC, Vellore, CSCR and SCTIMS, Thiruvananthapuram. Another program that appears close to clinical translation is the AAV vector based gene therapy program with an active collaboration between scientists at University of Florida, USA for the GMP technology, an industrial partner to scale-up, efficacy and toxicity studies at CSCR combined with the clinical expertise at CMC, Vellore for the trials. Another area that is developing towards translation over the last year is related to applications of induced pluripotent stem (iPS) cells. India, through the DBT, is part of an international consortium involving the development of a 'haplobank' – a bank of HLA haplotype homozygous individuals which could then be used to generate histocompatible cells /tissues for organ regeneration. Details are in the scientific write-ups. A further area of development is tissue engineering – both for flat organs such as skin and other external structures and hollow organs such as urogenital and intestinal tracts. This is however at a very nascent stage at present. Other individual scientific projects do cover a variety of translational goals. These include applications of mesenchymal stromal cells of different origins. Several clinical problems are also being evaluated in animal models. A few more early clinical trials with stem cells are also being undertaken utilizing the cell processing facility.

The core facilities are supporting scientific activities not only within CSCR but also for several scientists from CMC, Vellore. Scientists from more nearly 15 departments in CMC use the molecular biology and flowcytometry facilities at CSCR as also several other institutions from Vellore and outside.

PhD programs exist at CSCR through the Sri Chitra Thirunal Institute of Medical Sciences, Thiruvananthapuram and the Thiruvalluvar University, Vellore. Short term training programs are also offered to MSc students from different universities.

CSCR is attempting to fulfill the mandate for which it was created.

Alok Srivastava

TRANSLATIONAL STEM CELL RESEARCH AND REGENERATIVE MEDICINE PROGRAMS:

1. Gene therapy program: (**Group: At CSCR** – Alok Srivastava, RV Shaji, (Jayandharan G R); **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 disease applications – hemophilia and thalassemia major. The two major sub-themes of this program are:

A. HEMOPHILIA - USING ADENO-ASSOCIATED VIRUS (AAV) VECTORS –

Other Collaborators: Dr. Amit Nathwani, UCL, UK; Dr. Himanshu Gadgil, Intas Pharmaceuticals Industry partner in India. The clinical program will be developed with involvement of several colleagues from the departments of Haematology and Immunohematology and Transfusion Medicine in CMC, Vellore.

Vector Biology and Pre-clinical studies: This area of work was led by Dr. G. Jayandharan, who had developed several novel AAV based delivery systems by a through novel capsid modifications for the different AAV serotypes. These capsid modified forms were aimed at enhancing their transduction efficiency and reducing their immunogenicity. Early work done in some with some of these vectors showed promising results in animal models. However, as of October, 2014, Dr. Jayandharan left CMC, Vellore and CSCR and has closed his laboratory here. This part of the work now is therefore being done in collaboration with Dr. Arun Srivastava's laboratory in University of Florida, USA. Further details of this program are in Dr. Alok Srivastava's report.

B. LENTI VIRAL VECTORS FOR THALASSEMIA GENE THERAPY –

Other collaborators: Trent Spencer, Emory University, USA and Dr. Arun Srivastava, UFL.

The collaboration with Dr. Trent Spencer at Emory University continues. Several constructs of the lenti viral vectors have been prepared in Emory and evaluated by Dr. R. V. Shaji in CSCR. Optimization of the vector remains a goal. While this approach requires exvivo transduction of hematopoietic stem cells and autologous transduced HSC transplantation, we are also exploring the possibility of AAV mediated direct gene transfer to HSC in collaboration with Dr. Arun Srivastava.

This is another area where several clinical trials are ongoing in the world. Updated data presented at the ASH meeting in December showed improved results with 5-6gm elevation of hemoglobin in 3-6 months after transplant. A genome editing based approach to gene correction in hemoglobin disorders is also being developed by other groups.

2. Musculoskeletal Regeneration Program – **(Group: At CSCR** – Dr. Vrisha Madhuri, Dr. Sanjay Kumar, Dr. Vikram Mathews, Dr. Alok Srivastava; Dr. Shaji RV, Dr. Pratheesh, Dr. Soundararajan **in CMC, Vellore**- Noel M Walter, Dr. Sanjay K Chilbule, Dr Abhay Gahukambale, Dr. Sridhar Gibikote Dr. Albert Abhinay Kota, Dr. Sukria Nayak, Dr. Nitin S Kekre, Dr. Jessie Lionel, Dr. Lilly Varghese **Other collaborators – (National)** Dr. Prabha D. Nair, Dr. H K Varma and Dr. Annie John, from SCTIMST, Trivandrum. Dr. Dhirendra S. Katti and Dr. Amitabha Bhandhopadyay, IIT Kanpur, **(International)**, and Dr. Henrik Daa Schroder, Aarhus University, Denmark, Dr. Jorgen Kjems, Aarhus University, Denmark, Dr. Lars Savendahl, Karolinska Institute, Sweden.

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 led by Dr. Vrisha Madhuri at CSCR and CMC, Vellore with several collaborators in CSCR and CMC along with many external collaborators. This group's focus is on musculoskeletal regeneration with the major targets being articular and physeal cartilage replacement, bone and muscle regeneration in different clinical conditions with otherwise limited therapeutic options.

Bone regeneration – Patient recruitment for phase 1 trial on the treatment of large bone defects (gap non-union) in human using hydroxyapatite scaffold loaded with Mesenchymal stem cells differentiated to osteogenic lineage is initiated after approvals from DBT, CTRI and DCGA.

Physeal cartilage regeneration – Study for treatment of physeal growth arrest in children using autologous chondrocyte transplantation has been completed with remarkable success. Large animal study on physeal replacement using chondrocytes seeded over gel scaffold is complete which will now be taken up for human translation.

Growth modulation –Modality of shock waves is being assessed for their effect over the growth plate with possible therapeutic implications for management of deformities and length related issues. Interim analysis of the ex vivo effect of shock waves in an ex-vivo model of cultured rat metatarsal bones has led us to start further studies over effect of shock waves on growth plate cartilage and bone growth in young rabbits and in vitro effect over human growth plate chondrocytes and MSCs.

Articular cartilage- Large animal study is being conducted to regenerate the articular cartilage over the goat hip using autologous MSCs seeded over the electrospun scaffold. 3D multilayered composite scaffold is being evaluated in vitro and in vivo over rabbit knee model using MSCs for the regeneration of articular cartilage.

Muscle regeneration- After successful isolation and characterisation of the muscle satellite cells from human donors, we plan to use them for the regeneration of muscle sphincters for management of stool and urine incontinence. Vascular interface of the satellite cells is being evaluated at our lab by co culture with endothelial cells.

Ongoing studies include long term follow-up children treated for physeal defects as well as a new large animal model for evaluation of a novel combination of polycaprolactone and CHDA scaffold for treating articular cartilage defect on the femoral head after obtaining final regulatory approvals. Another area of evolving work is with human muscle satellite cell isolation, culture and characterisation. We have developed the GMP protocols for the same and have also planned for studies on sphincter muscle regeneration (urinary continence, fecal incontinence).

3. Stem cell niche program – Bone marrow niche: At CSCR – Aparna Venkatraman, 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). **Gastrointestinal tract niche: At CSCR** - Aparna Venkatraman, In CMC, Vellore – Ebby Simon, Anna Pulimood and Anup Ramachandran (GI Sciences).

This program evaluates the niche in two organs – bone marrow and the gastrointestinal tract and attempts to correlate alterations in the niche with disease processes.

1. Niche in bone marrow failure – Myelodysplastic syndromes (MDSs) represent clonal disorders that are characterized by bone marrow failure leading to ineffective hematopoiesis and an increased risk for malignancy. Increasing evidence suggest pathogenesis of MDS might emerge from accumulation and selection of specific genetic or epigenetic events which could modulate both hematopoietic stem/progenitor (HSPCs) cells and their surrounding microenvironment. Further more aberrant interactions of bone marrow stroma and HSPC can also contribute to its associated pathologies. Nearly 25% of patients with MDS in India are below 30 years of age with many in the pediatric age group which is different from reports from the Western countries. In this part of the program, samples from patients with a range of severities of the condition were included to initially screen for defects in de novo HSC and its surrounding niche components. In parallel, phenotypic characterization and differentiation abilities of cultured mesenchymal stem cells were also evaluated.

Our preliminary screening and phenotypic enumeration of bone marrow aspirates by flow cytometry revealed aberrant frequency of HSPCs, mesenchymal and endothelial cells. Qualitative analysis by confocal microscopy further confirmed these changes. Mechanistically, activation of *wnt*

signaling in vessels and stem cells were evident in marrow trephine biopsies. In vitro mesenchymal stem cell analysis of low and high risk MDS samples showed similar growth kinetics, phenotypic enumeration and tri-lineage differentiation ability when compared with MSC from control patients. Cell cycle and apoptotic patterns were also comparable with control.

2. Niche in Inflammatory bowel disease –The second part of this program, is the assessment of the gastrointestinal stem cell niche in Ulcerative Colitis (UC), a form of inflammatory bowel disease (IBD).Ulcerative colitis (UC) is a chronic, relapsing inflammatory disease with complex etiology. In the past decades, the incidence of UC, which was generally considered uncommon in India, is showing a steep increase. This, combined with the increased rate of discordance in monozygotic twins is suggestive of causative factors independent of the genetic code. Although several lines of evidence have given insight into later events in pathogenesis, early events which initiate the disease are poorly understood. Alterations in notch signaling, loss of goblet cells, Paneth cell metaplasia and development of cancer in long-standing UC implicate a stem cell defect in the disease. An increased expression of *wnt* ligands in colonic myo-fibroblasts at sites away from inflammation in patients suggests involvement of the surrounding mesenchyme in disease etio-pathogenesis. Hence in this program we plan to systematically investigate alterations in crypt and mesenchymal sub-compartments in patient samples. Our ongoing studies reveal defects in colonic stem cell, cell cycle and differentiation at sites independent of inflammation, which are unique to UC. Temporal analysis of the epithelial compartment in an animal model of colitis revealed that these defects are associated with aberrant notch and wnt signaling. To gain mechanistic insight we propose to investigate these changes using transgenic mice. These finding are of clinical relevance, since current therapy which mainly targets the inflammatory component is not curative and significant number of patients are refractory to therapy. Thus, a more comprehensive understanding of colonic stem cell-niche biology in the context of but not limited to, Ulcerative Colitis, could translate into novel therapeutic strategies for epithelial regeneration.

4.Vascular Biology Program – Group: CSCR -: Rekha Samuel, Sanjay Kumar; **CMC Vellore**: Jiji Elizabeth Mathews and Santhosh Benjamin, (Obstetrics and Gynecology) and MS Seshadri (Professor and retired Head, 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); **Others**: 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 (Indian Institute of Technology, IIT, Madras and 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. The major focus of the program involves dissecting the pathophysiology and molecular mechanisms of Diabetic microvasculopathy using in vivo imaging and Severe Combined Immunodeficient murine models. Using placental hyperglycemia as a model to extrapolate vascular defects of Type 2 diabetes (T2D), we also examine the blood placental barrier using ultrastructural studies, in vitro and in vivo murine models. Other areas of interest of the lab include exploring signaling pathways that influence the interaction of vascular and epithelial progenitor cells with immune cells and engineering functional blood vessels in vivo.

1. Microvascular defects in Type 2 Diabetes - India ranks second to China in the global prevalence of Type 2 Diabetes (T2D). In India, 0.7% T2D will become blind due to retinopathy. Up to 10-70% of Indian Gestational Diabetes Mellitus (GDM) women and babies develop T2D. Our lab has noted structural and functional abnormalities of foetal GDM pericytes and endothelial cells that resemble Adult T2D retinopathy, suggesting that T2D might have developmental origins *in utero*. We are exploring the mechanisms that mediate aberrant cross talk between foetal placental endothelial cells and pericytes in GDM vasculature.

2.Generating Functional blood vessels- The holy grail of vascular regenerative medicine is creating stable and functional blood vessels *in vivo* to treat vascular disease. We isolate vascular progenitor cells from sources such as adipose tissue and walls of blood vessels and substitute animal products with human platelet lysate in culture conditions to facilitate clinical translation.

3. 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 (Colin Jamora's lab) 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.

Utilizing autologous vascular progenitor cells to treat vascular disease in a translational setting remains a significant challenge due to inherent endothelial dysfunction. A basic science approach is necessary to examine defects of microvasculature in diseases such as T2D in biologically relevant animal models and perform preclinical studies first, before envisaging targeting of specific cytokines, using autologous vascular cell therapy, or vascularization of engineered tissues at the clinic.

Research Profile

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

RESEARCH PROGRAM: Human Mesenchymal Stem cells (hMSCs): Biology and SCID mice models for evaluating hMSCs therapeutic potentials:

My core scientific investigations are based on mesenchymal stem cells (MSCs). Despite hundreds of clinical trials using MSCs for wide range of intractable diseases, reasons for the beneficial effects are frequently unclear. The success of future clinical applications will depend on an exhaustive understanding of the biology of the hMSCs and, more importantly, the biological consequences of isolation, expansion, and manipulation of the stem cell for therapeutic use. Mesenchymal Stem Cells (MSC) are found virtually in all tissues (Tuan et al. 2003) and play an important role in maintaining homeostasis and repair in case of injury or during disease, through the renovation of cell repertoire. MSC are today's promise to regenerative medicine, due to their easy culture *in vitro*, their high proliferation rates, and their versatility of differentiation in many cell types, including the well established osteoblasts, chondrocytes and adipocytes (Pittenger et al. 1999), as well as hepatocytes, neurons, and glial cells. Further, substantial ambiguities still persist in the mesenchymal biology field regarding functional identity, mode of isolation, their nature and experimental handling of MSCs. Thus, research focus is on role of various tissue-derived mesenchymal stromal cells in normal and pathological tissue homeostasis. Ongoing studies on cellular interactions among different components of the multicellular tissue will provide significant knowledge towards how does MSCs modulates tissues niche functions, their maintenance and regeneration.

Study 1. Therapeutic applications of human perinatal-tissue derived Mesenchymal Stem Cells (MSC) eg placenta and wharton jelly of human umbilical cord.

Human bone marrow derived MSCs have limited proliferative capability consequently it is challenging to use in tissue engineering and regenerative medicine applications. Hence, perinatal tissues such as human Wharton jelly and placenta-derived MSCs, which serves as one of richest sources of MSCs, were chosen to establish long-term culture from the clinical waste materials of full-term human placenta. Over all analysis of our data indicated that MSCs derived from the perinatal tissues exhibits optimal mesenchymal characteristics such as phenotypic attributes, multilineage differentiation, cell cycle, cytokine expression profile, immunomodulatory compound secretion and apoptotic pattern than the adult MSCs for regenerative medical applications.

Study 2. Generation of virus-free, integration-free human induced pluripotent (iPS) cells from hPD-MSCs using site-specific (AAVS1) targeted AAV vectors.

We have derived iPSC colonies from human placental MSC by virus-free, integration-free approach and currently characterized and validated the basic features of induced pluripotent (iPS) cells by *in vitro* differentiation assays, *in vivo* teratoma assays as well as evaluating therapeutic potential of neurospheres derived from hiPSCs *in vivo* in spinal cord injury SCID mice models. Some molecular studies were also undertaken for characterization and comparing the gene expression profiles, epigenetic signatures with starting material of placenta-derived MSCs.

