Annual Report 2015

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

Christian Medical College Campus
Bagayam, Vellore-632002

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

The Beginnings ..... 2005 - 2010.
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, to be established in collaboration with the Christian Medical College (CMC), Vellore in December, 2005.

As of July, 2011, CSCR (www.cscr.in) is integrated with inStem and exists as the translational research unit of inStem, Bengaluru (www.instem.res.in).

Mandate

The mandate of CSCR is to bring stem cell science to management of human diseases with unmet needs. This is to be done by developing research along clearly defined themes which will help enhance understanding of disease biology or help create innovative diagnostics and therapeutics that is relevant to the needs of the country. 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, 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 faculty from CMC and CSCR who meet regularly to resolve all matters at CSCR that require discussion and a Steering Committee, chaired by the Director, CMC, Vellore along with other administrative officers 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 sanctioned period of CSCR as a project, CSCR has integrated with inStem from 1st July, 2011 through an MOA between DBT inStem and CMC, Vellore. It continues to function at the Bagayam campus of CMC, Vellore with its emphasis on translational stem cell research and regenerative medicine. 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.

Translational Research Themes at CSCR

1. Gene therapy: This program is coordinated by Alok Srivastava with RV Shaji, Saravanabhavan Thangavel, Mohankumar Murugesan and Srujan Marepally and involves two major areas at present – The first is directed towards a clinical trial for AAV vector based gene therapy for haemophilia B in collaboration with Emory University, Atlanta, USA and the Powell Gene Therapy Centre as well as scientist at the University of Florida, Gainesville, USA. Given the success of AAV based gene therapy reported in the last 4 years, the plan here is to apply a similar yet innovative approach to initiate a clinical trial in India with a novel AAV. Towards this end, apart from these scientific elements, regulatory processes are being established through ICMR, CDSCO and DBT in India. The possibility of vector production at an industrial level is also being explored through a pharmaceutical partner in India. The second part of the gene therapy program involves pre-clinical models for lentiviral vector based gene therapy through hematopoietic stem cell for the major haemoglobin disorders. This is in collaboration with the Emory University, USA. Lentiviral vectors carrying the beta globin gene are tested in human *ex-vivo* erythropietic systems developed at CSCR. Work towards using genome editing technologies towards therapeutic gene corrections in stem cells has also been initiated. Other non vector mediated gene transfer technologies are also being explored.

2. Osteoarticular regeneration:

This program is coordinated by Vrisha Madhuri with her team. The major focus is on clinical translations related to physis, articular cartilage and bone regeneration. For articular cartilage regeneration, ongoing small and large animal studies have articular defect reconstruction with differentiated MSCs on indigenous scaffolds. The continued

follow up for human physeal regeneration with culture expanded autologous chondrocytes has shown success at 2.5 years follow-up and work is ongoing for similar physeal regeneration using 3D scaffolds in large animals. In a first of its kind study, reconstructions of bone defects in children with MSCs differentiated to osteoblasts on ceramic scaffolds have shown good outcome in the first 5 cases. A larger trial is being planned.

3. Cellular Reprogramming and its applications - Disease modeling and Haplobanking

The cellular reprogramming technology has been established by R.V. Shaji at CSCR. This is now being applied to two areas of disease modeling and haplobanking. Towards understanding the mechanisms of reprogramming, a shRNA library is being used to investigate the role of epigenetic factors in different stages of reprogramming. Results so far have identified specific histone methylases and protein arginine methylases involved in the late stages of reprogramming. The reprogramming technology is also being applied to the development of disease models of various bone marrow failure syndromes – Fanconi anemia, Diamond Blackfan anemia and congenital dyserythropoietic anemia. A major translational effort has also been initiated towards establishing a "haplobank", where the field and clinical aspects are being coordinated by Dolly Daniel and Alok Srivastava. This involves obtaining blood mononuclear cells from HLA haplotype homozygous normal individuals and creating a bank of these cells from which iPSCs are generated in a GMP compliant manner. This is part of an international consortium called the Global Alliance for iPSC Therapies (GAiT) for potential use in regenerative medicine in the future.

Other areas of Scientific Research:

Given the translational mandate at CSCR and the clinical needs and interests at the Christian Medical College, Vellore, there are several other areas of translational research that are also being pursued at CSCR. These include work on human mesenchymal stromal cells (hMSCs), with its immense possibilities of translational applications. This work in Sanjay Kumar's laboratory is aimed at exploring the biology of hMSCs from different sources with regard to their isolation, expansion, and manipulation for therapeutic use which are being evaluated in mouse models. Neuronally differentiated cells have shown promising results in a spinal cord injury model. Another area of research is vascular biology. This work is done in Rekha Samuel's laboratory and focuses on the cellular and molecular events involved in the interaction of human endothelial progenitor cells and perivascular cells, with a special interest in the diabetic pericyte. Fetal reprogramming in utero involving the gestational diabetes mellitus placental microvasculature is being evaluated. Given the wide possibilities for tissue engineering using scaffolds, Murugan Ramalingam's laboratory aims to develop biomaterials and scaffolds for stem cell delivery and to engineer physiologically functional tissues, in particular bone, cartilage and soft-to-hard interfacial organs. They are developing nanofiber and hydrogel-based 3D scaffolding system, both in injectable and implantable forms, with biomimetic characteristics of native tissue microenvironment, which could also be used in control/regulate cellular behavior.

Other Translational Stem Cell Research projects

This year CSCR was able to re-invigorate several projects related to specific areas of clinical needs. These included the following projects with different CMC faculty:

» "Efficacy of placenta derived mesenchymal stem cell in reducing corneal scarring in an ex-vivo organ culture model of post mortem human corneas"- Jeyanth Rose, Department of Ophthalmology.

» "Establishing an animal model of keloid to evaluate its pathogenesis and treatment options" Blessed Winston, Department of Pharmacology.

» "Study of human keloid fibroblasts in culture and effects of novel drugs" Aniket Kumar, Department of Pharmacology

» β chemokine expression and HIV-1 infection in CD34+ haematopoetic stem cells from HIV-1 positive patients – A pilot study - Ravikar Raphel, Department of Infectious Diseases.

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

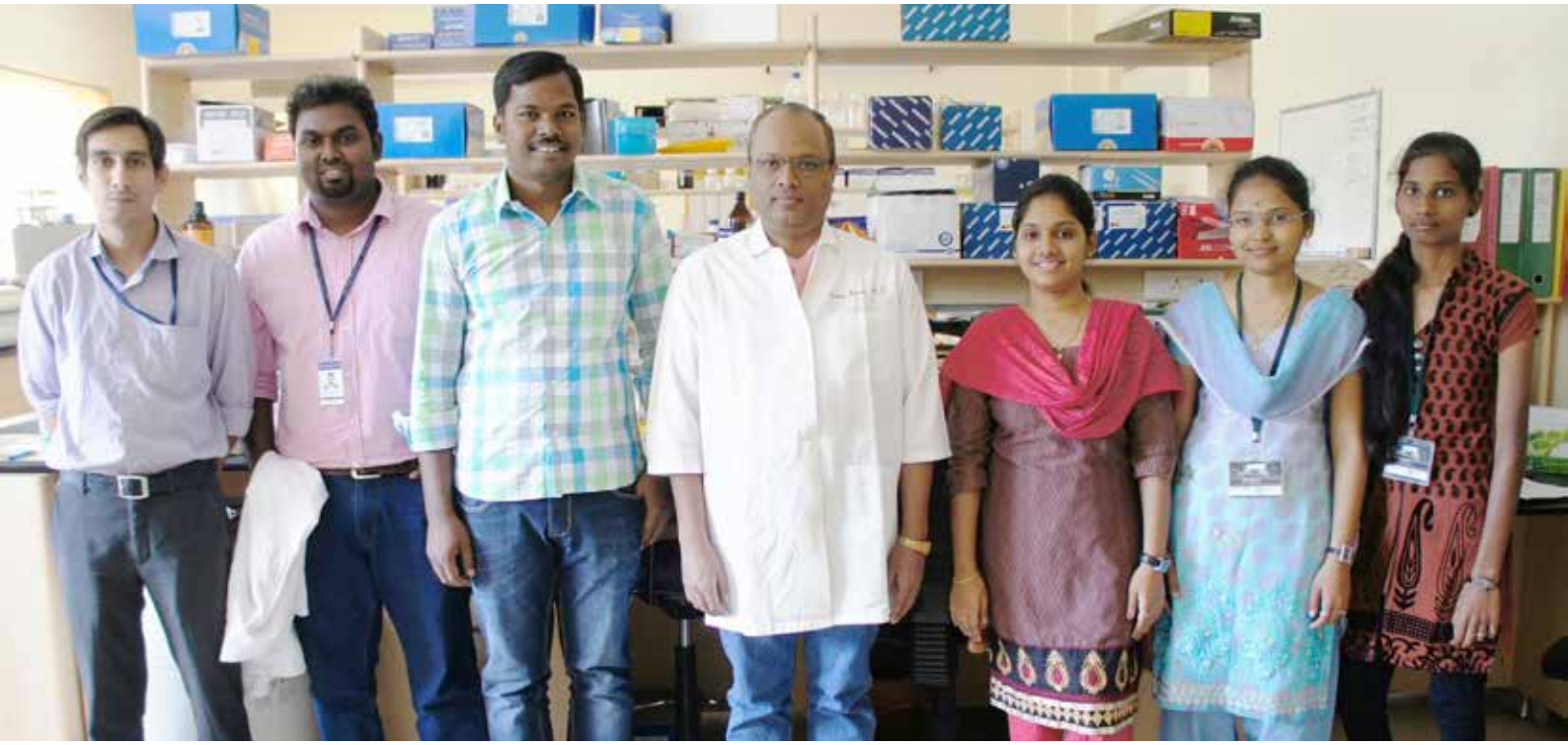
Training continues at CSCR through the PhD programs affiliated to the Sree Chitra Tirunal Institute of Medical Sciences and Technology, Thiruvananthapuram and the Thiruvalluvar University, Vellore. Short term training programs are also offered to MSc students from different universities.

CSCR continues to evolve and attempts to fulfill the mandate for which it was created.

Alok Srivastava

Research Profile

Sanjay Kumar, PhD
Scientist, Ramalingaswami Fellow



Preclinical work & Ongoing studies:

Hypothesis related to hMSCs Study Program: Primary human Mesenchymal Stem cell cultures more closely mimic the physiological state of these cells in vivo and thus may help generate more relevant data representing living systems.

My core scientific investigations are based on hMSCs. Despite hundreds of clinical trials using MSCs for a 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. Keeping the focus of the institution on translational medicine,I have started developing projects related to three fundamental aspects of MSCs based human clinical translation, which are:

- Efficiency of hMSCs based therapeutic approaches in preclinical models
- Augmentation in desired therapeutic outcome in preclinical models
- Safety and tracking fate of the transplanted hMSCs in mice models.

Mesenchymal Stem Cells (MSCs) 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. MSCs 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 (Lee et al. 2004), neurons (Resende et al 2010, Sanchez-Ramos et al. 2000), and glial cells (Tohill et al. 2004).Further, substantial ambiguities persist in the mesenchymal

biology field regarding functional identity, mode of isolation, their nature and experimental handling of MSCs. Thus, my research focus is on the role of tissue-derived mesenchymal stromal cells in normal and pathological tissue homeostasis. Ongoing studies on cellular interactions among niche components (HSCs and MSCs) of the human bone marrow will provide significant knowledge towards how, MSCs modulate tissues niche functions, their maintenance, and regeneration.

The stem cell, as a product, falls under the stringent quality control requirements imposed on a therapeutic product by industry regulators. These include validated measurements of purity, potency, efficacy, and stability. Problematically, there is no current measurement system that can completely define a cell using either an individual or a set of assays. Each cell type has different properties, and the mechanisms for their therapeutic influence on tissue homeostasis *in vivo* is frequently unclear and mostly attributed to paracrine factors and other MSCs derived factors influencing the homed tissue microenvironment.

Once adapted to *in vitro* culture conditions, primary cells undergo a limited, predetermined number of cell divisions before entering senescence. There are several challenges associated with the use of primary cells. One of the greatest hurdles primary cell culturists face is limited cell accessibility due to issues with donor tissue supply, difficulty with cell isolation/purification, quality assurance, and consistency, and contamination risks. Data comparability is also a serious problem with primary cell use and it arises out of variability among reagents used and the procedures implemented by individual laboratories to isolate and culture primary cells. The efficacy of the isolation and propagation depends on the source of the cells, the mode of collection and isolation, type of culture media, culture supplements and culture conditions. Among this, developing optimal culture conditions plays a significant role in defining the quality and quantity of the cell harvest. Also, the variation between different laboratories and individual scientists is a major obstacle that most often leads to difficulty and failure in isolating primary cells from published protocols. Even FBS from different batches in the same laboratory has a profound effect on primary cultures. The end-result from such variability leads to unreliable, non-reproducible results that are tough to reconcile and compare between laboratories. Even within the same laboratory variation between different preparations of primary cells is often an issue due to the variability of materials used in the media and serum preparation, forcing researchers to spend hours standardizing protocols. The ultimate solution for many of these problems is the creation of a standardized cell culture system that includes all the reagents and protocols leaving only the origin of the tissue as the major source of variability.