Study 3. Human Wharton's Jelly Mesenchymal Stem Cells Plasticity Augments Scar-Free Skin Wound Healing with Hair Growth.

Human mesenchymal stem cells (MSCs) are a promising candidate for cell-based transplantation and regenerative medicine therapies. Thus in the present study Wharton's Jelly Mesenchymal Stem Cells (WJ-MSCs) have been derived from extra embryonic umbilical cord matrix following removal of both arteries and vein. WJMSCs seeded on decellularized amniotic membrane scaffold transplantation on the skin injury of SCID mice model demonstrates that combination of WJ-MSCs and decellularized amniotic membrane scaffold exhibited significantly better wound-healing capabilities, having reduced scar formation with hair growth and improved biomechanical properties of regenerated skin compared to WJ-MSCs alone. Further, our experimental data indicate that indocyanin green (ICG) at optimal concentration can be resourcefully used for labeling of stem cells and *in vivo* tracking by near infrared fluorescence non-invasive live cell imaging of labelled transplanted cells , thus proving its utility for therapeutic applications.

Study 4. 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 (BRNS Grant).

Short Project Introduction: The present study aims to provide best possible experimental

combinations of stem cells based-therapy for radiation-accident injuries in the events of catastrophic incidences after evaluating the experimental results in mice models.

Specific Aim1: Transplantation of genetically manipulated human term-placenta derived MSC expressing SCF, FLT3 ligand, TPO and IL3 to mitigate radiation induced apoptosis and secondary tissue damages after ARS in a SCID mice models.

Specific Aim2: Transplantation of hPD-MSC cultured in 3D under physiological oxygen conditions in a SCID ARS model to assess reduction of inflammatory response, support of tissue regeneration and angiogenesis.

Study 5. Genetically-engineered human umbilical cord-derived mesenchymal stem cells (UC-MSC) / engineered UC-MSC derived exosomes as therapeutic delivery vehicles for tumor-targeted therapy or maintaining tissue-homeostasis.

Abstract: The current study describes the development of a nano-vesicle based cancer therapeutic product, derived from engineered human umbilical cord mesenchymal stem cells (hUC-MSC). The produced nano-vesicles will have both tumor-specific targeting capacity and will deliver highly bio-active therapeutic RNAi molecules (si/shRNA) directly to cancer cells. Specific Aims and objectives are:

Specific Aim 1: Genetically engineer hUC-MSC to produce NVs for tumor specific targeting.

Specific Aim 2: Genetically engineer hUC-MSC to produce highly efficient RNAi content in NVs to block tumor cell growth.

Specific Aim 3: Determine which product is most efficient; natural exosomes or artificial nano- vesicles

Specific Aim 4: Test the therapeutic NVs in vitro in tumor cell lines, and in vivo in mouse models of human tumors, to establish an efficient RNAi containing NV-based cancer product.

Study 6. 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 (DBT-Grant)

Short Project Introduction: The use of small animal models with syngeneic/autologous cells as research tools has contributed immensely to biomedical science. Cell therapy has shown augmentation in functional activities in spinal cord injury of SCID mice.

Specific Aims and Objectives:

Specific Aim 1: To test if early intervention approaches using the immunomodulatory compounds ethyl pyruvate, Rolipram, neuropeptide substanceP (SP) or liposomal encapsulated clodronate widens therapeutic window by attenuating spinal cord ischemic injury in a SCID mouse model.

Specific Aim 2: Assess the therapeutic applications of early invention in SCI using mesenchymal stem cells (MSC) alone or in combination with virus-free, integration-free human iPS derived neurospheres

and/or immunomodulatory compounds in a SCID mice model.

Specific Aim 3: To evaluate if viral vector expressing inducible PTEN shRNA expression in injured scid mice axon increases the axonal regeneration after early phase injury stabilization by reducing acute inflammatory responses.

Study 7. Do perinatal tissue-derived MSCs maintain their phenotypic attributes and retain intrinsic characteristics during long-term in vitro cultures.

Short project Introduction: What is the best possible approach for MSC in vitro culture that retains many of the intrinsic MSC features and MSC biological properties *in vivo*?

Another important feature is that human MSCs, as distinct from mouse MSCs, are not immortal in culture but senesced very early in their population doublings; for this reason they are unlikely to cause tumors *in vivo* (Prockop and Keating, 2012). However, if early passage cells are replated at low density, the cells re-acquired the characteristics of the initial early progenitor cells.

Specific Aim 1: Extensively evaluate every alternated passage of *in vitro* cultured MSC in terms of their phenotypic attributes, cell proliferation rate, cytokines and growth factors secretion profile, cell migration, gene expression analysis, cell cycle maintainance, apoptosis pattern, multilineage differentiation, soft agar invasion assays, population doubling time calculations.

Specific Aim 2: Test therapeutic potentials of passage 1, passage 5, passage 10, passage 15, passage 20, passage 25 and passage 30 in SCID mice models.

Study 8. Generation of an epigenetic factor shRNA library for studying the mechanisms of stem cell differentiation, disease pathogenesis and drug resistance. (DBT Grant, Co-PI: Shaji RV)

Study 9: Non-invasive in vivo imaging of indocyanin green (ICG) labeled human mesenchymal stem cells (MSC) in SCID mice. Real-time tracking and monitoring the fate of the cells is essential to realize and validate the full potential of cell transplantations therapy in clinical applications. At present, there is no cost effective and efficient labeling technique for tracking the cells under in vivo conditions. Indocyanine green is a safer, economical and superior labeling technique to track the cells *in vivo*.

Study 10: Studies on molecular characterization of different native MSCs, comparisons with in vitro 2D and 3D cultures in normoxia and physiological oxygen concentrations by transcriptome analysis, small RNA sequencing, glycomics, lipidomic, metabolomics, proteomic and in vivo transplantation experiments.

Short Project Introduction: To predict drug response or toxicity, the pharmaceutical industry is increasingly performing smaller-scale validation studies of experimental drug compounds in novel 3D cell culture models intended to mimic more closely the structure, activity, and extracellular environment of tissues *in vivo.*

Specific Aims and Objectives:

Specific Aim 1: Evaluate phenotypic attributes, growth characteristics, gene expression analysis, cell cycle status, apoptosis pattern, multilineage differentiation potential of Mesenchymal stem cells (MSC) cultured in 3D matrix under physiological oxygen culture conditions and compare that to 2D grown MSC.

Specific Aim 2: Comparative study of gene expression, surface glycolytic expression pattern, proteomic, total kinome, metabolome and lipidom of 3D cultured MSC under physiological conditions versus 2D normoxia grown MSCs.

Specific Aim 3: Test and compare 3D vs 2D cultured MSC's therapeutic potentials in SCID mice models.

Study 11. Biological studies on chromatin modulators for MSC osteogenic fate choices in metabolic bone diseases.

Short project Introduction: Histone lysine demethylases are chromatin modifiers that play important roles in many pathological processes such as inflammation and cancer, making them potentially attractive drug targets. While significant progress has been made in understanding transcriptional controls of MSC fate, little is known about how MSC differentiation is epigenetically regulated.

Aim1: Overexpression of histone demethylases KDM4B and KDM6B to promote osteogenic differentiation in MSCs isolated from normal and pathological conditions.

Aim2: Promote osteogenic fate choices by inducible shRNA approach to modulate adipogenic differentiation pathway genes.

Aim3: Screen for small molecule inhibitors for modulating the adipogenic pathways and promoting osteogenesis lineage differentiation in abberent differentiating MSCs.

Study 12: Evaluating mesenchymal compartment of bone marrow stem cells niche in pathological bone marrow samples of myelodysplastic syndrome.

Short project Introduction: Our knowledge remains limited about the stromal cells that orchestrate the complex balance between supply and demand that regulates HSCs differentiation and their release in the circulation. Mesenchymal stromal cells (MSC) work plan to study mesenchymal compartment from MDS bone marrow samples: Establish Bone marrow MSC culture, Phenotypic characterization of cultured MSC by flow analysis, In vitro tri-lineage differentiation assays (adipogenesis, osteogenesis and chondrogenesis) from cultured BM MSC, Cell cycle status of cultured bone marrow MSC, Apoptosis analysis of cultured bone marrow MSC, Cultured MSCs for karyotyping.

PUBLICATIONS / PATENTS:

Patents:

» Indian Provisional patent application No. 5171/CHE/2012. Dated 15th June 2014. METHOD OF PREPARATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS.

Principal Inventor: *Sanjay Kumar.*

» ii. Indian complete Patent Application No. 57/CHE/2014. Dated 6th January 2014. A PROCESS OF LABELLING CELLS AND A METHOD OF TRACKING THEREOF. Principal Inventor: *Sanjay Kumar.*

MTCC Deposition: Deposited the reprogramming plasmid vector to Microbial Type Culture and Gene Bank (MTCC).

Book Chapter contribution: Bone Defect Repair in Mice by Mesenchymal Stem Cells Humana Press, USA part of the Springer Publishing Group.

Scientific Journal Publications (12) since October 2010 with cumulative Journal Impact Factor (JIF): 40.454

Peer Reviewed Scientific Publications (2013-2014):

» Human Wharton's Jelly Mesenchymal Stem Cells Plasticity Augments Scar-Free Skin Wound Healing with Hair Growth. PLoS One. 2014 Apr 15; 9(4):e93726. Sabapathy V, Sundaram B, Sreelakshmi VM, Mankuzhy P, Kumar S. (Impact Factor: 3.730)

» Bone defect repair in mice by mesenchymal stem cells. Methods Mol Biol. 2014; 1213: 193-207. Kumar S. (Impact Factor: 1.29)

» Adeno-associated virus (AAV) vectors in gene therapy: immune challenges and strategies to circumvent them. Rev Med Virol - 2013. Hareendran S, Balakrishnan B, Sen D, Kumar S, Srivastava A and Jayandharan GR. (Impact Factor: 7.615)

» Comparative Studies to Evaluate Relative in vitro Potency of Luteolin in Inducing Cell Cycle Arrest and Apoptosis in HaCaT and A375 Cells. Asian Pacific J Cancer Prev. (2013) 14 (2), 631-637. George VC, Ragupathi D, Kumar N, Suresh PK, Kumar S, Ashok Kumar R. (Impact Factor: 1.270)

» Detection of Merkel cell polyomavirus in formalin-fixed, paraffin-embedded tissue of Merkel cell carcinoma and correlation with prognosis. Rom J Morphol Embryol, 2014, 55 (3 Suppl):3-6. Andea AA, Patel R, Ponnazhagan S, Isayeva T, Kumar S, Siegal GP. (Impact Factor: 0.723)

Manuscripts Submitted for Peer Review:

» Neurospheres derived from integration-free iPSCs of human placental MSC augments motor function in SCID mice following spinal cord injury. (PLOS ONE) Sabapathy V, Murugan D, Samuel R, Tharion G, Kumar S*.

» Non-invasive in vivo imaging of indocyanin green (ICG) labeled human stem cells in SCID mice injury models. (Stem Cell Research and Therapy) Sabapathy V, Jyothsna M, Paul MJ, Kumar S*

» Natural decellularized amniotic membrane scaffold packs better tissue engineering features compared to synthetic PLGA and hybrid scaffolds. (J of Tissue Engineering & Regenerative Medicine) Sabapathy V, Hurakadli M, Kumar S*.

» Plasticity of long-term cultured human perinatal tissue-derived Mesenchymal Stem Cells provides a unique therapeutic opportunity. (Current Stem Cell Research and Therapy) Sabapathy V and Kumar S*.

» Cell therapy Augments the Functional Recovery following Spinal Cord Injury. (Stem Cell Int.) Sabapathy V and Kumar S*.

GRANTS:

a. Successful:

- 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. Total Funding: 34.23 Lakhs
- Foot-print free iPSC technology (2011-2015) Ramalingaswami fellowhip project, DBT. Grant # BT/HRD/35/02/14/2009. Total Funding: 76.75 Lakhs
- 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). DBT Grant # BT/PR8527/MED/31/234/2013. Total Funding: 36.19 Lakhs

Pending Sanction orders after successful grant presentations:

» 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). Total Funding: 99.372 Lakhs

» 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). Funding: 30 Lakhs

Grants preparations for submission Process:

» Genetically-engineered human umbilical cord-derived mesenchymal stem cells (UC-MSC) / engineered UC-MSC derived exosomes as therapeutic delivery vehicles for tumor-targeted therapy or maintaining tissue-homeostasis.

» Biological studies on chromatin modulators for MSC osteogenic fate choices in metabolic bone diseases.

» Evaluating 3D cultured perinatal tissue derived-mesenchymal stem cells under physiological oxygen conditions and comparison with 2D MSC characteristics and therapeutic applications.

INTERNATIONAL MEETINGS: POSTER PRESENTATIONS

I. International Society of Stem Cell Research (ISSCR) Annual Meeting, Boston, USA-2013

II. International Society of Stem Cell Research (ISSCR) Annual Meeting, Vancouver-Canada-2014

COURSES TAUGHT:

(i) Stem Cell Module (ii) Cell Biology (iii) Gene Therapy courses for Graduate students (iv) Conducted workshops for mouse mesenchymal stem cell isolation, *in vitro* culture, imunophenotypic characterization and therapeutic applications in SCID mice.

OTHER ACADEMIC ACTIVITIES:

Human Resource Development:

» Trained 16 students for partial fulfilment of their M. Tech., B.Tech., M. Sc.(Biotech) and B. Sc. (Bioech) degree.

» Conducted 4 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: 1. Flow Cytometry-FACS Core Facility; 2. *In vivo* small animal whole body imaging system 3. Also, organizing PhD pre-registration course work for Stem Cell Module as a course co-ordinator.

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, Tamilanadu.

» Cell therapy potential of adult bone marrow-derived mesenchymal stem cells. Vellore Institute of Technology (VIT), Tamilnadu.

» Evaluating cell-based therapeutic approaches in mice models of bone regeneration. Sri Venkateswara College of Engineering, Pennalur, Irungattukottai - 602117, Tamilnadu.

International and National Scientific Meetings attended:

» International Society of Stem Cell Research (ISSCR) Annual Meeting, Boston-2013

» International Society of Stem Cell Research (ISSCR) Annual Meeting, Vancouver-Canada-2014

» Stem Cell Biology Symposium, Lifeline Hospital, Chennai, TN-2013

» Advances in Stem Cell Research, Sri Venkateswara College of Engineering, Pennalur, TN

Invited as Peer Reviewer for evaluating scientific journal manuscripts:

Cancer Research; Human Gene Therapy; Gene Therapy; Molecular Therapy; Stem cells; Cytotherapy; PLOS ONE; Stem Cell Research & Therapy; Burn & Trauma; Cell Transplantation; Wound Healing; Indian J of Biophysics & Biochemistry

Evaluated scientific contents of several submitted grants of different funding agencies: *DBT; DBT (BIRAC); ICMR; CSIR*

Membership of other academic bodies and professional Societies: American Society of Gene & Cell Therapy (ASGCT); American Association of Advancement of Science; International Society for Stem

Cell Research (ISSCR)

GROUP MEMBERS (Students, JRFs, PostDocs, Trainees, etc. for the period in review)

» Two Graduate Student.