Following are scientific leads and research findings obtained from the projects in my laboratory related to human MSCs biology and understanding the role of MSCs in regeneration and pathological conditions:

Finding 1 for the question "Do perinatal tissue-derived hMSCs maintain their phenotypic attributes and retain intrinsic characteristics during long-term in vitro cultures?"

Publication 1: Long-term cultured human term placenta-derived mesenchymal stem cells of maternal origin displays plasticity.Sabapathy V, Ravi S, Srivastava V, Srivastava A, Kumar S. Stem Cells Int. 2012;2012:174328. doi:10.1155/2012/174328.

Publication 2: Quest for the alternate personalized clinical source of MSCs: Advancing towards hiPSCs

derived Quest for the alternate personalized clinical source of MSCs: Advancing towards hiPSCs derived iMSCs. Sabapathy V, Kumar S; Current Stem Cells Research and Therapy, Vol. 11, No. 2, 2016.

Publication 3: hiPSCs derived iMSCs: NextGen MSCs as an advanced therapeutically active cell resource for regenerative medicine. (in press, J of Cellular and Mol Med) Sabapathy V, Kumar S*.

Finding 2 for the question "Can human stem cell therapy augment the spinal cord injury healing in SCID mice following spinal cord injury?"

Project: A novel multifaceted approach to widening 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.

* Clinical trials using stem cells for spinal cord disease have been met with limited success as early and timely intervention is critical for functional recovery (Lu P et al. 2012; Zillai Z et al. 2012; Ianotti CA et al. 2011; Jiang MH et al. 2012)

Publication 1: Cell Therapy Augments Functional Recovery Subsequent to Spinal Cord Injury under Experimental Conditions.Sabapathy V, Tharion G, Kumar S. Stem Cells Int. 2015; 2015: 132172. doi: 10.1155/2015/132172

Publication 2: hMSCs / neurospheres derived from hiPSCs Augment functional recovery following spinal cord injury in SCID. manuscript in review. Sabapathy V, Murugan D, Tharion G, Ojha R, Samuel R, Kumar S*

Short Project Introduction: We have previously demonstrated that syngeneic rat olfactory ensheathing cells and bone marrow Mesenchymal stem cells (BM-MSC) were effective in motor function recovery (Tharion et al. 2011; Clinical Collaborator from Christian Medical College Vellore hospital for the spinal cord injury study) following spinal cord injury (SCI). Our recent finding of the plasticity of the human placenta-derived MSC also supports potential therapeutic applications of MSCs in allogeneic settings (Sabapathy and Kumar S et al. 2012 & 2014). We propose to test whether early interventions by immunomodulatory drugs and/or applications of mesenchymal stem cells (MSCs) / neurospheres derived from footprint-free human induced pluripotent Stem cells (iPSCs) will augment functional recovery following spinal cord injury in small animal models. Or, Since, Spinal Cord Injury in PTEN knockout mice have shown spontaneous regeneration of axons; we conceive this idea that forced up-regulation of mTOR activity in corticospinal neurons by conditional deletion of PTEN, a negative regulator of mTOR, may enhance compensatory sprouting of uninjured CST axons, therefore, should enable successful regeneration of a cohort of injured CST axons past a spinal cord lesion. As a proof of principle study in mice model, viral vectors expressing inducible shRNA for PTEN modulation will be tested for controlling the regenerative capacity of mice corticospinal neurons. The regrowth potential of CST axons will be tested, and this will be accompanied by a downregulation of mTOR activity in corticospinal neurons. We will also determine whether axonal injury further diminished neuronal mTOR activity in these neurons. Furthermore, we will assess if these regenerating CST axons have the ability to reform synapses in spinal segments distal to the injury. Thus, modulating neuronal intrinsic PTEN/mTOR activity for a transient period by small molecule inhibitors or siRNA might represent a potential therapeutic strategy for promoting axon regeneration and functional repair after adult spinal cord injury.

Specific Aims and Objectives:

Specific Aim 1: Assess the therapeutic applications of the early invention in SCI using mesenchymal stem cells (MSC) or in combination with virus-free, integration-free human iPS-derived neurospheres and/or immunomodulatory compounds in an SCID mice model.

Specific Aim 2: To evaluate, if viral vector expressing inducible PTEN shRNA expression in injured axon increases the axonal regeneration after early phase injury stabilization by reducing acute inflammatory responses using immune modulatory compounds.

Finding 3 for the question "what are the molecular characteristics of different native MSCs, *in vitro* cultured in 2D normoxia (21% oxygen) or 3D cultures with physiological oxygen (5% Oxygen) concentrations by transcriptome analysis, small RNA sequencing, secretome (cytokines, chemokines and growth factors), glycomics, lipidomic, metabolomics, proteomic and in vivo transplantation experiments?"

Short Project Introduction: The oxygen tension (or partial pressure) in most in vitro settings (~140 mm Hg) is considerably higher than that found in most mammalian and avian tissues. For example, the partial pressure of oxygen (pO2) in arterial blood has been measured at 60-90 mm Hg by Grant and Smith. These reports, and that of Kofoed et al. (1985) place the pO2 of bone marrow in the 27-49 mm Hg range. These tensions correspond to an oxygen concentration of approximately 4-6%. While organisms have evolved sophisticated mechanisms, including the enzymes glutathione peroxidase, catalase, superoxide dismutase, and use the antioxidants ascorbate and vitamin E to defend themselves against the toxic effects of free radicals derived from oxygen (Frank and Massaro, 1980; Halliwell, 1984), it is possible that these mechanisms are inadequate to protect cells when oxygen concentrations are unusually high.Thus, it is conceivable that many primary stem cells would function more normally *in vitro* at oxygen concentrations lower than 20%.

Finding 4 for the question "Can human perinatal tissue-derived MSCs (Placenta-derived MSCs or Wharton jelly-derived MSCs) augment therapeutic outcome in SCID mice models?"

BOOK Chapters:

» Bone defect repair in mice by mesenchymal stem cells. 2014; Kumar S. Humana Press, USA, part of the Springer Publishing Group.

» Therapeutic Application of Placental Mesenchymal Stem Cells Reprogrammed Neurospheres in Spinal Cord Injury. (Springer Publishing Press) Sabapathy V, Herbert FJ, Kumar S*.

» Therapeutic application of human Wharton Jelly Mesenchymal Stem Cells in skin injury. (Springer Publishing Press) Sabapathy V, Sundaram B, Kumar S*.

Publication 1: Bone defect repair in mice by mesenchymal stem cells.Methods Mol Biol. 2014; 1213: 193-207. Kumar S.

Publication 2: Decellularized amniotic membrane scaffold compared to synthetic PLGA and hybrid scaffolds exhibit superlative biomechanical properties for tissue engineering applications. (in Press, J of Biomaterials and Tissue Engineering) Sabapathy V, Hurakadli M, Rana D, Ramalingam M, Kumar S*.

Publication 3: Therapeutic Application of Placental Mesenchymal Stem Cells Reprogrammed Neurospheres in Spinal Cord Injury. (Invited Article, Methods MolBiol) Sabapathy V, Herbert FJ, Kumar S*.

Publication 4: Therapeutic application of human Wharton Jelly Mesenchymal Stem Cells in skin injury. (Invited Article, Methods MolBiol) Sabapathy V, Sundaram B, Kumar S*.

Research Finding 5: 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)

Finding 6 for the question "Can we track indocyanine green (ICG) labeled human mesenchymal stem cells (MSC) in SCID mice by non-invasive optical *in vivo* imaging using Xenogen Live-animal imaging system?"

Publication 1: Non-invasive optical imaging and *in vivo* cell tracking of indocyanine green (ICG) labeled human stem cells transplanted into thesuperficial or in-depth tissue of SCID mice. Sabapathy V, Jyothsana M, Paul MJ and Kumar S (Stem Cells Int. 2015: 606415).

Finding 7 for the question "How can we arm hMSCs with a cargo of our choice and deliver them for specific therapeutic purposes?"

Project Model: 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.

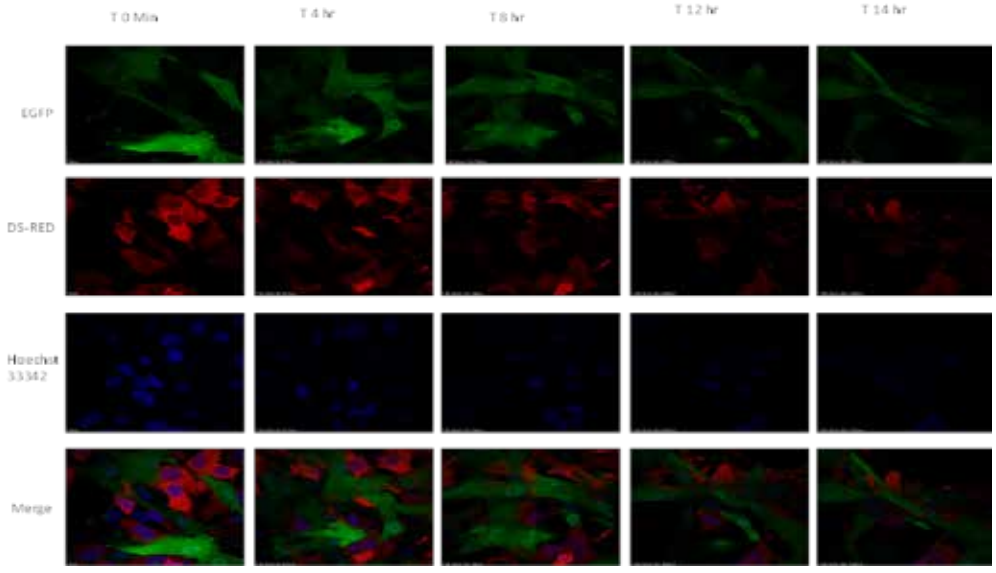


Figure: Confocal live cell time-lapse imaging of hWJ-MSC is expressing EGFP, suicide gene and co-cultured with cancer cells MCF-7 expressing dsRed Express. Showing apoptotic death of cancer cells by a reduction in red fluorescence with time mainly due to loss of dsRedlabeled MCF-7 cells

Finding 8 for the question "How MSCs differentiation is epigenetically regulated?"

Project: Biological studies on chromatin modulators for MSC osteogenic fate choices in metabolic bone diseases. (Proposal in preparation for DST funding)

LABORATORY HIGHLIGHTS

External Grants:

» Generation of a novel epigenetic factor shRNA library for studying the mechanisms of stem cell differentiation, disease pathogenesis and drug resistance. (Co-PI: Shaji RV) Total Funding: 79.04 Lakhs DBT Grant No. BT/PR8742/AGR/36/773/2013

» 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 # BT/PR8527/MED/31/234/2013. Total Funding: 36.19 Lakhs

» Foot-print free iPSC technology (2010-2016) Ramalingaswamifellowhip project. DBT Grant # BT/HRD/35/02/14/2009. Total Funding: 91.95 Lakhs

Grants preparations for submission Process:

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

» 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.

Completed studies & Funding:

» 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

Awaiting funding:

» 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. Funding: 30 Lakhs (successfully defended)

Publications since last report:

BOOK Chapters:

» Therapeutic Application of Placental Mesenchymal Stem Cells Reprogrammed Neurospheres in Spinal Cord Injury. (Springer Publishing Press) Sabapathy V, Herbert FJ, Kumar S*.

» Therapeutic application of human Wharton Jelly Mesenchymal Stem Cells in skin injury. Sabapathy V, Sundaram B, Kumar S*(Springer Publishing Press).

» Bone defect repair in mice by mesenchymal stem cells. 2014; Kumar S. Humana Press, USA, part of the Springer Publishing Group.

Scientific Journal Publications (18) since October 2010 with cumulative Journal Impact Factor (JIF): 54.167

» Non-invasive optical imaging and *in vivo* cell tracking of indocyanine green (ICG) labeled human stem cells transplanted at superficial or in-depth tissue of SCID mice. Sabapathy V, Jyothsana M, Paul MJ and Kumar S (Stem Cells Int. 2015: 606415) [Impact Factor:- 2.813]

» Quest for personalized source of MSCs: Advancing towards hiPSCs derived iMSCs. Sabapathy V and Kumar S (Current Stem Cells Research and Therapy, Vol. 11, No. 2, 2016) [Impact Factor:- 2.861]

» Decellularized amniotic membrane scaffold compared to synthetic PLGA and hybrid scaffolds exhibit superlative biomechanical properties for tissue engineering applications. (in Press, J of Biomaterials and Tissue Engineering) Sabapathy V, Hurakadli M, Rana D, Ramalingam M, Kumar S*.[Impact Factor:- 2.07]

» hiPSCs derived iMSCs: NextGen MSCs as an advanced therapeutically active cell resource for regenerative medicine. (in press, J of Cellular and Mol Med) Sabapathy V Kumar S*. [Impact Factor:- 5.8]