» One Laboratory technician on my Ramalingaswami Fellowship Grant.

» 16 Short-term trainees students from M.Tech, M.Sc (Biotech), B.Tech (Biotech) from various universities from across the country.

COLLABORATIONS

International-Selvarangan Ponnazhagan, Professor, Dept. of Pathology, UAB.

CSCR/CMC-Dr. RV Shaji, Dr. Rekha Samuel, Dr. Vrisha Madhuri, Dr. Alok Srivastava, Dr. George Tharion, Dr. Suresh Devhsayam, Dr. Paul MJ, Dr. Antony Devasia, Dr Ashish Gupta, Dr. Margaret.

inStem Collaborations: Dr. Colin Jamora, Dr. Pravin Vemula.

Dr V. Madhuri, MS Orth, MCh Orth, Prof and Adjunct scientist

RESEARCH PROGRAMS – Paediatric orthopaedics Unit – Lab 5

We are working on several projects which relate to our overall theme of musculoskeletal regeneration. Our work is mainly focused on clinical translations which are related to articular and physeal cartilage replacement, bone and muscle regeneration.

Completed studies:

Since the submission of our last report, a large animal study has been completed on physeal replacement using chondrocytes with chitosan hyaluronic acid aldehyde (CHDA) gel; based on our outcomes, we are planning to take this forward for human translation.

In our human translation we have completed 2 years of follow-up for the physeal translation.

On-going studies:

» 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 – 50 lakh

» Project - Musculoskeletal stem cell in tissue regeneration.

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

Funding- 100,000 Euro

Projects under Indo- Danish funding-

- Cartilage regeneration in an osteoarthritis rat knee model.
- Biology of Cartilage regeneration in childhood articular disorders.

- Isolation and characterization of cancer stem cells from human osteosarcoma tissue
- Generation of a mouse model of congenital pseudarthrosis of tibia (CPT) by injection of Mesenchymal stem cells (MSCs) derived from human lipofibromatosis tissue isolated from CPT biopsies in children.
- Efficacy of cultured mesenchymal stem cells (MSCs) loaded over the PCL electrospun scaffolds for regeneration of articular cartilage defects in goat hip joint.
- To study the effect of shock wave on growth plate in rat metatarsal organ culture
- To study the effect of shock waves on young rabbit physis.

» 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.

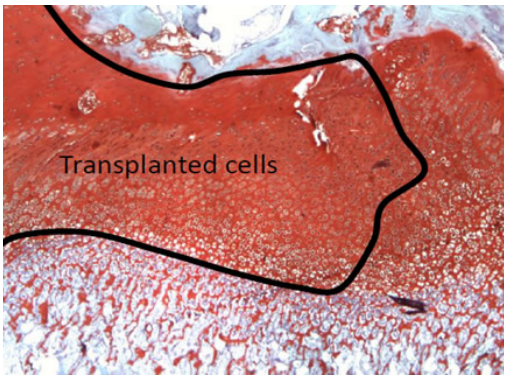
Budget - Rs 49.02 Lakh

Studies ongoing under this project

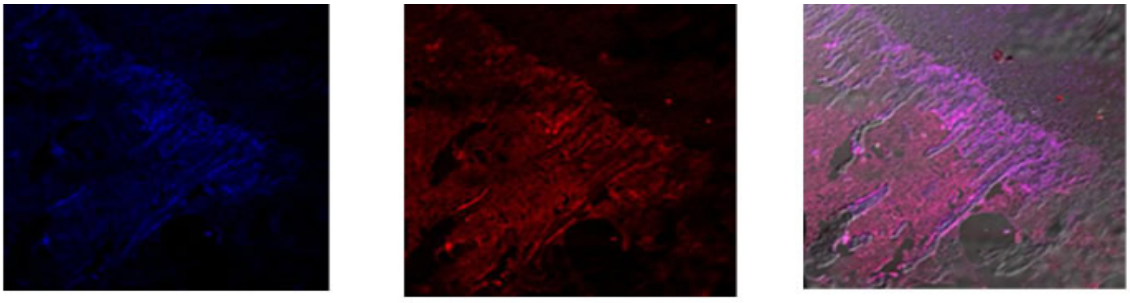
A. Isolation, characterization of Rabbit MSCs and differentiation of MSCs to chondrocytes.
B. Rabbit MSCs miRNA profile during chondrogenic differentiation in the presence of PTHrp/TGF beta supplemented environment.
C. Effect of Mechanical environment on the articular cartilage tissue engineering.
D. Chitosan based scaffolds for Cartilage Tissue Engineering

Figures: Physeal regeneration in the goat model using chitosan hydrogel scaffolds loaded with Iliac crest chondrocytes

a)



b)



DAPI | **Transplanted cells stained with Anti-GFP** | **DAPI and Anti- GFP merged image**

Figure: a) Safranin-O staining showing the region of transplantation where regenerated growth plate (treated) and the regenerated cartilage structure and GAG staining is similar to normal; b) transplanted cells were tracked using anti-GFP antibody which shows that the regenerated cartilage is stained positive for GFP.

Awards:

» Dr. Vrisha Madhuri has been appointed as the President of Paediatric Orthopaedics society of India.

» Dr. Vrisha Madhuri was an invited speaker for Annual conference of Limb reconstruction society meeting in Osaka, Japan (March 2014).

» Dr. Vrisha Madhuri was invited to speak at Paediatric orthopaedic department, Astrid, Lindgren hospital, Karolinska University, Sweden October 2013.

Publications:

» Ramesh S, Rajagopal K, Vaikkath D, Nair PD, Madhuri V. Enhanced encapsulation of chondrocytes within a chitosan/hyaluronic acid hydrogel: a new technique. Biotechnol Lett. 2014 May;36(5):1107-11.

» Rajagopal K, Chilbule SK, Madhuri V. Viability, proliferation and phenotype maintenance in

» cryopreserved human iliac apophyseal chondrocytes. Cell Tissue Bank. 2014 Mar;15(1): 153-63.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.

Manuscripts in preparation-

- Classical and Atypical Fibrodysplasia Ossificans Progressiva in India (Revision)
- Parathyroid hormone related peptide (1-34) does not prevent hypertrophy of periosteal derived mesenchymal stem cells during in vitro chondrogenesis – (Revision)
- Isolation, in vitro expansion and characterisation of human muscle satellite cells from the rectus abdominus muscle (Submitted)
- Genetic analysis of Progressive pseudorheumatoid dysplasia patients for WISP3 gene in Indian population (Submitted)
- Autologous chondrocyte transplantation for physeal bars – From bench to bedside.
- Autologous cultured chondrocyte loaded on chitosan hydrogel gel for replacement of physeal defects in goats
- Suitability of chitosan gelatin scaffold for articular cartilage defects: A preliminary in vitro and in vivo study
- Pamidronate negatively regulates the osteogenesis in mesenchymal stem cells derived from fibrous hamartoma in congenital pseudoarthrosis of tibia

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

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

Brief outline of completed and ongoing research:

Pharmacogenetics of cytarabine and daunorubicin resistance in the leukemic stem cell compartment in acute myeloid leukemia

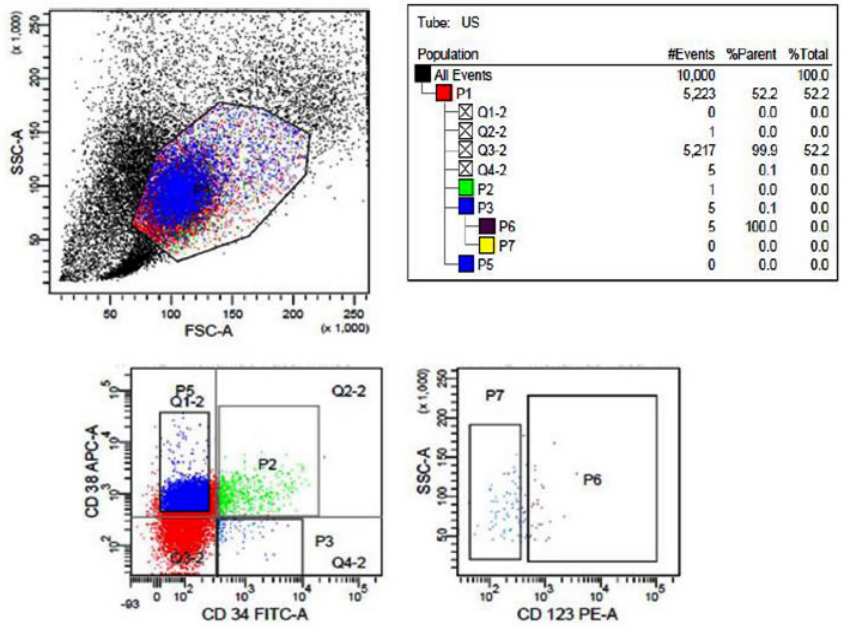
Acute myeloid leukemia (AML) is clinically and biologically heterogeneous disease. The backbone of AML treatment for the last 30 years has been the combination of Daunorubicin (DNR) and Cytosine Arabinoside (Ara-C). Even though AML treatment has made incremental advances in the last few decades, combination chemotherapy is successful only in 25% of the AML patients, the majority remaining non-responsive. Drug resistance and relapses are the major reasons for treatment failure. Although these drugs effectively eradicate the blast population it has minimal activity on the leukemia initiating cells or leukemic stem cells (LSC) that generate the blasts. Qualitative differences (constitutive or acquired during leukemogenesis) in LSCs among patients can explain the differences in the chemotherapeutic treatment outcome. Such differences can be recognized by the specific expression pattern of genes (DCK, CDA, NT5C2, RR, ENTI, MRP8, ABCG2, ABCB1, ABCA1, CBR1, CBR3) encoding the enzymes/proteins involved in the metabolic pathway of these drugs. Identification of these factors may not only provide biomarkers predictive of treatment outcome but also allow designing rationale patient-specific treatment options. The aim of this study is to identify the mechanisms involved in the drug resistance phenomenon shown by LSC compartment by looking at the pattern of expression of the enzymes and transporters involved in resistance to ara-c and daunorubicin.

Bone marrow samples from patients with AML (n=25) at diagnosis were collected after obtaining informed consent from the patients. Prospective or retrospectively cryopreserved samples which satisfy the criteria of high blast and high CD34 percentage were subjected to sorting. These samples were sorted based on the expression of cell surface markers CD34, CD38 and CD123. The volume of each antibody to be added was decided based on antibody titration experiment. The samples were sorted based on serial gating strategies and CD34+CD38+, CD34-CD38+, and CD34+CD38-CD123+ (pLSC fraction) were collected. Qiagen method of RNA extraction was opted for samples with cell count ranging from 5*104 to 5*105 and for samples/fractions with less than this cell count, a MiniRNA extraction kit from ZYMORESEARCH was used. cDNA synthesis was done using ABI high capacity cDNA synthesis kit. As the RNA yield from pLSC fraction is low, we performed Taqman pre- amplification to amplify the target genes of interest in an unbiased way. mRNA expression of the enzymes and transporters involved in AraC [Deoxycytidine Kinase (dCK), Cytidine deaminase (CDA), 5’ Nucleotidase (NT5C2), Ribonucleotide reductase (RRM1), Equilibrative nucleoside transporter

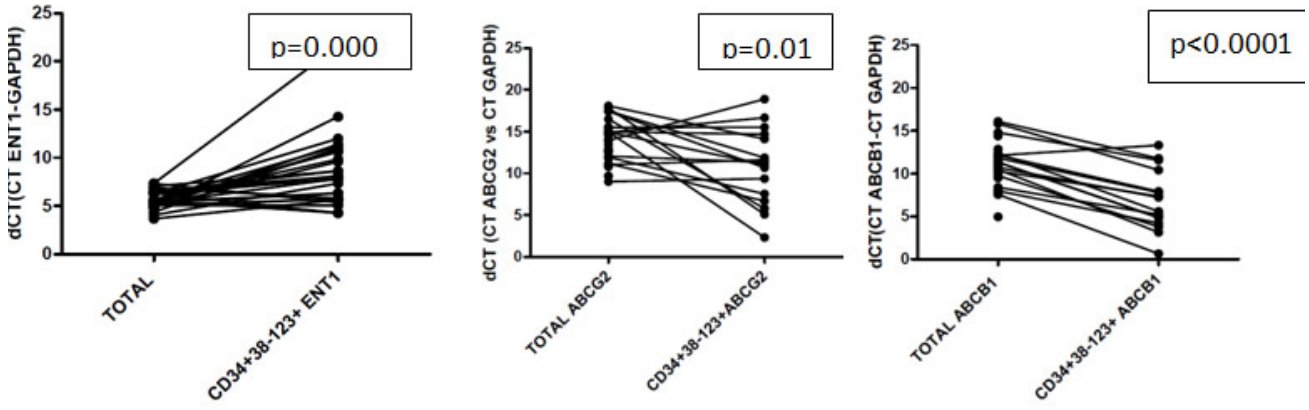
1(ENT1) Multidrug Resistance Protein 8 (MRP8)] and daunorubicin [ABCG2 (BCRP), ABCB1 (MDR1), ABCA1, Carbonyl reductase-1 (CBR1), carbonyl reductase-3 (CBR3)] metabolism and transport was analyzed using real time quantitative PCR (Q-RTPCR) with Taqmanbased gene expression assays (Life Technologies).The target gene expression was normalized to the expression of the house keeping gene GAPDH. The expression of these genes in the pLSC fraction was compared with the expression from total cells and the statistical significance was identified by paired t test using graph pad prism software.

Results

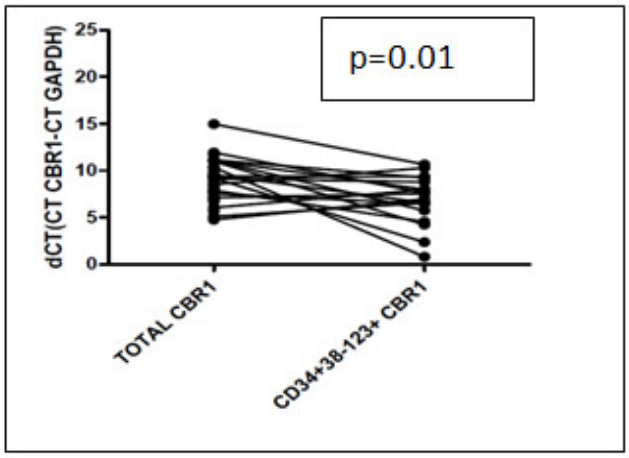
Representative flow sort of AML bone marrow MNCs conjugated with flurochrome tagged antibodies CD34 FITC, CD38 APC, CD123 PE.

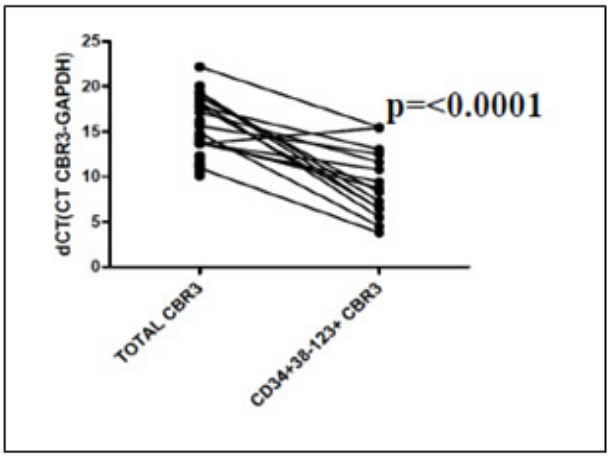


Expression of Ara–C influx transporter hENT1 was identified to be significantly lower (p=0.0003) in pLSC fraction compared to the total cells. Meanwhile the expression of efflux transporters ABCG2, ABCB1 were significantly higher in pLSC fraction compared to the total cells.



Daunorubicin metabolising genes CBR1, CBR3 expression in the CD34+38- fraction was found to be significantly higher with p value of 0.01 and <0.0001 respectively when compared with the total expression.





The functional role of these differentially expressed genes in pLSC which contribute to resistance need to be further confirmed by additional studies. This study suggests that identification of these factors may not only provide the biomarkers predictive of treatment outcome but also allow designing patient specific treatment strategies.

3. RELEVANT PUBLICATIONS IN 2013-14

Abraham A, Devasia AJ, Varatharajan S, Karathedath S, Balasubramanian P, Mathews V. Effect of cytosine arabinoside metabolizing enzyme expression on drug toxicity in acute myeloid leukemia. Ann Hematol. 2014; Nov 13. [Epub ahead of print]

Abraham A, Karathedath S, Kumaraswamy V, Jayavelu AK, M S, Srivastava VM, Zhang W, Zhou T, George B, Srivastava A, Mathews V, Balasubramanian P. Novel NPM1 mutation in the 3'-untranslated region identified in two patients with acute myeloid leukemia. Leuk Lymphoma. 2014 Jun; 55 (6):1421-4

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. 2014 Mar; 93(3):509-12.

LABORATORY HIGHLIGHTS OF YEAR 2013-14.

Mr. Ajay Abraham

» Three minute thesis presentation- 2nd prize

» Abstract Achievement Award Recipient American Society of Haematology (ASH) annual meeting-2012 & 2013

» Best poster Award in "Annual Research Day"- Christian Medical College, Vellore. 2012

» DBT travel award to attend American Society for Haematology meeting 2012.

Ms. Sreeja Karathedath

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

» Best poster award- First prize, Annual Research day CMC, 2014

» Best poster award- First prize, Winter Symposium, CMC, 2014

Ms. Savitha Varatharajan

» Abstract Achievement Award Recipient American Society of Haematology (ASH) annual meeting-2014

» DBT and ICMR travel award to attend American Society for Haematology meeting 2014.

» Charpak Fellowship to work in France for 6 months 2012.

ONGOING RESEARCH SUPPORT

S. No.	Title of Project	Funding Agency	Amount (Lakhs)	Date of sanction and Duration
1.	Pharmacogenetic and pharmacodynamic analysis of fludarabine based conditioning regimen for HSCT – **Principal Investigator**	DBT	61.87	March 2013 3 years
2.	Modulation of drug resistance in acute myelogenous leukemia: role of Nrf2 and ABCB6- – **Principal Investigator**	ICMR	32.0	December 2014 2 years

PATENTS: None

RESEARCH GRANTS AWAITING REVIEW

» "Personalizing conditioning regimen in Hematopoietic Stem cell Transplantation"- Submitted to Wellcome-DBT India Alliance, 2014 (PI: Poonkuzhali B; Co-I: Biju George, Auro Viswabandya, Vikram Mathews, Alok Srivastava)

» "Identification of novel nuclear receptor drug targets using short hairpin RNA (ShRNA) screen in myeloid leukemias" Submitted to DBT (PI: Poonkuzhali B; Co-I: Shaji R.V, Vikram Mathews, Biju George, Alok Srivastava)

» "Exploring the mechanisms of disease progression, tyrosine kinase inhibitor resistance, and intolerance in Chronic Myeloid Leukemia"- Submitted to DBT. (PI: Poonkuzhali Balasubramanian; Co-I: Vikram Mathews, Shaji R.V, Biju George, Alok Srivastava)

» "Proposal for ICMR Advanced centre for Clinical pharmacology in haematological diseases - Aiming at Personalized Medicine"- submitted to ICMR. (PI: Poonkuzhali Balasubramanian; Co-I: Vikram Mathews, Biju George, Auro Viswabandya, Alok Srivastava)

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

RESEARCH PROGRAM:

Area: Biomaterials and Tissue Engineering

Our lab focuses on synthesis, design and characterization of biomaterials and scaffolds suitable for controlling/regulating stem cell fate and function, and for engineering tissues and organs for regenerative medicine.

Surgical reconstructive procedures often require the use of additional tissues, such as autograft, allograft or xenograft, in order to restore normal anatomical and functional tissue configurations. However, these grafts are often associated with complications such as donor site morbidity, limited availability, risk of disease transmission and host tissue reactivity. Tissue engineering has emerged as a promising approach to overcome these limitations, as it enables the fabrication of functional tissues or organs that could be used for reparative procedures in patients. The basic idea behind is to create bioengineered tissues or organs by combining patient's own cells with engineered matrices called scaffolds. The 3D microenvironments are one of the key factors to engineer a physiologically functional tissue and organ in vitro, which are generated in our lab using conventional-, micro- and nano-technologies. Such types of designed tissue scaffolds are briefly discussed in the following three sections.

1. High-Throughput Screening of Stem Cells using Nanomaterials:

This project involves the development of nanofiber scaffolds with composition gradient libraries suitable for high-throughput screening of stem cells response in terms of adhesion, migration, proliferation, differentiation and tissue organization. Scaffolds made up 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 composition and characteristics towards stem cell behavior to optimize tissue organization is the key selection criteria for scaffolding systems in tissue engineering and regenerative medicine. Therefore, this project aims to develop gradient nanofiber scaffold libraries, made of poly(caprolactone) (PCL) nanofibers with composition gradients of nano hydroxyapatite (nHA), suitable for high-throughput screening of human bone marrow-derived mesenchymal stem cells (hBMSCs) under a defined 3D microenvironment(see Figure 1), which 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.

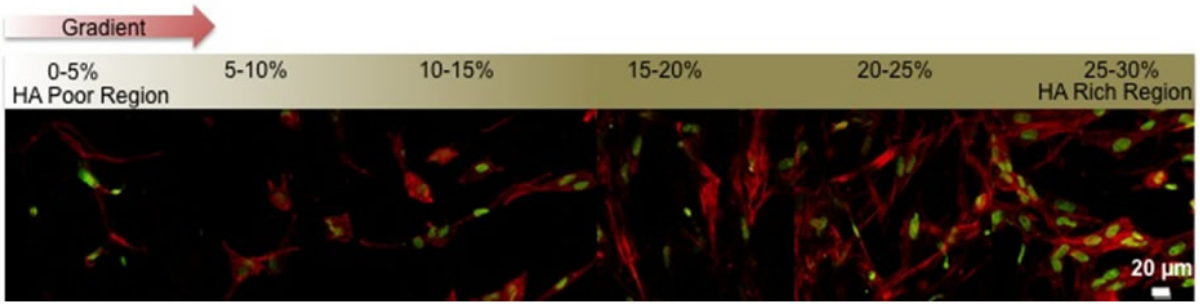


Figure.1. A representative confocal image of hBMSCsresponses to gradient microenvironment. HA denotes hydroxyapatite, a major bone mineral substance.

2. Cell-Laden Hydrogels for Stem Cell Delivery and Tissue Engineering:

Hydrogel systems have been investigated for cell and drug delivery applications. However, cell encapsulation properties of hydrogel is a milestone in stem cell delivery and regenerative medicine applications, which requires optimal 3D microenvironment with physical, chemical and biological properties quite similar to native extracellular matrix (ECM). Encapsulation of cells in biodegradable hydrogels offer numerous attractive features for stem cell delivery and tissue engineering, which includes ease of handling, a highly hydrated tissue-like 3D microenvironment for cell and tissue growth, and their ability to perform in vivo. In a pilot study, we have demonstrated synthes is and characterization of polyacrylamide/alginate (PAM/Alginate) hydrogel systemand their ability to culture of hBMSCs. For comparison of the cell culture systems (2D Vs 3D), hBMSCs were cultured under defined conditions using three different culture systems i.e., on the tissue culture plate (2D system), on the gel (OG 3D system) and in the gel (IG 3D system). The results of the cell culture experiments demonstrated that 2D and OG systems were found to be comparable in terms of cell viability, attachment and proliferation and material's compatibility with stem cells. In contrast, IG systems showed slightly less viability but exhibited significant proliferation in 7 days during the course of study. The stem cells cultured in IG system show morphology and behavior in resemblance to the native tissue-like microenvironment. For instance, hBMSCs exhibits round shape morphology when cultured in IG 3D systems whereas the same cells look like a spindle shape when cultured in both OG 3D systems as well as 2D systems. These results suggest that PAM/Alginate hydrogels may be a promising candidate for stem cell delivery and tissue engineering.

3. Optimizing Engineered Hydrogels for Cardiomyogenesis:

The aim of this project is to develop and optimize the preparation of polyethylene glycol (PEG) based hydrogels with gradients in stiffness to support attachment, growth and differentiation of adipose derived stem cells into cardiomyocytes. The use of adult stem cell therapy for cardiac repair has shown enormous potential in experimental settings. In particular, in experimental models of myocardial infarction (MI) the administration of adult mesenchymal stem cells (MSCs) limits infarct size, prevents ventricular remodelling and improves cardiac function. There are still several issues that need to be clarified in order to efficiently translate stem cell therapy to the clinic. A major obstacle is represented by the low efficiency of cell engraftment due to immediate washout and low

survival rate. Another crucial point is represented by the low differentiation efficiency of MSCs to cardiomyocytes.

Hydrogels are considered as an efficient delivery system for stem cells. PEG is a promising candidate and often used when designing 3D microenvironment for the culture of different types of cells, including stem cells. However, there is no standard method and measurement tool for making PEG gels with gradients in mechanical and biochemical cues. Mechanical and biochemical cues of cellular microenvironment play a significant role in regulating a variety of critical cell behaviours with stiffness having been recently shown to aid in directing stem cell lineage specification. It is thus opportunity to investigate both the optimal hydrogel stiffness and chemical composition required for cardiomyocyte differentiation from MSCs and their ability to enhance cardiogenesis.

Funding

» DST India-South Africa extramural grant on "Optimising Engineered Hydrogels for Stem Cell Delivery and Differentiation in the Heart". Role: Principal Investigator (June 2014 – May 2017).

» CSCR start-up grant to initiate a "Biomaterials and Tissue Engineering" Lab.

Publications (2013-2014)

Journals:

» DeeptiRana, T.S. Sampath Kumar and Murugan Ramalingam. Cell-laden hydrogels for tissue engineering. J. Biomater. Tissue Eng. 4 (2014) 507-535.

» KaarunyaSampathkumar, ShylajaArulkumar and Murugan Ramalingam. Advances in stimuli responsive nanobiomaterials for cancer therapy. J. Biomed. Nanotech. 10 (2014) 367-382.

» N. Varadarajan, R. Balu, DeeptiRana, Murugan Ramalingam, and T. S. Sampath Kumar. Accelerated sonochemical synthesis of calcium deficient hydroxyapatite nanoparticles: Structural and morphological evolution. J. Biomater. Tissue Eng. 4 (2014) 295-299.

» J. Ramón-Azcón, S. Ahadian, R. Obregon, H. Shiku, R. Murugan and T. Matsue. Applications of carbon nanotubes in stem cell research. J. Biomed. Nanotech. 10 (2014) 2539-2561.

» S. Ostrovidov, X. Shi, L. Zhang, X. Liang, S. B. Kim, T. Fujie, R. Murugan, M. Chen, K. Nakajima, F. Al-Hazmi, H. Bae, A. Memic and A. Khademhosseini. Myotube formation on gelatin nanofibers-multiwalled carbon nanotubes hybrid scaffolds. Biomaterials 35 (2014) 6268-6277.

» S. Ahadian, J. Ramón-Azcón, H. Chang, X. Liang, H. Kaji, H. Shiku, K. Nakajima, R. Murugan, H. Wu, T. Matsue and A. Khademhosseini. Electrically regulated differentiation of skeletal muscle cells on ultrathin graphene-based films. RSC Advances 4 (2014) 9534-9541.

» R. Obregon, J. Ramón-Azcón, S. Ahadian, H. Shiku, H. Bae, R. Murugan, and T. Matsue. The use of microtechnology and nanotechnology to fabricate vascularized tissues. J. Nanosci. Nanotech. 14 (2014) 487-500.

» S. Ostrovidov, V. Hosseini, S. Ahadian, T. Fujie, S. P. Parthiban, R. Murugan, H. Bae, H. Kaji and A. Khademhosseini. Skeletal muscle tissue engineering: Methods to form skeletal myotubes and

their applications. Tissue Engineering 20 (2014) 403-436.

» S. Ahadian, J. Ramón-Azcón, M. Estili, X. Liang, H. Shiku, R. Murugan, K. Nakajima, Y. Sakka, H. Bae, T. Matsue and A. Khademhosseini. Hybrid hydrogels containing vertically aligned carbon nanotubes with anisotropic electrical conductivity for muscle myofiber fabrication. Scientific Reports 4 (2014) 4271, 1-11.

» S. Ostrovidov, S. Ahadian, J. Ramón-Azcón, V. Hosseini, T. Fujie, S. P. Parthiban, H. Shiku, T. Matsue, H. Kaji, R. Murugan, H. Bae and A. Khademhosseini. Three-dimensional co-culture of C2C12/PC12 cells improves skeletal muscle tissueformation and function. J. Tissue Eng. Reg. Med. (2014).

» J. Ramón-Azcón, S. Ahadian, M. Estili, X. Liang, S. Ostrovidov, H. Kaji,H. Shiku, R. Murugan, K. Nakajima, Y. Sakka, A. Khademhosseini, T. Matsue. Dielectrophoretically aligned carbon nanotubes to control electrical and mechanical properties of hydrogels for muscle tissue engineering. Advanced Materials 25 (2013) 4028-4034.

» S. Ahadian, S. Ostrovidov, V. Hosseini, H. Kaji,R. Murugan, H. Bae and A. Khademhosseini. Electrical simulation as a biomimicry tool for regulating muscle cell behavior. Organogenesis 9 (2013) 87-91.

» Seidi, K. Sampathkumar, A. Srivastava, S. Ramakrishna and R. Murugan. Gradient nanofiber scaffolds for tissue engineering. J. Nanosci. Nanotech. 13 (2013) 4647-4655.

» DeeptiRana and Murugan Ramalingam. Decellularizedbiomaterialsfor stem cell research and therapy (in process).

» DeeptiRana and Murugan Ramalingam. Synthesis and characterization of polyacrylamide/ alginate hydrogels as a carrier for human bone marrow-derived mesenchymal stem cells (in process).

Text Books:

» Vishwakarma, X-P. Wang, P.T. Sharpe, S. Shi and R. Murugan. Stem Cell Biology and Tissue Engineering in Dental Sciences. Elsevier Publication, USA (2014) 792 pages.

» R. Murugan and S. Ramakrishna. Nanofiber Composites: Biomedical Perspectives, Elsevier Publication, USA (2014, in process)

» R. Murugan, E. Jabbari, S. Ramakrishna and A. Khademhosseini.Micro and Nanotechnologies in Engineering Stem Cells and Tissues.IEEE Press, USA (2013) 328 pages.