» Cell Therapy Augments Functional Recovery after Spinal Cord Injury under Experimental Conditions. (Stem Cells Int. 2015: 132172) Sabapathy V, Tharion G and Kumar S. [Impact Factor:- 2.813]

» Bone defect repair in mice by mesenchymal stem cells.Methods Mol Biol. 2014; 1213: 193-207. Kumar S.[Impact Factor:- 1.290]

» Therapeutic Application of Placental Mesenchymal Stem Cells Reprogrammed Neurospheres in Spinal Cord Injury. (Invited Article, Methods MolBiol) Sabapathy V, Herbert FJ, Kumar S*.[Impact Factor:- 1.290]

» Therapeutic application of human Wharton Jelly Mesenchymal Stem Cells in skin injury. (Invited Article, Methods MolBiol) Sabapathy V, Sundaram B, Kumar S*.[Impact Factor:- 1.290]

» 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)

Manuscripts submitted for peer review / in preparation if any:

» hMSCs or neurospheres derived from hiPSCs Augment functional recovery following spinal cord injury in SCID (in review) Sabapathy V, Murugan D, Tharion G, Ojha R, Samuel R, Kumar S*.

Presentations since last report: International Scientific Meetings (Poster Presentations):

» International Society of Stem Cell Research (ISSCR) Annual Meeting, Stockholm, Sweden-2015

» World Stem Cell Summit, Regenerative Medicine Capital, Atlanta, GA- 2015

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

SEMINARS GIVEN:

» Stem/Progenitor cells analysis, cells sorting and theirin vivo tracking and functional evaluations. JNCASR, Near Mahatma Gandhi Institute, Jakkur P.O., Bengaluru, Karnataka 560064.

» Biology and therapeutic applications of human MSCs- Goa University, Goa India.

» Mesenchymal Stem cells (MSCs) based therapeutic approaches in mice models. Thiruvalluvar University, Serkkadu, Vellore, Tamil Nadu.

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

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.

» 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 and co-inventor: Vikram Sabapathy.

COURSES TAUGHT:

» Stem Cell Biology Module courses for Graduate students

» Cell & Molecular Biology courses for Graduate students

» Gene Therapy courses for Graduate students.

Human Resource Development:

Students trained

» Trained 19 students for partial fulfillment of their M. Tech., B.Tech., M. Sc.(Biotech) and B. Sc. (Biotech) degree from across the country.

» Conducted 5 Stem cell workshops/courses and demonstrated mouse bone marrow mesenchymal stem cell isolation, in vitro characterization and immunophenotypic assays.

Honors& Awards

Ramalingaswami Fellowship (2010-Till Date)

Other Academic Activities:

Other Academic Activities:

Managing Core facilities as a Faculty in Charge:

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

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, DST, DBT (BIRAC), ICMR

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)

*Other Faculty Responsibilities:

Total University Grants Commission (UGC) Academic Performance Index (API) SCORE: 1201 Points

Nature of Activity:

- Research Publications, Scientific Abstracts in international and national meetings, Research Grants, Research collaborations and Use of participatory and innovative teaching-learning methodologies; updating of subject content, course improvement,etc.
- Examination duties (Question paper setting, Evaluation/Assessment of Seminars & answer scripts) as per allotment.

» Contribution and management of the department and institution through participation in academic activity faculty in charge and administrative committees and responsibilities.

» Preparation and Imparting of knowledge/instruction as per curriculum; syllabus enrichment by providing additional resources to students. Professional Development activities (such as participation in seminars, conferences, short term, training courses, talks, lectures, membership of associations, dissemination, and general articles).

» National Conferences: 1. National Stem Cell Symposium, 2011 2. International Stem Cell Biology Conference, 2012. 3. Stem Cell Biology Symposium, 2013 4. Developments in Stem Cell Technologies, 2014. 5. Recent advances in Biomedical Technology, 2015 6. Goa University, Goa

» Regional State Level: 1. VIT University Vellore, TN 2. Dhanlaxmi College Trichy, TN 3. Venkateshwara College, Pennalur, TN 4. Thiruvalluvar University, Serkkadu, TN 5. VIT University, Vellore, TN

Lab member Information

GROUP MEMBERS: (Students, JRFs, PostDocs, Trainees, etc.)

» Three Ph.D. Students. (One has completed his Ph.D.; Vikram Sabapathy; who happens to be the first Ph.D. student from Centre for Stem Cell Research, A unit of inStem Bengaluru, CMC Vellore, Bagayam, Vellore.

» One Laboratory technician on my Ramalingaswami Fellowship Grant.

» Ninteen (19) 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. R V Shaji, Dr. Rekha Samuel, Dr. Vrisha Madhuri, Dr. Alok Srivastava, Dr. George Tharion, Dr. Suresh Devhsayam, Dr. Paul MJ, Dr. Antony Devasia, Dr. Asha Abraham, Dr. Ashish Gupta, Dr. Margaret.

» inStem Collaborations: After organising robust grant resources to investigate mechanism of action for augmented therapeutic outcome obtained from the existing research leads, intend to collaborate with Dr. Pravin Vemula and Dr. Colin Jamora.

Vrisha Madhuri, MS Orth
Prof and Adjunct scientist

Work in Progress:

Bone regeneration – Patient recruitment for phase 1 trial for the treatment of large bone defects (gap non-union) in human using hydroxyapatite scaffold loaded with Mesenchymal stem cells is on-going. Five patients have undergone transplantation. First four children have shown radiological evidence of union. Fifth one has less than two months follow up. First child in addition to assessment of union with radiographs has also undergone CT evaluation and has shown good integration at both proximal and distal ends. There have been no serious adverse effects relating to transplants in any patient.

Articular cartilage:

Goat femoral head articular cartilage regeneration:

In this study the bone marrow MSCs were harvested and seeded on PCL electrospun scaffold and Chitosan hyaluronic dialdehyde scaffold. Cells loaded on the scaffolds were differentiated into chondrocytes and were transplanted into the goat femur articular cartilage defect. One of the goats was transplanted with GFP labeled cells to track the fate of the transplanted cells. The histology at 2 months post-operation shows evidence of regeneration. However we are awaiting 1 year follow up results to be able to get a conclusive data.