» R. Murugan, Xiumei Wang, Peter Yang, Guoping Chen and Fu-Zhai Cui. Biomimetics: Advancing Nanobiomaterials and Tissue Engineering. Wiley-Scrivener Publishing, USA (2013) 354 pages.

» R. Murugan, P. Vallittu, U. Ripamonti and Wan-Ju Li. Tissue Engineering and Regenerative Medicine: A Nano Approach. CRC Press, USA (2013) 592 pages.

Book Chapters:

» SergeOstrovidov, AzadehSeidi, DeeptiRana, KaarunyaSampathkumar, QueenyDasgupta, AlokSrivastava, A. Khademhosseini and MuruganRamalingam. Introduction to nanobioscience: A tissue engineering perspective. In Encyclopedia of Life Support Systems, UNESCO Project, EOLSS Publications, France (2014, in press).

» DeeptiRana, ShylajaArulkumar, Akshyaa Ganesh and MuruganRamalingam. Biological and Pharaceutical Applications of Nanomaterials. In Nanomaterials in Drug Delivery, PolinaProkopovich (Ed.), CRC Press, USA (2014, in press).

» DeeptiRana, ShylajaArulkumar, AjaykumarVishwakarma and MuruganRamalingam. Considerations on designing scaffold for tissue engineering. In Stem Cell Biology and Tissue Engineering in Dental Science, Pg 133-148, AjaykumarVishwakarma, Paul Sharpe, Songtao Shi, Xiu-Ping Wang and Murugan Ramalingam(Eds.), Elsevier, USA (2014).

» AjaykumarVishwakarma, Paul Sharpe, Songtao Shi, Xiu-Ping Wang and MuruganRamalingam. An introduction to Stem Cell Biology and Tissue Engineering. In Stem Cell Biology and Tissue Engineering in Dental Science, Pg 1-13, AjaykumarVishwakarma, Paul Sharpe, Songtao Shi, Xiu-Ping Wang and Murugan Ramalingam(Eds.), Elsevier, USA (2014).

» S. Ahadian, S. Ostrovidov, T. Fujie, P. P. Selvakumar, H. Kaji, K. Sampathkumar, R. Murugan and A. Khademhosseini. Microfabrication and nanofabrication techniques for dental tissue engineering and regeneration. In Stem Cell Biology and Tissue Engineering in Dental Science, Pg 207-219, AjaykumarVishwakarma, Paul Sharpe, Songtao Shi, Xiu-Ping Wang and Murugan Ramalingam(Eds.), Elsevier, USA (2014).

» R. Obregón, J. Ramón-Azcón, S. Ahadian, H. Shiku, R. Murugan, A. Khademhosseini and T. Matsue. Gradient biomaterials as tissue scaffolds. In Stem Cell Biology and Tissue Engineering in Dental Science, Pg 175-186, AjaykumarVishwakarma, Paul Sharpe, Songtao Shi, Xiu-Ping Wang and Murugan Ramalingam(Eds.), Elsevier, USA (2014).

» KaarunyaSampathkumar, AzadehSeidi, AlokSrivastava, T.S. Sampath Kumar, S. Ramakrishna and MuruganRamalingam. Biomimetic materials for engineering stem cells and tissues. In Biomimetics: Advancing Nanobiomaterials and Tissue Engineering, J R. Murugan, X. Wang, G. Chen, P. Ma and F-Z. Cui (Eds), Wiley-Scrivener Publication, USA (2013) 329-344.

» S. Ahadian, R. Murugan and A. Khademhosseini. The emerging applications of graphene oxide and graphene in tissue engineering. In Biomimetics: Advancing Nanobiomaterials and Tissue Engineering, J R. Murugan, X. Wang, G. Chen, P. Ma and F-Z. Cui (Eds), Wiley-Scrivener Publication, USA (2013) 279-299.

» S. Ostrovidov, A. Seidi, S. Ahadian, R. Murugan and A. Khademhosseini. Micro- and nanoengineering approaches for advancing gradient biomaterials suitable for interface tissue engineering. In Micro and Nanotechnologies in Engineering Stem cells and Tissues, R. Murugan, E. Jabbari, S. Ramakrishna and A. Khademhosseini (Eds), Wiley-IEEE Press, USA (2013) 52-80.

» M. Shayanthi, J. Venugopal, R. Rajeswari, R. Murugan, M. Raghunath and S. Ramakrishna. Nanofiber technology for controlling stem cell functions and tissue engineering.In Micro and

Nanotechnologies in Engineering Stem cells and Tissues, R. Murugan, J. E. Jabbari, S. Ram akrishna and A. Khademhosseini (Eds.) Wiley-IEEE Press, USA (2013) 27-52.

» Peter Molnar, MuruganMurugan, and James J. Hickman. Surface chemical determination of placement, growth and differentiation of cells for drug screening, toxin detection and lab-on-a- chip applications. In Tissue Engineering and Regenerative Medicine: A Nano Approach, R.Murugan, P. Vallittu. U. Ripamonti and Wan-Ju Li (Eds.), CRC Press, USA (2013) 205-228.

» Y.N. Cho,R. Murugan and Z.S. Haidar. Nanobiomaterial-based siRNA delivery systems.In Tissue Engineering and Regenerative Medicine: A Nano Approach, R. Murugan, P. Vallittu. U. Ripamonti and Wan-Ju Li (Eds.), CRC Press, USA (2013) 473-498.

Honors and awards

» Adjunct Professor, Tohoku University, Japan
» Fellow, Royal Society of Chemistry, UK
» Fellow, Institute of Nanotechnology, UK
» Editor-in-Chief, Journal of Biomaterials and Tissue Engineering, USA
» Editor-in-Chief, Journal of Bionanoscience, USA
» Chief Editor, Biomedical Science, Engineering and Technology Series, Wiley-Scrivener Publishing, USA
» Associate Editor of Journal of Nanoscience and Nanotechnology, USA
» Advisory Board Member of Stem Cell Research and Therapy, USA
» Editorial Board Member ofJournal of Stem Cell Research and Therapy, USA
» Editorial Board Member of Journal of Biomimetics, Biomaterials, Tissue Engineering
» Advisory Board Member, Int. Workshop on Advanced Nanomaterials 2013, India
» Organizing Committee Member, 4th International Congress on Cell Science and Stem Cell Research, Spain
» Co-Organizer, 5th International Congress on Ceramics, China
» Scientific Committee Member of ICTE, Portugal
» Advisory Board Member, Stem Cell Research, WebmedCentral, UK
» Advisory Board Member, The European Society for Biomaterials

Invited talks

» Nanotechnology in Stem Cell Research, VIT University, Vellore (January 2014)

Course taught

» Stem Cell Nanotechnology, Stem Cell Course Module, PhD Programme at CSCR

List of team members (postdocs, students, JRFs, short-term trainees, others)

» Deepti Rana, JRF (September 2014-To date)

Collaborators

- AlokSrivastava, Centre for Stem Cell Research/CMC, Vellore
- Sanjay Kumar, Centre for Stem Cell Research, Vellore
- Manasseh Nithyananth, Christian Medical College, Vellore
- Ashish Gupta, Christian Medical College, Vellore
- InianSamarasam, Christian Medical College, Vellore
- GeethaManivasakam, VIT University, Vellore
- Sampath Kumar, Indian Institute of Technology Madras, Chennai
- Praveen Vemula, inStem, Bangalore
- Ali Khademhosseini, Harvard University and MIT, USA
- Seeram Ramakrishna, National University of Singapore, Singapore
- ZiyadHaidar, Universidad de los Andes,Chile
- Xiumei Wang, Tsinghua University, China
- Serge Ostrovido, Tohoku University, Japan
- Tomokazu Matsue, Tohoku University, Japan
- Neil Davies, University of Cape Town, South Africa
- Thomas Franz, University of Cape Town, South Africa

Dr. Rekha Samuel, MD, Professor of Pathology. July 13th 2011-present.

RESEARCH PROGRAM: Vascular Biology Program

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. The major focus of the program is dissecting the pathophysiology and molecular mechanisms of Diabetic microvasculopathy and is discussed in detail below. Other areas of interest in the program involves exploring signaling pathways that influence the interaction of vascular cells with non vascular cells such as epithelial and immune cells in Diffuse Scleroderma and utilizing adult sources (e.g. adipose tissue) to engineer functional blood vessels.

The basis of Type 2 diabetic (T2D) micro vascular pathology is unresolved, worldwide. A third of newly diagnosed Diabetic patients present with micro vascular complications. Targeting early vascular changes that precede the catastrophic events culminating in Proliferative T2D retinopathy is therefore the key to an understanding the biology of early T2D microvasculopathy and ultimately, preventing blindness. Among young Indian diabetics (age <25 years), 48% have Type 2 Diabetes (T2D) and 4.5% have Gestational Diabetes Mellitus (GDM). GDM women, and their babies have a 10- 70% chance of developing T2D postpartum in India. Understanding the association between fetal reprogramming in utero and the development of adult diabetic vasculopathy is of particular relevance in India, where Diabetes and vascular disease are prevalent.

Ongoing projects:

1. Placental Pericytes & Microvascular Dysfunction in Type 2 Diabetes, *DBT (Stem Cell Research). 2012-2014. BT/PR5915/MED/31/172/2012: Rs.42, 22,200. Funds received.*

We have optimized protocols for the isolation, characterization, in vitro and in vivo functional analyses of placental foetal endothelial progenitor cells and pericytes. We have noted structural and functional abnormalities of GDM pericytes and endothelial cells that resemble Adult T2D retinopathy. (Back to the future: Examining Type 2 Diabetic vasculature using the gestational diabetic placenta. Samuel R, Ramanathan K, Mathews JE, Seshadri MS. Diab Vasc Dis Res. 2014 Sep;11(5):363-5. We are now exploring key signaling pathways that mediate cross talk between foetal placental endothelial cells and pericytes in healthy and GDM cases.

2. Blood placental barrier in Hyperglycemia of pregnancy. *Fast track Scheme, DST, Science and Engineering Research Council SB/FT/LS-196/2012. 2012-2015: Rs. 24,79,000. Funds received.*

Examination of the ultrastructure of the placenta is a tool to study the vascular and non-vascular components of the Blood placental barrier (BPB), namely fetal endothelial cells, pericytes,

syncytiotrophoblasts, immune cells, extracellular matrix, accessory cells and organelles. We focus on interactions of the pericyte in maintenance of normal BPB, and a plausible breach in the same in hyperglycemia of pregnancy.

3. Non-invasive long-term in vivo vascular imaging using Multi photon Laser Scanning Microscopy. *DBT (Bioengineering). BT/PR7990/MED/32/282/2013. 2013-2016: Rs. 50,00,000. Funds received.*

We have shown that pericyte coverage in the GDM placenta is normal, however the GDM pericytes show structural or functional abnormalities in vitro.. Endothelial cells from diabetic placenta show functional abnormalities in vitro..This proposal involves engineering blood vessels using endothelial progenitor cells and pericytes derived from GDM placenta in vivo in immune deficient mouse models. Functionality of the engineered vessels will be evaluated using multiphoton laser scanning microscopy, when the specialized hood procured from funds for this project will arrive.

4. SNAIL associated microvascular defects in hyperglycemia of pregnancy. *Research Society for the Study of Diabetes, India: 2014-2016. Rs. 500,000. Funds received.*

Snail deficient mice show abnormalities in vasculogenesis, germ line differentiation and specification to endothelial cells. This proposal evaluates for the first time the role of the transcription factor SNAIL in mediating microvascular defects in hyperglycemia of pregnancy.

5. Generating functional blood vessels using adult vascular stem cells. *ICMR 2012-0803. 2012-2014:Rs. 29,55,080. Sanctioned, awaiting release of funds.*

This study examines the potential of adipose tissue derived vascular/ mural progenitors, cultured with non-animal human platelet lysate to form functional blood vessels in vivo, in an attempt to move to clinical translation.

6. Back to the future: Placental vascular progenitor cells predict adult type 2 diabetic microvascular complications. *ICMR 2013-2825. Invited full submission of proposal on 30.06.14. Awaiting acceptance. Requested Rs. 89 lakhs.*

Placental whole genome analysis studies, transcriptome or epigenetic signatures might shed mechanistic clues on defects in tissue engineered GDM blood vessels in vivo. Our research could potentially lead to the discovery of novel molecular signatures that could explain defects in microvasculature of, or novel placental vascular specific signaling pathways that predict T2D microvasculopathy

7. i)Vasculopathy in Scleroderma: *IRB approved, preliminary data generated and submission of proposal to DBT by end of 2014.*

Systemic sclerosis (SSc) or scleroderma is a chronic connective tissue disorder characterized by microvascular damage, immune defects and progressive fibrosis. The access to human SSc samples and the potential to manipulate specific proteins in the mouse system that mimics human SSc

(Colin Jamora) provides an innovative approach to examine vasculopathy in SSc.

ii) Elucidating the role of PAI-1 mediated signaling in cutaneous fibrosis: in collaboration *with Dr. Colin Jamora, PhD, Principal Investigator, inStem, Bangalore.*

Translational relevance of studying T2D microvascular pathology: The GDM placenta is a model to examine functionality of T2D vasculature. **The hope** is that understanding the evolution of vasculopathy in the GDM placental model might permit the identification of new signaling molecules that could either predict vascular complications of T2D, or even serve as targets for therapy in the future. **The reality** is that inherent endothelial dysfunction and metabolic memory are concerns for direct application/ infusion of autologous T2D vascular progenitor cells to humans at this time. **What's critical** is early intervention of T2D to control and prevent T2D-associated vascular complications.

In summary, the aim of the vascular biology program keeps with the mandate of the Centre for Stem Cell Research: to establish a lab for translational research with stem cells (endothelial cells and pericytes) within a medical institution (CMC) that will use the knowledge of the basic biology of stem/ progenitor cells to develop a better understanding of human disease, primarily, Diabetes. Besides involving Medical fraternity, collaborations also include bioengineering faculty (IIT-M) and basic researchers (NCBS/inStem) to achieve this mandate.

Intramural Funding Received: I. From CMC (Fluid Research Grants)

1. Isolation of placental perivascular cells and endothelial progenitor cells from Gestational Diabetes to explore early microvascular functional abnormalities. 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. IRB Min 7846, 2012-2014, Rs.80,000.

II.CSCR Core funding in 2014: 5 lakhs (March)+ 1 lakh (August)

Publications 2014

Accepted: 1.Back to the future: Examining Type 2 Diabetic vasculature using the gestational diabetic placenta. Samuel R, Ramanathan K, Mathews JE, Seshadri MS. Diab Vasc Dis Res. 2014 Sep;11(5): 363-5.

2. Spontaneous Development of Neoplasms in Severe Combined Immunodeficient (SCID) mice. Samuel R. SAGE open medical case reports. (Accepted 24.12.14)

Submitted:1. Engineering blood vessels from human induced pluripotent stem cells: Implications for modeling vascular disease and clinical translation. Samuel R, Duda DG, Fukumura D and Jain RK. Science Translational Medicine. In review.

2.Fetal Metabolic reprogramming in vascular progenitor cells of Gestational Diabetes Mellitus. Samuel R, Benjamin SJ, Mathews JE and Seshadri MS. Diabetologica, Journal. Accepted

presubmission.

In progress

1.Diabetes, Stem cells and Vascular Tissue Engineering. Selected chapter to be included in book to be published by Springer at the end of 2014.

2. Vascular progenitor cells in Diabetes. Selected chapter to be included in “Diabetes Up date” Book by Research Society of Diabetes in India, 2014.

Invited talks

- 2015 February. Clinical Applications of Stem Cells. Singapore Bioimaging Consortium and Select Biosciences South East Asia.
- 2015 February. All India Ophthalmological Society, Delhi. (Speaker, and Course instructor).
- 2014 December. XXIII Annual Conference of Vitreo Retina Society of India, Agra.
- 2014 October. Biomaterials 2014. International Conference on Polymeric Biomaterials, Bioenegineering and Biodesign, Delhi.
- 2014 October. 6th International Symposium on Diabetic Retinopathy and Vascular Disorders. Organized by Aravind Eye Hospital and LV Prasad Eye Institute, Pondicherry.
- 2014 September. XIth Congress of the SAARC Academy of Opthalmology, 23rd Annual Con gress of the College of Opthalmologists.
- 2014 February. Vellore Institute of Technology.

Posters

- 2014 February. EM: Frontiers in Bioimaging Conference, National Centre for Biological Sciences, Bangalore.
- 2014 February. Diabetes in Pregnancy Study Group of India, Conference.

Training

Laser capture Microscopy on the Zeiss Palm system at Advanced Molecular Pathology, A-Star Institute, Biopolis, Singapore 2nd-3rd September 2014

Lab Members

1. Chitra Premkumar, Graduate Technician. BSc Zoology, employed on DST, Science and Engineering Research Council SB/FT/LS-196/2012.

2. Saranya Rajendran, Graduate Technician. BSc Microbiology, employed on DBT project BT/PR7990/MED/32/282/2013.

Collaborators Vascular Biology Team

- Dr. Jiji Elizabeth Mathews, DGO, MD. Professor and Head, V, Obstetrics and Gynecology, CMC
- Santhosh Benjamin, MS. Assistant Professor, Obstetrics and Gynecology, V, CMC.
- MS Seshadri, MD, PhD, FRCP. Professor & Retired Head, Endocrinology, Diabetes and

Metabolism, CMC.

» Niranjan Joshi, PhD. Researcher, Healthcare Technology and Innovation Centre (HITC).

» Mohanasankar Sivaprakasam, PhD. Assistant Professor, IIT-Madras and Director, HITC.

» Colin Jamora, PhD. Associate Professor, IFOM-inStem Joint Research Laboratory.

» H. Krishnamurthy, PhD. Director of Flow Cytometry, CCAMP, NCBS.

» Sanjay Kumar, PhD. CSCR.

» Sukria Nayak, MS, FRCS. Professor and Head, Surgery IV, CMC.

» Indrani Sen, MCh, Vascular Surgery, CMC.

» Paul MJ, MS. Professor and Head, Endocrine Surgery, CMC.

» Renu George, MD. Professor and Head Dermatology 1, CMC.

» Debashish Danda, MD, DM, FRCP. Professor and Head, Clinical Immunology& Rheumatology, CMC.

Future Directions

We propose to examine specific molecular signatures that may give clues to mechanisms involved with dysfunctional Diabetic vasculature. We will establish the cranial window model that would permit in vivo longitudinal imaging of engineered blood vessels using multiphoton laser scanning microscopy as we await the arrival of the specialized biosafety laminar flow hood. Alternate animal models to evaluate blood vessel functionality in vivo, controlled release of growth factors within biocompatible materials and the use of 3D-culture systems are planned as we look for appropriate extramural funding.

Projected 5-year work plan, vascular biology theme (2012-2016)

	YEAR				
Aims	1	2	3	4	5
Isolation, characterization and in vitro functional analysis of vascular progenitor cells	■	■			
Establishing the SCID mouse colony in Animal Facility		■	■		
Establishing serum free media for vascular progenitor cells				■	
Establishing in vivo models in mice	■	■			
Establishing in vivo cranial window model in SCID mice and Multiphoton laser scanning microscopy				■	
Long term in vivo imaging of tissue engineered vascular constructs in SCID mice					■
Image analysis of engineered blood vessels					■
Transcriptome analysis of vascular progenitor cells				■	■

Core Faculty responsibilities at CSCR: Faculty in charge of Imaging and Histopathology. Faculty in charge, Histopathology.

RV Shaji, PhD Professor, Department of Haematology / Adjunct Scientist, Centre for Stem Cell Research

Area of research: Molecular mechanisms of human erythropoiesis and somatic cell reprogramming.

I. Molecular mechanisms of human erythropoiesis:

We are currently investigating the role of small RNAs and epigenetic factors in human erythropoiesis. The progress achieved in different aims in this research area is described below.

Ex-vivo erythropoiesis systems: To answer our questions in human erythropoiesis we established a robust ex-vivo erythropoiesis system using which we could obtain a large number of cultured erythroid cells from normal individuals and patients with β-thalassaemia. As the cells in liquid culture go through all the stages of in vivo erythropoiesis they are suitable for studying the dynamics of transcriptional changes that occur during erythroid differentiation. For establishing this method this we used purified CD34+ cells from donors who were treated with GCSF and peripheral blood mononuclear cells from patients with β-thalassaemia and the cells were cultured in serum free medium containing appropriate cytokines. The cells were analyzed for morphology, surface marker expression, activation of globin gene transcription and haemoglobinization.

miRNAs in human erythropoiesis: For the first time, we carried out the comprehensive screening of small RNAs in human erythropoiesis. Small RNA sequencing in the cultured erythroid cells at six different time points in ex-vivo erythropoiesis revealed several miRNAs which were significantly upregulated or downregulated during erythroid differentiation. We observed several novel miRNAs and miRNA clusters that are regulated by erythroid transcription factors. We are currently carrying out overexpression and knockdown experiments to validate the functions of these miRNAs. We also

plan to extend this study on cultured foetal liver and cord blood derived erythroid cells to identify the miRNAs that are involved in developmental stage regulation of human erythropoiesis.

Understanding the role of transcription co-factors in human erythropoiesis using ChIP-Seq: Though systematic genome wide association studies have been carried out for master transcription factors involved in erythropoiesis, such studies have not been carried out for transcription cofactors. We are interested in the role of transcription cofactors that are involved in epigenetic modifications and chromatin remodeling in human erythropoiesis. In the first step we performed ChIP-Sequencing experiments to understand the genome wide binding of histone acetyl transferase proteins, CBP and p300, which bind at the transcriptional regulatory sequences, mainly enhancers. Our data showed distinct regulatory DNA elements where these two proteins bind in haematopoietic stem cells and the differentiated erythroid cells. We are currently doing comprehensive bioinformatics analysis of our data with the help of Dr. Sreenivasulu Kurukuti at University of Hyderabad. While the bioinformatics analysis is going on, we are performing the ChIP-seq experiments for other cofactors EZH2, EZH1 and BRG1, which showed significant difference in their expression in ex-vivo erythropoiesis. The data from these experiments will help us understand the role of transcription cofactors in the transcription networks involved in maintenance of human haematopoietic stem cells and in erythropoiesis.

RNAi to identify the epigenetic regulators of erythropoiesis: Currently, there are no validated shRNA libraries available for human epigenetic factors. Several experiments carried out in our laboratory have identified the most efficient lentiviral back bone and the promoters for shRNA expression in stem cells and progenitors. We have so far validated shRNAs for 31 epigenetic factors with knock down efficiency of more than 70% and we will be completing the generation of validated shRNAs for 100 epigenetic factors in the middle of next year. These shRNAs will be used for studying the role of epigenetic factors in different stages of erythropoiesis using the ex-vivo generated erythroid cells.

Identification of transcriptional regulatory sequences in the beta globin cluster: Haplotype analysis of human beta globin cluster in several populations has shown that there are undetermined regulatory sequences in this locus that can modulate the expression of gamma and beta globin genes. We screened patients with mild and severe beta thalassaemia and sickle cell disease for their haplotypes in the beta globin cluster. We sequenced the entire 100KB region in 10 patients with different phenotypes using next generation sequencing based technologies and identified specific genetic variations present in this region that are associated with milder phenotype of the disease. To identify the regulatory role of these variations we are carrying out experiments using cultured erythroid cells obtained from patients with different phenotypes.

Evaluation of lentiviral vectors for gene therapy: In collaboration with Dr. Alok Srivastava, CSCR and Trent Spencer, Emory University we are testing lentiviral beta globin vectors for evaluation of their

efficiency in stable beta globin expression in cultured erythroid cells. We transduce the CD34+ cells with lenti viral particles and estimate the expression of transgene in differentiated erythroid cells. We have so far evaluated 4 vectors and found that our ex-vivo erythropoiesis system is an useful tool and we will be testing more vectors in the following year to identify the most efficient vector suitable for gene therapy.

II. Somatic Cell Reprogramming: Mechanisms and disease modelling

Establishing technology: We have successfully generated human induced pluripotent stem cells (hiPSCs) from adult skin fibroblasts and peripheral blood mononuclear cells. Robust protocols for generation of hiPSCs have been established using retroviral vectors, lentiviral vectors, episomal plasmids and Sendai virus for expression of OSKM. The generated clones have been well characterized for pluripotency tests including teratoma formation.

Identification of factors that are involved in the final stages of reprogramming: During reprogramming several colonies achieve part of the pluripotency network and they fail to transform to fully pluripotent state. To understand the molecular mechanisms and barriers in the final stages of reprograming we isolated partially reprogrammed iPSC colonies, also called as pre-iPSCs. Analysis of retroviral silencing kinetics, morphology, gene expression, surface marker expression, differentiation potential identified and response to small molecules showed that pre-iPSCs have high molecular heterogeneity. These clones were highly heterogeneous with variable levels of expression of E-cadherin and CD44 expression and they also showed variable response to small molecules that affect small molecules. These cells expressed high levels of OCT4 and we found an OCT4+NANOG to OCT4+NANOG+ barrier in the late stage of reprogramming, for the first time. We found that systematic stage wise treatment of small molecules increases reprogramming efficiency significantly. The findings helped us to understand the molecular mechanism in the late stage of reprogramming and in addition helped us devise a method to manage the culture

Epigenetic factors in reprogramming: To understand the role of epigenetic factors involved in different stages of reprogramming, we are currently performing RNAi experiments using inducible lentiviral vectors. We have identified several epigenetic factors which are differentially regulated at different stages of reprogramming including those which are exclusively expressed in pluripotent stem cells. As explained above in the project heading on Human Erythropoiesis, we have generated validated shRNAs for 31 human epigenetic factors and we hope to complete 100 more validated shRNA vectors in the following year. The validated shRNAs are used for transducing human skin fibroblasts and their expression is induced in different stages of reprogramming. Initial inducible knock down and over expression identified two PRMTs that regulate somatic cell reprogramming in the early and late stages of reprogramming. Further analysis using more shRNAs will help us identify additional epigenetic factors in different stages of reprogramming.

Disease modelling: We have successfully generated hiPSCs from fibroblasts from patients with Fanconi anaemia (FA), a disease caused by the mutations affect FA pathway. Previous reports suggested that generation of hiPSCs for this disease is challenging due to the defective DNA repair in the cells. Using our modified protocols we could obtain large number of FA-hiPSC lines with morphology and gene expression similar to hiPSCs generated from normal individuals. The clones generated are being tested for their differentiation potential and chromosome stability. These clones will be extremely useful for understanding the role of FA pathway proteins in haematopoietic differentiation and early development.

COLLABORATIONS:

- Alok Srivastava, CSCR: 1. Gene therapy programme in CSCR is led by Dr. Alok Srivastava. Dr. Srivastava and Dr. Trent Spencer, Emory University are involved in the design of new lentiviral vectors for gene therapy. 2. In other projects involving patients with beta thalassaemia, sickle cell disease and Fanconi anemia Dr. Alok Srivastava is involved in the design of the study and in the recruitment of patients.
- Sanjay Kumar, CSCR: Role of epigenetic factors in stem cell differentiation by RNAi
- Dasaradhi Palakodeti, inStem: Small RNA sequencing analysis.
- Sreenivasulu Kurukuti, University of Hyderabad: ChIP-sequencing analysis

GROUP MEMBERS:

I. Kannan VM: SRF/PhD student
II. Musheer Aalam Syed Mohammed: SRF/PhD student
III. Sumitha P Bharathna: SRF/PhD student
IV. Janakiram Rayabram: SRF/PhD student
V. Thiyagaraj Mayuranathan: SRF/PhD student
VI. Nancy Beril Jannette: SRF/PhD student (In collaboration with Dr. Alok Srivastava
VII. Dhava Priya: Technician
VIII. Aneesha Nath: JRF/PhD student
IX. Saravanan Ramakrishnana: JRF

Research Projects:

» Generation of human induced pluripotent stem cells (DBT, 2010-2012 (Completed))
» Molecular Basis of Human globin gene regulation (DBT, 2009-2014).
» Molecular Basis of Fanconi Anaemia in Indian population (DBT, 2009-2014)
» RNAi screen to identify the novel regulators of somatic cell reprogramming. Funded by Department of Biotechnology ((DBT, 2012-2015)
» Generation of human induced pluripotent stem cells for studying the mechanisms of haematological diseases (ICMR, Approved).
» Generation of an epigenetic factor shRNA library for studying the mechanisms of stem cell

differentiation, disease pathogenesis and drug resistance (DBT, Approved).

Publications (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 (δβ)0-thalassaemia and hereditary persistence of foetal haemoglobin in Indian population.Eur J Haematol. 2014 Jun;92(6):514-20.

» Mayuranathan T, Rayabaram J, Edison ES, Srivastava A, Velayudhan SR. 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. A novel δ-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 β-globin gene mutation HBB.c.22 G>C produces a hemoglobin variant (Hb Vellore) mimicking HbS in HPLC.Int J Lab Hematol. 2012 Oct;34(5):556-8.

» Edison ES, Venkatesan RS, Govindanattar SD, George B, Shaji RV. A novel 26 bp deletion [HBB: c.20_45del26bp] in exon 1 of the β-globin gene causing β-thalassemia major. Hemoglobin. 2012;36(1):98-102.

Manuscript under preparation:

» Efficient generation of Fanconi anaemia induced pluripotent stem cells for Fanconi Anaemia

» Molecular heterogeneity in induced pluripotent stem cells

» Small RNA analysis in human erythropoiesis

» Genome wide association of CBP and p300 in human erythroid cells.

Alok Srivastava, MD, FRACP, FRCPA, FRCP

RESEARCH PROGRAM:

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

1. Clinical stem cell transplantation – This involves a program of clinical hematopoietic stem cell transplantation as a service for patients with hematological diseases and includes studies evaluating mobilization of stem cells from the bone marrow as well as new protocols for transplantation or post transplant management of these patients.

As coordinator of the Indian Stem Cell Transplant Registry with a team of colleagues in the department of Haematology in CMC, Vellore, I am also involved with collection and analysis of national data for this registry and reporting to the Asia Pacific Blood and Marrow Transplant registry. I have been elected Vice Chair of the Asia Pacific Blood and Marrow Transplant Group executive board and continue to be the Vice Chair (Asia, Africa, Australasia) for the Center for International Blood and Marrow Transplant Research (CIBMTR). These positions also require involvement with various educational and research activities of these groups.