Treatment of Osteochondral defects in rabbits:

Articular cartilage is an avascular tissue present at the surface of the knee joints. It does not have the ability to regenerate upon injury or infection and eventually leads to osteoarthritis. An alternative strategy to treat articular cartilage defects is required. In collaboration with Dr. Dhirendra Katti, IIT, Kanpur funded by the Department

of Science and Technology, three hydrogel based scaffolds have been designed to test for articular cartilage regeneration in an osteochondral defect (rabbit) model. One month follow up shows evidence of hyaline like cartilage regeneration at the site of cell-seeded constructs; the gross appearance of the test groups are close to the native cartilage. However this needs to be confirmed by further analysis and we are awaiting the long term follow up results. Success of this hydrogel based scaffold will be further evaluated in the large animal study before the clinical translation.

Muscle Satellite Cell Co-Culture studies:

The muscle satellite cells which are located between the sarcolemma of myofibers and basal lamina possess a remarkable ability for muscle regeneration in case of muscle injury. However depletion of muscle satellite cells fails to regenerate in case of muscle injury and muscle related diseases. Since there is no specific treatment, cell therapy using muscle satellite cells offers a hope for muscle regeneration. The differentiated (myoblast) and cultured muscle satellite cells have been transplanted in humans and animals and proven not to be very effective as hoped to be in long term muscle regeneration. This was attributed to inability of the transplanted cells to maintain its quiescent state there by contributing to the long term muscle regeneration. Hence it is necessary to understand the niche which harbors satellite cells and helps in maintaining its stemness. Muscle satellite cells residing in niche are always found in close proximity to the endothelial cells. We planned to look at the cellular components that may help in maintaining the stemness and help in proliferation of the muscle derived stem cells

In these experiments we attempt to study the effects of endothelial cells and mesenchymal stromal cells on the proliferation, phenotype maintenance of muscle derived satellite cells/myoblast. Based on the outcome, we infer that co-culturing satellite cells with endothelial cells, affects the rate of proliferation of satellite cells whereas with the MSCs, it promote myogenesis; however no significant effect was observed on cell proliferation.

Cancer stem cells:

Osteosarcoma is the most common primary tumor of the bone in children characterized by heterogeneity and variability in the chemo sensitivity due to number of mutations and high rate of recurrence. Cancer stem like cells (CSCs) have been isolated from the osteosarcoma cell lines but characterization of human osteosarcoma tissues has not been performed.

In this study, tumour cells have been isolated from tissue of patients with osteosarcoma. Aldefluor and tumor sphere methods have been used to study cancer stem like cells. The tumour cells and spheroids are being characterized in vitro for embryonic and stemness markers (CD 133, CD 117, Stro1, SOX 2, OCT4, NANOG, ABCG2, CXCR4 and ABCB1 assay). In vivo tumorigenicity or "virulence" of these cells is being assessed through the xenograft by using the SCID mouse model. In vitro chemo sensitivity is being performed using methotrexate, doxorubicin and cisplatin.

Chemo sensitivity test in future may form a laboratory tool to know the aggressiveness of the tumor before the surgical intervention and availability of the tumor necrosis data. This may have an impact over the future management of the osteosarcoma.

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. A pilot study with six rabbits was conducted and histological changes were were observed. Both low and high energy shock wave induces increase in growth plate height by 14% and 26% respectively after 1 month follow up. We also observed

that shockwave increases cell proliferation and collagen X secretion. Based on the short term follow up, we observe that shockwave is inducing histological changes within the growth plate. In order to see an actual difference in bone length, the animals will have to be followed up until fusion after treatment. In parallel, additional experiments will be performed to explore the mechanism behind shockwave treatment on growth plate and bone.

LABORATORY HIGHLIGHTS OF YEAR

Grants, Ongoing studies & Funding

Project 1:

Title: In vitro and In vivo testing of a layered 3D Composite Scaffold for Articular Cartilage Tissue Engineering
Funding agency: Department of Science and Technology
Budget: Rs. 48.82 lakhs

Project 2:

Title: Treatment of large segmental bone defects with custom made triphasic hydroxyapaptite scaffolds loaded with mesenchymal stem cells in children
Funding agency: Department of Biotechnology
Budget: Rs. 56, 87,000

Project 3:

Title: Mutation analysis of WISP 3 gene in patients with Progressive Pseudorheumatoid Dysplasia
Funding agency: Institutional fluid grant
Budget: Rs. 75, 000

Project 4:

Title: Muscle satellite cells on scaffolds from human tissue
Funding agency: Institutional fluid grant
Budget: Rs.1.46 lakhs

Project 5:

Title: Comparison of different coating substrates for muscle satellite cell adherence, proliferation and self-renewal
Funding agency: Institutional fluid grant
Budget: Rs. 50,000

Project 6:

Title: In vivo effect of shockwave on rabbit growth plate
Funding agency: Institutional fluid grant
Budget: Rs.1, 00,000

Project 7:

Title: Wharton jelly mesenchymal stem cells on scaffolds for the treatment of second degree burns: Animal model
Funding agency: Institutional fluid grant
Budget: Rs.2, 00,000

Project 8:

Title: Isolation and in vitro and in vivo characterization of cancer stem like cells (CSCs) from human osteosarcoma tissue and assessment of chemo sensitivity
Investigator: Dr Sanjay K Chilbule
Supervisor: Dr Vrisha Madhuri
Funding agency: Wellcome trust DBT India Alliance
Budget: Rs 34.9 Lakh

Project 9 (funds awaited):

Title: Differentiation of mesenchymal stem cells (MSCs) into chondrocytes by sustained delivery of miRNAs using chitosan hydrogel.
Funding agency: Department of Science and Technology
Budget: Rs. 74 lakhs
Completed studies & Funding

Title: Musculoskeletal stem cell in tissue regeneration
Funding agency: Danish council for strategic research, Denmark and Department of Biotechnology, India (Indo Danish collaborative program)
Budget: 100,000 euros

Awaiting approval:

The following projects have been submitted for funding and it is under review.

Project 1:

Title: Molecular genetic analysis of Osteogenesis imperfecta in Indian Children
Funding agency: Indian Council of Medical Research
Budget: Rs. 69.00 lakhs

Project 2:

Title: Culture expanded satellite cells/myoblasts for the treatment of urinary incontinence in female patients with urethral sphincter insufficiency
In collaboration with Urology II, Gynecology unit II, Radiology
Funding agency: Indian Council of Medical Research
Budget: Rs. 88.3 lakhs

Publications since last report

» Madhuri V, Santhanam M, Sugumar LK, Rajagopal K, Chilbule SK. Classical and atypical Fibrodysplasia Ossificans Progressiva in India. Ann Hum Genet. 2015 Jul; 79(4):245-52.

» B Balakumar, Rajagopal K, Madhuri V. Bone marrow extract as a growth supplement for human growth plate chondrocyte culture. Indian Journal of Medical Research (accepted for publication)

Patents:

"Padmapada"– a sensor equipped brace for clubfoot is patented and applied for international patent and is selected for "Innovations in Science and Biotechnology" at Rashtrapati Bhavan on March 11, 2015.

Students trained

S.No	Student	Qualification	Place
1.	Mr. Santhosh kumar	B.Tech Biotechnology	Arunai Engineering College
2.	Ms. Shikha Nishith	12th grade	London
3.	Dr. Raj Bharath	MBBS	Coimbatore Medical College
4.	Dr. Sasank	MS(Surgery)	CMC Vellore

Proposed Clinical work

Allogeneic mesenchymal stem cells for critical size bone defects:

Based on the (5 patients) results of our ongoing clinical trial for bone regeneration using autologous MSCs on customized HASi scaffolds, we are in the process of initiating a new study for small size bone defects using allogeneic MSCs. Several preclinical studies have shown that allogeneic MSCs on scaffolds have the potential to heal critical size bone defects without immunosuppressive therapy. In this we propose to use allogeneic MSCs on HASi for non-unions less than 3 cm. This can eliminate the need for autologous bone marrow harvest, expansion and differentiation.

Autologous chondrocyte transplantation for physeal injury:

Five children with eight physeal bars (5 distal femurs and 3 proximaltibias) of a size greater than 30% of the physis were treated with autologous chondrocyte transplantation. After a mean follow-up of 3 years 5 months, six out of eight physis were continuously growing without any bony bar formation. Following success of this project, we are in the process of submitting application for funding, and ethical clearance for phase 2 clinical trials with objective of treating 10 children.

Muscle Sphincter Regeneration:

Translational use of the satellite cells to develop new skeletal muscle for clinical application has been promising and there is tremendous potential in treating various muscle related disorders. The use of satellite cells for treatment of muscular dystrophy diseases has been postulated and studied in detail. They are also being investigated for debilitating conditions like urinary incontinence in animal models and anal sphincter injuries. It's estimated that

more than 200 million people suffer from sphincter injuries worldwide, current treatments like use of bulking agents, tapes and other invasive methods though offering a temporary solution does not improve the conditions of the disease. In our hospital, we have several patients being treated every year for the same. In an effort to improve the condition of these patients, we have proposed to use autologous culture expanded muscle derived stem cells/ satellite cells for the treatment of damaged sphincter injuries for conditions such as urinary incontinence and fecal incontinence in collaboration with other related departments in CMC. A proposal in regeneration of sphincter muscle using satellite cells has been submitted to ICMR and awaiting for the ethical and financial clearance.

Any major scientific breakthroughs not captured above.

Five children have undergone bone transplantation using autologous bone marrow mesenchymal stem cells differentiated on customized hydroxyapatite scaffolds for large segmental bone defects. The results show union at 2 months with no adverse effects so far.

Honours & Awards

» Picture award- Dr Sanjay K Chilbule received Best research image prize at 1st India Alliance Research Image Competition 2015 at Hyderabad in November 2015.

» Dr. Vrisha Madhuri was awarded travel grant to present the poster titled "Autologous culture expanded chondrocytes from the iliac crest apophysis in the treatment of physeal bars in children" at the Annual meeting of International Society for Stem Cell Research (ISSCR) at Stockholm, Sweden in June 2015.

» Dr Vrisha Madhuri was honoured to present the Padma Pada -Compliance monitored clubfoot brace at Exhibition on "Developments in Medical Technology" at Rashtrapati Bhavan in March 2015.

» Ms. Sowmya Ramesh was awarded a scholarship from Sällskapet Barnavård, Sweden as a part of her PhD project – March 2015.

» Mr. David Livingstone won the consolation prize for the poster titled "Isolation, culture and characterization of skeletal muscle satellite cells for human rectus abdominis" at CMC Annual Research Day, October 2014.

Poster / Conference

» Paper titled "Isolation and characterization of the cancer stem cells (CSCs) from wild type osteosarcomas and comparison with Saos2 cell line" has been selected for presentation in the Best paper category in 21st annual national conference of Paediatric Orthopaedics Society of India at Bangalore, Hyderabad in January 2016. (Dr Sanjay Chilbule, Karthikeyan & Dr Vrisha Madhuri)

» Dr. Sanjay K Chilbule presented poster on update of "Isolation and in vitro and in vivo characterization of cancer stem like cells (CSCs) from human osteosarcoma tissue and assessment of chemo sensitivity" at the annual meeting of Wellcome trust DBT India Alliance fellow's meeting at Hyderabad in Nov 2015

» Ms. Sowmya Ramesh presented poster titled "Tissue engineered bone from lime, sand and bone mineral: A new treatment for segmental bone loss at the Annual Research day – October 2015.

» Dr. Vrisha presented poster titled "Autologous culture expanded chondrocytes from the iliac crest apophysis in the treatment of physeal bars in children" at the annual meeting of international society for stem cell research (ISSCR) at Stockholm, Sweden in June 2015

» Dr. Vrisha presented poster titled "Pamidronate negatively regulates the osteogenesis in MSCs of fibrous hamartoma in congenital pseudarthrosis of the tibia" at the annual meeting of international society for stem cell research (ISSCR) at Stockholm, Sweden in June 2015

» Mr. Karthikeyan Rajagopal presented poster titled "Effect of endothelial cells on the muscle satellite culture" at the CMC Annual Research Day,2014

» Mr. David Livingston presented poster titled "Isolation, culture and characterization of skeletal muscle satellite cells for human rectus abdominis" at the CMC Annual Research Day, 2014

» Ms. Sowmya Ramesh presented poster titled "Role of parathyroid hormone related peptide (1-34) during periosteal mesenchymal stem cell differentiation to chondroctyes" at the CMC Annual Research Day, 2014

Other Academic Activities:

Dr. Vrisha Madhuri – Lecture on "Cell-based therapies - Bone & Cartilage" for PhD students.

Lab member Information

S.No	Name	Designation
1.	Prof. Vrisha Madhuri	Principal Investigator, Prof. & Head Paediatric orthopaedics
2.	Dr. Sanjay K Chilbule	Clinical Research Fellow
3.	Karthikeyan R.	Senior Research Fellow
4.	Sowmya R.	Junior Research Fellow
5.	David Livingstone I.	Junior Research Fellow

Collaborations:

International Collaboration:

» Dr. Henrik Daa Schrøder, Professor Pathology, University of Southern Denmark, Denmark

» Dr. Jørgen Kjems, Professor Department of Molecular Biology, University of Aarhus, Denmark

» Prof. Lars Savendahl, Professor of Pediatric Endocrinology, Karolinska University Hospital, Stockholm, Sweden

National collaboration (Both Inter and intra institutional Collaboration):

» Dr. Prabha D.Nair, Scientist G, Tissue Engineering and Regeneration Technologies Division, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Trivandrum.