2. Translational stem cell research and novel therapies –

This involves four areas of work–

I. The gene therapy program

A major thrust in the last year has been on developing gene therapy towards clinical trials. Two areas are being developed in collaboration with different groups:

a. The first is the gene therapy program for hemophilia using AAV vectors. Current experience in the world has shown that while many so far, all the successful clinical trials have only been done with wild type and not capsid modified vectors. Wild type scAAV8 has been used with a codon optimized FIX gene construct successfully by Dr. Amit Nathwani and updated results on 10 patients with follow-ups of more than 5 years has shown response in all of them, the best with the highest dose cohorts. Since the success of this trial, several others have started using the same or different AAV vectors with other FIX gene constructs. However, in spite of the success of the Phase 1 / 2 trials, no major phase 3 trials have been undertaken predominantly related to issues of efficient vector production among several other reasons in a complex field where effective therapies are available to patients in developed countries already. Recent data from Dr. Arun Srivastava's lab has shown that AAV3 has much higher tropism for the human hepatocyte than AAV8, a fact that had been missed in the field because it had only been tested in the mouse models where it has very poor tropism. AAV3 is also free of any intellectual property restrictions. This is therefore a very good candidate for us to

develop and that is what we are doing in collaboration with the University of Florida which has done the largest number of AAV based gene therapy trials in USA. The current plan is to seek their help in developing the processes to produce the first batch of AAV vectors for a phase 1 clinical study. In this process scientists from INTAS pharmaceuticals will also be involved in learning all aspects the production process and CSCR will partner with them is doing all the toxicity, safety and efficacy studies with the GMP grade vectors. The major goal here therefore is that of product and process development along a successful model that seems to exist already and move to larger study depending on the results of the phase 1.

b. The second gene therapy program is towards hemoglobin disorders – thalassemia major in particular. There are reports of successful gene therapy using autologous hematopoietic stem cells transduced with lentiviral vectors carrying the beta globin gene. This work is being done collaboration with Dr. Trent Spencer of Emory University, USA. They have a lentiviral vector which has NIH RAC / per IND FDA approval for moving to a clinical trial for hemophilia A (FVIII deficiency). We are using the same vector with some modifications to carry the beta globin gene. This is being evaluated in the ex-vivo human HSC erythroid differentiation model that Dr. R. V. Shaji has established at CSCR. Initial data with a set of these vectors is promising with 70-80% transduction efficiency. Beta globin expression and its translation to hemoglobin needs to be optimized further. Once this is achieved, animal toxicity studies need to be carried out as well as evaluation of the pattern of random integration of this vector in the genome. If results from all these studies are acceptable, the possibility of moving towards a clinical study can be considered. This is very which is likely, given the status of the field at present where several studies with bith lentiviral and retroviral vectors have been started for hemoglobin disorders. Expertise will then need to be developed for GMP production of lenti viral vectors which is more challenging than AAV vectors. However, there is clear path to be followed in this development process and that is what we have initiated.

In parallel, a process for review, approval and monitoring of such research activities in the country is being developed. A joint working group of the DBT and ICMR was constituted a year ago and has met to discuss the path for this development. Documents are being prepared for the next meeting which is likely to be early in 2015.

II. Translational stem cell research:

a. An exciting area of development in translational stem cell research is the rapidly evolving understanding of the niche particularly in the bone marrow with all its components and complex interactions. One of the disease conditions where this knowledge has shed considerable new light is in bone marrow failure states such as the myelodysplastic syndrome. We have been able to put together a group which can comprehensively evaluate several aspects of this problem from clinical characterization (Dr. Biju George), histological features including vascularity (Dr. Marie Therese), cytogenetics on monnuclear cells and MSCs (Dr. Vivi Srivastava), molecular genetics /

mutations (Dr. Eunice Sindhuvi) hematopoietic and other de novo cellular elements (Dr. Aparna Venkatraman), mesenchymal stromal elements (Dr. Sanjay Kumar). Unique data is being identified but larger numbers need to be done before any definite conclusions can be established. Recently, collaboration has also been established with Dr. David Scadden from Harvard University, USA particularly for evaluating certain stromal elements.

b. Another area of translational stem cell research that we are engaged with is the creation of an induced pluripotent stem cell "haplobank". The idea here is to identify individuals who are homozygous for a HLA haplotype. The profile of HLA haplotypes in any population has shown that iPSCs derived from a relatively small number of such individuals can provide histocompatible cells for a large section of the population. For example, in USA, the top 10 haplotypes would cover 30%, the next 45 about 50%,the next 361 about 80% and about 863 would cover 90% of the population. In collaboration with DATRI (www.datriworld.org) and the department of Immunohematology and Transfusion Medicine, we are trying to analyze the haplotype frequencies among approximately 80,000 donors. Several homozygous individuals have already been identified. A collaboration has also been established with the University of California, San Francisco for this.

III. Stem cell transplantation and tissue engineering:

In this area, I am working with colleagues in different disciplines to test therapeutic strategies in animal models or human stem cell transplantation studies. Currently these studies include trials of engineered bone and cartilage (collaboration with Dr. V. Madhuri). An evolving area of development here is that of tissue engineering using epithelial cells – uroepithelium and squamous epithelium towards generating urological and other hollow organs as well as skin on scaffolds. This is an area of interest of several clinical departments in CMC, Vellore including urology (Dr. Anthony Devasia / Dr. George Tharion), plastic surgery for burns (Dr. Ashish Gupta) as well as gastrointestinal surgery for esophagus (Dr. I. Samaresan). Scientists from CSCR (Dr. Murugan Ramalingam and Dr. Sanjay Kumar) as well as inStem (Dr. Praveen K V) are also helping develop this area with support from core scientific staff (Mr. Augustine Thambaiah). The aim is to grow flat tissues and hollow organs on scaffolds seeded with appropriate cells.

IV. Policies and regulations for clinical translation of stem cell research in India –

Currently, I chair the National Apex Committee for Stem Cell Research and Therapy, Department of Health Research, Ministry of Health. (http://bmi.icmr.org.in/nacscrt/), which has the mandate to oversee all aspects of human stem cell research and therapy. These include formulating policies and guidelines (http://bmi.icmr.org.in/nacscrt/Downloads.html) for stem cell research as well as reviewing certain areas of research activities, monitoring the clinical translation process as well as therapies being offered.

PhD Students:

» Salar Abbas works on the BM niche (collaboration with Dr. Aparna Venkatraman and Dr. Sanjay Kumar).

» Sangeeta Hareendran works on AAV vector modifications (in collaboration with Dr. G. Jayandharan – work completed / thesis to be submitted Jan 2015).

» Nishant Gabriel works on AAV vector modifications (in collaboration with Dr. G. Jayandharan – thesis submitted Nov 2014).

» Nancy Beryl Janet A - Fanconi anemia (in collaboration with Dr. R.V. Shaji)

Selected publications (2013-14):

» Mathews V, Srivastava A, Chandy M. Allogeneic Stem Cell Transplantation for Thalassemia Major. Hematol Oncol Clin North Am. 2014 Dec;28(6):1187-1200.

» George B, Mathews V, Lakshmi KM, Melinkeri S, Sharma A, Viswabandya A, Sharma S, Das S, Ahmed R, Abraham A, Nair V, Apte S, Chandy M, Srivastava A. The use of a fludarabine-based conditioning regimen in patients with severe aplasticanemia--a retrospective analysis from three Indian centers. Clin Transplant. 2013Nov-Dec;27(6):923-9

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

» 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.

» 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.

» 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.

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

RESEARCH PROGRAM: STEM CELL NICHE IN DISEASE DEVELOPMENT

Stem cells receive signals from the surrounding environment to self- renew or to differentiate for tissue regeneration during homeostasis and tissue injury. Dys-regulation 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 niche cells 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 like goblet cells depletion are the central defect that underlies the development of UC. Identification of the local or niche factors responsible for such an abnormality in the epithelium may contribute to understanding of the aetio-pathogenesis of this disease.Since the surrounding mesenchymemaintains the functions of the different sub- populations of the colonic crypt, we performed phenotypic enumeration of sub-populations of colonic epithelium and mesenchyme in an animal model of colitis and patient mucosal biopsy samples. Even though total colonic epithelial cell number was unaltered prior to inflammation, there was a significant reduction in the number of immature epithelial cells in the lower crypt with a concomitant increase in the mature upper crypt cells in an animal model of colitis. Along with this decrease in number of immature crypt cells, aberrant cell migration, block of cell differentiation and cell cycle arrest were also noted. In parallel, an alteration of phenotypic number and migration in the surrounding mesenchyme was seen. Similar results were obtained in the endoscopically and microscopically uninvolved regions of colonic biopsies from UC patients. Mechanistically, alterations in notch and wnt signaling pathways were observed. Our results thus provide new insights into the development of UC and suggest that the stem cell niche in the colon may influence pathogenesis of the disease.

Role of niche cells in development of myelodysplatic syndrome (MDS)

Myelodysplastic syndromes (MDS) are a group of heterogeneous disorders characterized by ineffective hematopoiesis and frequent progression to acute myeloidleukemia. Early events driving

the initiation of bone marrow failure or hematological cancer are poorly understood. Emerging evidence from animal models suggest that the etio-pathogenesis of the disease is likely to depend on anterplay between aberrant hematopoietic cells and their microenvironment. Our lab is interested in elucidating the role of hematopoietic stem cells (HSCs) and their associated niche in development of MDS. Phenotypic enumeration of different HSC populations and their committed progenitors from bone marrow aspirate revealed a block in differentiation among different grades of MDS patients. In parallel, in situ analysis of bone marrow trephine of low risk MDS samples for different niche components revealed altered vasculature and clustering of HSPCs around vessels. At the molecular level, an increased expression of membrane bound β-catenin in vessels interacting with cadherin expressing HSPCs was noted. These data reveal that aberrant *wnt* signaling in the vessels and HSPCs could be involved in the etio-pathogenesis of MDS.

LABORATORY HIGHLIGHTS OF YEAR 2013-14

Publications:

» Amirtharaj GJ, Thangaraj KR, Kini A, Raghupathy V, Goel A, Eapen CE, Venkatraman A, Pulimood AB, Balasubramanian KA and Ramachandran A. Toxicology Reports (2014)1:707-717

» Zhao M, Perry JM, Marshall H, Venkatraman A, Qian P et al.Megakaryocytes maintain homeostatic quiescence and promote post-injury regeneration of hematopoietic stem cells. Nature Medicine. (2014) 20: 1321–1326

Ms. ArchanaKini

Best poster award- Second prize, Annual Research day CMC, 2014

Group Members:

» T. Archana Kini: JRF

» Salar Abbas: SRF/PhD student (In collaboration with Dr Alok Srivastava)

» Abhishek Sivappa Gowdar: Short term Trainee (December 2013- May 2014)

Collaborators

Ulcerative colitis project

» Dr Ebby Simon, MD,DM, Professor of Gastrointestinal Sciences, CMC, Vellore

» Dr Anna Pulimood, MD, PhD, Professor of Pathology, Dept of Gastrointestinal Sciences, Wellcome research unit, CMC, Vellore

» Dr Anup Ramachandran, PhD, Professor of Biochemistry, Wellcome research unit, Dept of Gastrointestinal sciences CMC, Vellore

Myelodysplastic syndrome project

» Dr Alok Srivastava, MD, FRACP,FRCPA, Professor of Hematology, Head CSCR, CMC, Vellore.

» Dr Biju George, MD, Professor of Hematology, CMC, Vellore.

» Dr Marie Theresa, MD, Professor of Pathology, CMC Vellore.

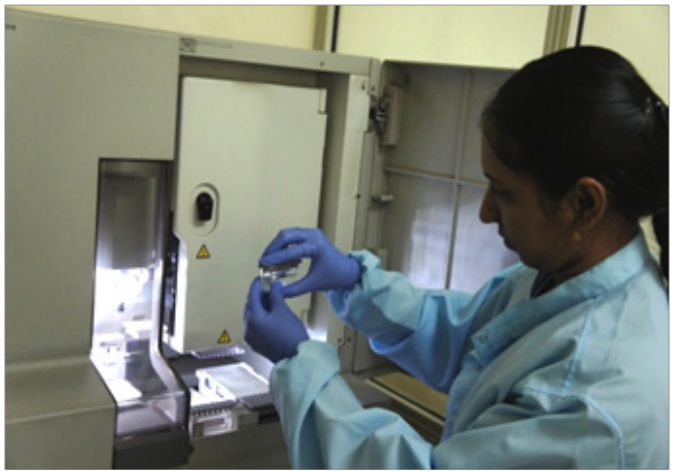
Core Facilities and Instrumentation

A. 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
- Graduate Technician: 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). 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.

II.Flow Cytometry Core Facility:

- Faculty In-Charge : Dr. Sanjay Kumar, PhD.
- Technical Officers : Mr. Vaidyanathan. S and Ms. Samrajyam. N
- Graduate Technician : 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 III cell sorter with a 5 laser 11 colour setup, BD FACSAria I SORP cell sorter with a 3 laser 9 colour setup for sorting applications and a BD FACSCalibur cell analyzer for analysis. The BD FACSAria III system has a throughput of 70,000 events per second and can do 4-way sorting and single cell sorting. The BD FACSCalibur has a 2 laser 4 colour system and is routinely used for intracellular and surface marker analyses 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 and experiment design. An offline workstation with a FlowJo license is also available and networked for data sharing and post-acquisition data analysis.

III. Imaging Core Facility:

» Faculty In-Charge : Dr. Rekha Samuel, MD.

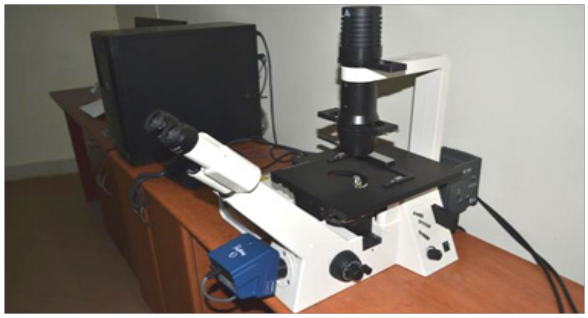
» Technical Officer : Mr. Vaidyanathan. S.

1. Leica DMI6000B Inverted Fluorescence Microscope

The Leica DMI6000B is an inverted fluorescence microscope comprising of 6 interchangeable filters for detecting various fluorochromes. It has two independent cameras eras – DFC295 for high resolution brightfield imaging and DFC360 FX for high frame rate fluorescence imaging. It is also equipped with a fluorescence intensity manager and programmable function keys for easy access to functions.

2.Leica Light Microscopes

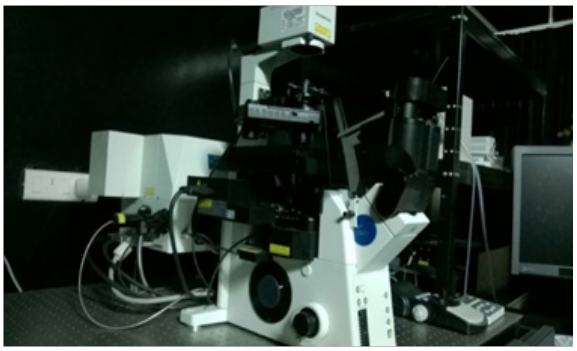
Leica DMIL (upright) and Leica DMI1000 (inverted) are available for users to perform routine light microscopy imaging. Both microscopes are provided with an inter changeable Leica DFC290 camera for high resolution bright field imaging. The Leica DMI1000 is also installed in the tissue culture facilities of individual labs and the Core tissue culture area.



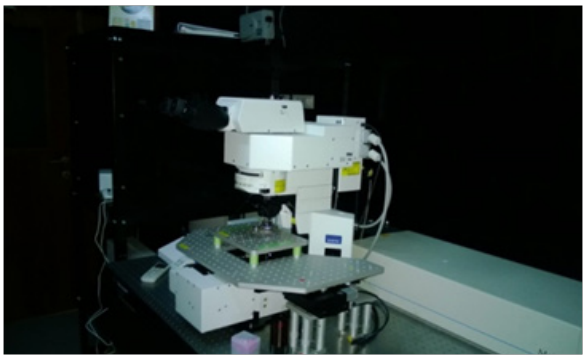
3. Zeiss Inverted Fluorescence Microscope

A Carl Ziess Axiovert 40 CFL equipped with 3 filters (DAPI,FITC and TRITC) for routine fluorescence imaging is available, along with a ProgRes C3 camera module for image acquisition.

4. 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 for live cell imaging. 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 be used to perform Multi-Area Time Lapse, FRET, FRAP, FLIM and diffusion experiments



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

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

Training Sessions

The Imaging Core Facility conducts training sessions regularly for both first time and experienced users. The training sessions comprise of specifically designed modules which include theory and practical sessions. The final authorization is given to the user upon successfully completing the required modules. The hands-on training sessions are tailored to the specific application requirement of each user so that they get the maximum benefit out of these systems. Apart from in-house training, the imaging core organizes sessions by application specialists from Leica and Olympus. Till date, 2 sessions for the Olympus FV1000 confocal microscope and 3 sessions for the Leica DMI6000B were conducted.

IV. Histopathology Core Facility:

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

Special stains standardized in 2012-2013:

1. 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.

2. Cytology: Cell block preparation.

V. 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

B. CSCR Laboratory Animal Facility:

» Faculty In-Charge: Aparna Venkatraman, PhD

» Scientific Officer: M.D Pratheesh. M.V.Sc ,PhD

» Veterinary Officer: Hemanta Kumar Maity. MVsc

» 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 and CMC, 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/PO/bc/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. CSCR-LAF maintains records of daily activities as well as breeding, maintenance and experimentation as per the statutory requirement of CPCSEA. Qualified veterinarians supervise all the animal health concerns, and provide all necessary veterinary care to ensure that healthy animals are available for research. Ad libitum supply of UV treated autoclaved R.O water and irradiated commercial diets were given to

animals. The CSCR-LAF is equipped with Small animal live imaging system, Multi photon microscope and Small animal irradiator with Co-60 as source in addition to a couple of Isoflurane anesthesia machines and Leica zoom microscopes.

The Scientific staff of the CSCR-LAF also conducts an orientation course for all animal users including PhD students and Project Assistants on mouse and rat bio-methodologies, principles of three R's, ethics, laws and guidelines on the regulation of scientific experiments on animals, hematological parameters, husbandry and care, animal identification techniques, sex differentiation, handling and restraint, and IACUC approved techniques for anesthesia and monitoring, drug administrations, blood collection, humane euthanasia etc.

Strains

The CSCR-LAF maintains ten different strains of mice - including knock out and SCID strains and a single strain of rat. The majority of rodent strains are bred under strictly inbred conditions.

	Strain	Description	Disease Model	Source
1	C57BL/6J	Inbred strain	Multi- Purpose model	Jax Lab, US
2	BALB c/J mice	Inbred strain	Inbred strain	Jax Lab, US
3	FVB/NCrl mice	Inbred strain	Mouse leukemia model	Charles River, UK
4	CD-1	Out bred strain	Sentinel animals, Pseudopregnancy	Charles River, UK
5	B6.129S4-F8tm1Kaz/J	Mutant Stock; Targeted Mutation	Hemophilia A	Jax Lab, US
6	B6.129P2-F9tm1Dws/J	Congenic; Mutant Strain	Hemophilia B	Jax Lab, US
7	B6;129S4 Pou5f1tm1Jae/J.			
	Mutant Stock; Targeted Mutation	OCT-GFP model	Jax Lab, US	
8	B6.129-Adamts13tm1Dgi/J	Congenic; Mutant Strain	Thrombotic Thrombocytic Purpura	Jax Lab, US
9	B6.CB17-Prkdcscid/SzJ	SCID	Transplantation studies	Jax Lab, US
10	C.B-17/Icr-Prkdc<Scid>IcrIcoCrl	SCID	Xeno Graft Research	Charles River, UK
11	Sprague Dawley	Rat- Outbred strain	Orthopedic surgery	Jax Lab, US

Quality control (QC)

Strict QC tests are performed routinely for microbiology, clinical pathology and genotyping to supply quality animals to in-house researchers. Routine sentinel animal sampling is being done in every three months to ensure the health status of breeding and experimental animals stock. Animal skin and hair samples are checked for ectoparasites. Fecal samples are checked for the endoparasites by

sedimentation method. Microbiological examination of animal room air, animal feed, water, bedding material, fecal samples and throat swabs are also being carried out in every month. Furthermore randomly selected serum samples are screened microbiologically by ELISA-based kits for selected rodent pathogens such as Mouse Pneumonia Virus (MPV), Mouse Lymphocytic Choriomeningitis Virus (MLCV), Mouse Noro Virus (MNV) and Mouse Hepatitis Virus (MHV). Blood samples of sentinel animals are checked for Mycoplasma pulmonis by PCR method. Genetic monitoring of mutant and SCID strains are conducted often by PCR. All report of QC are maintained in CSCR-Laboratory Animal Facility.

Protocols established

SOP's for Sub capsular renal cell transplantation and Retro orbital injection were established.

Ongoing research work:

Functional hematopoietic stem cell analysis in the mouse model by bone marrow transplan tation (Institution Fluid Research Funding)

C. 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. AleyaTabasum, 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 57 bone marrow samples (total yield of ~ 6.6x 10^9 MSC) and 7 placenta samples (total yield of ~2.5x 10^9 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. We plan to expand MSC 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.SukriaNayak (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.VrishaMadhuri (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 Work:

Treatment of large segmental bone defects with custom made triphasic hydroxyapatite scaffolds loaded with mesenchymal stem cells in children – Dv. VrishaMadhuri, Department of Paediatrics Orthopaedics, CMC, Vellore.

Training:

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:

i. Short term student projects (Bi-annual)

SHORT TERM STUDENTS

S. No	Name	Duration	Qualification	University	Project title	PI /Lab
1.	Mr. Elumalai. R	Jan 14 - Mar 14	M.Sc - Biotech	Thiruvalluvar University	Analysis of FANCD2 Ubiquitination in Patients with Bone Marrow Failure	Dr. Shaji / Lab -2
2.	Ms. Gayathri. D	Jan 14 - Mar 14	M.Sc - Biotech	Thiruvalluvar University	Phenotypic Characterization and Labeling of Mesenchymal Stem Cells Derived from Human Placenta with Lentivirus Expressing Enhanced Green Floursent Protein	Dr. Sanjay/ Lab- 3
3.	Ms. Angela Devanboo	Jan 14 - Jun-14	B.Tech - Biotech	VIT University	Cloning and Over expression of Herpes simplex virus - Thymidine kinase in Mesenchymal Stem Cells	Dr. Sanjay/ Lab- 3
4.	Ms. Keerthana. V	Jan 14 - Jun-14	B.Tech - Biotech	VIT University	Cloning and overexpression of Herpes simplex virus-Thymidine kinase in Mesenchymal Stem Cells	Dr. Sanjay/ Lab- 3

5.	Mr. Rajini. G	Jan 14 - Mar 14	M.Sc - Biotech	Thiruvalluvar University	Stealth AAV Vectors	Dr. Jayandharan / Lab-4
6.	Ms. Anjana Chandrasekhar	Jan 14 - Jun-14	M.Sc - Biotech	VIT University	Gene Transfer into Leukemic Cells	Dr. Jayandharan / Lab-4
7.	Ms. Deeksha Varma	Jan 14 - Jun-14	B.Tech -Genetic Engineering	SRM University	Micro RNA regulated AAV Vetors	Dr. Jayandharan / Lab-4
8.	Ms. Deepti Rana	Jan 14 - Jun-14	B.Tech-M. Tech (Dual Degree)	Amity University	Development of Polyacrylamide/Alginate Hydrogels as Human Bone Marrow-derived Mesenchymal Stem Cell Carrier for Tissue Engineering Applications	Dr. Murugan / Lab-8
9.	Mr. Abhishek Gowdar	Jan 14 - Jun-14	M.Sc - Biotech	VIT University	Identification, Isolation and Characterization of Colonic Epithelium and its surrounding Niche in Mouse models	Dr. Aparna / Lab-9
10.	Ms. Paloma Rosy Gomendes	May 14 - Jun 14	M.Sc - Biotech	Goa university	Estimation of knock-down efficiency of inducible shRNAs in a third generation lentiviral vector	Dr. Shaji / Lab - 2

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.

a. Governing Council of inStem

Dr. K. Vijay Raghavan	Secretary, DBT, New Delhi	Chairman
Dr. Satyajit Mayor	Director, inStem Bengaluru	Member
Dr. S. Ramaswamy	Dean, inStem, Bengaluru	Member
Dr. Apurva Sarin	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

b. 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 Apurva Sarin	Dean inStem, Bengaluru	Member
Dr.Alfred Job Daniel	Principal, Christian Medical College	Member
Dr Alok Srivastava	Head CSCR	Member Secretary

c. CSCR Sub Committee (Finance)

Director, inStem, Bengaluru	Chairperson
J.S. & F.A, Department of Biotechnology, Govt. of India, New Delhi	Member
Deans inStem, Bengaluru	Member
Director, Christian Medical College, Vellore	Member
Associate Director (Finance), Christian Medical College, Vellore	Member
Advisor, Department of Biotechnology, Govt. of India, New Delhi	Member
Joint Director, Department of Biotechnology, Govt. of India, New Delhi	Member
Head, Centre for Stem Cell Stem Research, CMC , Vellore	Member Secretary

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

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. Robbie P Singh	Treasurer	Member
Mr Ebernezer Sunderrajan	General Superintendent (Ag)	Member
Dr. Nihal Thomas	Addi. Vice Principal (Research)	Member
Dr. Molly Jacob	-	Member
Dr. Vikram Mathews	-	Member
Dr Alok Srivastava	Head CSCR	Member Secretary

Scientist:

S. No.	Name	Designation	Position
1.	Dr. Sanjay Kumar	Ramalingaswamy Fellow	Scientist
2.	Dr. Vrisha Madhuri	Professor, Department of Paediatric Orthopaedics	Adjunct Scientist
3.	Dr. B. Poonkuzhali	Professor, Department of Haematology	Adjunct Scientist
4.	Dr. Murugan Ramalingam	Associate Professor, Centre for Stem Cell Research	Scientist
5.	Dr. Jayandharan	Associate Professor, Department of Haematology	Adjunct Scientist Till Oct 2014
6.	Dr. Rekha Samuel	Professor of Pathology, Centre for Stem Cell Research	Adjunct Scientist
7.	Dr. R.V. Shaji	Professor, Department of Haematology	Adjunct Scientist
8.	Dr. Alok Srivastava	Professor, Head, Centre for Stem Cell Research	Adjunct Scientist
9.	Dr. Aparna Venkatraman	Associate Professor, Centre for Stem Cell Research	Adjunct Scientist
10.	Dr. Vikram Mathews	Professor, Department of Haematology	Adjunct Scientist
11.	Dr. Ari Chacko	Professor, Department of Neurosurgery	Adjunct Scientist
12.	Dr. Thomas Kuriakose	Professor, Department of Opthalmology	Adjunct Scientist
Post Doctoral fellows			
13.	Dr. Dwaipayan Sen	Post Doctoral Fellow	Till May 2014
14.	Dr. Ruchita Selot	Post Doctoral Fellow	Till Aug 2014
15.	Dr. Sabna. C	Post Doctoral Fellow	Till Sep 2014

Scientific and Technical Officers			
16.	Dr. Pratheesh. M.D.	Scientific Officer	-
17.	Dr. Muralidharan	Veterinary Officer	Till Jul 2014
18.	Dr. Hemanta Kumar Maity	Veterinary Officer	-
19.	Mr. Augustine Thambaiah	Technical Officer	-
20.	Mr. Vaidyanathan Subramaniam	Technical Officer	-
21.	Samarajayam Nara	Technical Officer	Project
Research Fellows			
22.	Ms. Sangeetha Hareendran	SRF	-
23.	Mr. Salar Abbas	SRF	-
24.	Mr. Syed Mohammad Musheer Aalam	SRF	-
25.	Mr. Vikram Sabapathy	SRF	-
26.	Mr. Nishanth Gabriel	SRF	Till Oct 2014
27.	Mr. B. Balaji	SRF	Till Oct 2014
28.	Ms. P.B. Sumitha	SRF	-
29.	Mr. Kannan Thoopil	SRF	-
30.	Mr. M. Thyagarajan	SRF	-
31.	Mr. R. Janakiraman	SRF	-
32.	Mr. Ajay Abraham	SRF	-
33.	Ms. Sreeja Karathedath	SRF	-
34.	Ms. Savitha Varatharajan	SRF	-
35.	Ms. Nancy. A	ARO	-
36.	Mr. S. Balasubramanian	JRF	-
37.	Ms. Akshaya Krishnagopal	JRF	Till Aug 2014
38.	Ms. Archana Kini	JRF	-
39.	Ms. Elizabeth Jayex Panakkal	JRF	Till Jul 2014
40.	Ms. Shylaja Arulkumar	JRF	Till Mar 2014
41.	Ms. Deepti Rana	JRF	-
42.	Mr. Karthikeyan. R	SRF	-
43.	Ms. Sowmya	JRF	-
44.	Ms. Mona	JRF	-
45.	Mr. David Livingston	Project Assistant	-
Technical staff			
46.	Ms. Aleya Tabasum	Graduate Technician	-
47.	Mr. P. Sathish	Technician	-
48.	Ms. J. Esther Rani	Technician	-
49.	Ms. Dhavapriya	Graduate Technician	On Project
50.	Ms. G. Kalaivani	Graduate Technician	On Project
51.	Ms. R. Saranya	Graduate Technician	On Project
52.	Ms. J. Saranya	Graduate Technician	-
53.	Ms. R. Pavithra	Graduate Technician	-
54.	Ms. P. Chitra	Graduate Technician	On Project
55.	Ms. R. Saranya	Graduate Technician	On Project

Annual Report 2013 - 2014

CENTRE FOR STEM CELL RESEARCH
(A unit of inStem,Bengaluru)

Christian Medical College Campus,
Bagayam, Vellore-632002,
Tamil Nadu, India.

Phone: 91-416-3075101, 3075107, 3075120
Fax: +91- 416 307-5103
Email: cscr@cmcvellore.ac.in
website: www.cscr.in