» Dr. Harikrishna Varma, Scientist F, Tissue Engineering and Regeneration Technologies Division, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Trivandrum.

» Dr. Annie John, Scientist F, Tissue Engineering and Regeneration Technologies Division,Sree Chitra Tirunal Institute for Medical Sciences & Technology, Trivandrum.

» Dr. Dhirendra S. Katti, Professor Department of Biological Sciences & Bioengineering, Indian Institute of Technology, Kanpur

» Dr. Nihal Thomas, Professor Department of Endocrinology, CMC, Vellore

» Dr. Susan Jehangir, Associate Professor, Department of Paediatric Surgery, CMC Vellore

» Dr. Sukriya Naik, Professor, Department of General Surgery,Unit -4, CMC Vellore

» Dr. Nitin, Professor, Urology, Unit II – CMC Vellore

Srujan Kumar Marepally, PhD
Scientist

Our lab was started in October 2015. We aim to develop non-viral gene therapy approach for autoimmune disorders such as psoriasis and genetic disorders such as hemophilia.

Our research goals include:

Epidermal immune regulating gene therapy for psoriasis.

Psoriasis is a chronic autoimmune skin disorder with a substantial negative impact on the patient's quality of life. To date, a wide range of treatment options such as topical delivery of corticosteroids, vitamin D derivatives, phototherapy, and administration of immuno-modulators are available. However, these approaches have major limitations such as a plethora of side effects and skin develops resistance to treatments over time. In addition, therapeutic outcome of psoriasis treatments also can be unpredictable. Thus, need to develop efficient and safer psoriasis therapeutics exits. In psoriatic condition, overactive immune system triggers skin cells to grow which subsequently pile up on the surface to form psoriatic plaques. Basic characteristics of psoriatic plaques are

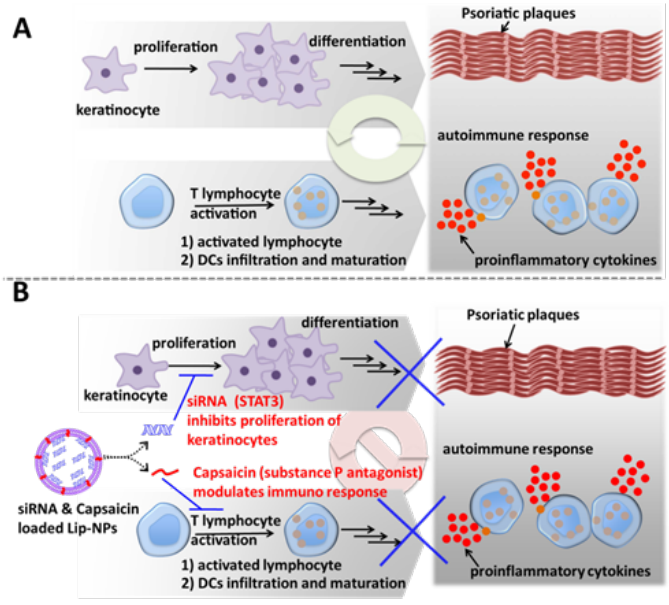


Fig.1. A) Two major pathological contributors for psoriasis disease, i) hyperproliferation of keratinocytes leads to psoriatic plaque formation and ii) activation of lymphocytes leads to autoimmune response. B) Our approach for combination therapy is co-delivery of siRNA (to inhibit hyperproliferation of keratinocytes) and capsaicin neuropeptide antagonist (to suppress the autoimmune response).

i) thick epidermis, due to increased proliferation of keratinocytes, and ii) significantly infiltrated mononuclear leukocytes (T cells and dendritic cells) in dermal region (Fig-1A). Thus, a rational combination treatment that targets epidermal alterations and inflammation may provide enhanced efficacy, in a synergistic manner. Recent research on neuro-cutaneous connections revealed that neuropeptides play a vital role through modulation of both keratinocyte proliferation and dendritic cell (DC) infiltration. Several factors influence the psoriasis disease pathology. Thus, targeting multiple factors such as simultaneous inhibition of keratinocyte hyperproliferation to prevent plaque formation while suppressing immuno-response could be an efficient strategy to combat against psoriasis. To best of our knowledge, till to date, no attempt has been made to target immune and neuro-cutaneous connections together to achieve therapeutic efficacy in psoriasis treatment. Towards that end, we aim to develope a combinatorial delivery system of a small interfering RNA (anti-STAT3 siRNA) to inhibit keratinocyte hyperproliferation, and a neuropeptide antagonist (capsaicin) to reduce the immune response (Fig-1B). The siRNA against STAT3 will be delivered in combination with capsaicin (substance P antagonist) to the skin lesions to knock-down the levels of STAT3 and substance P (SP), respectively. These factors involved in multiple aspects of psoriasis pathogenesis and dysfunctional crosstalk among antimicrobial peptides and cytokines such as TNF-∂.

Transdermal delivery of therapeutics through topical route is an effective approach for treatment of psoriasis. The main advantages of topical delivery system are to bypass first pass metabolism and minimal or no systemic toxicity. However, delivery of macromolecules such as peptides and nucleotides at higher concentrations into the deeper layers of skin remains as a major challenge till to date. In our preliminary effort, we have developed a new class of nanoparticles coated with cationic lipids with pyrrolidiniumheadgroup. These lipids breached skin barrier effectively and delivered macromolecules such as siRNA in deeper dermal milieu (unpublished work). Thus, taking cues from preliminary data, we aim to generate a library of fusogenic lipid based nanocarriers to load STAT3 siRNA and capsaicin with higher encapsulation efficiency and deliver the payload to the deeper layers of the skin with minimal or no skin damage, for an immuno therapy of psoriasis.
To test the hypothesis, specifically, we aim to :

Aim-1: Design and synthesis of novel skin penetrating lipids, and fabrication of lipid-nanoparticles (Lip-NPs) through encapsulation of anti-STAT3 siRNA&Capsaicin. Novel amide linker based keratinolytic cationic lipids varying acyl chains from palmitoyl to stearyl (Fig-2, Lipids LA-11, MA13, PA-15 and SA-17) are synthesized. Effect of chain length variation in hydrophobic tail on self-assembly will be systematically evaluated. We will optimize the formulations to achieve high payload (siRNA and neuropeptide antagonist) controllable particle size, sustained drug release profile and extended

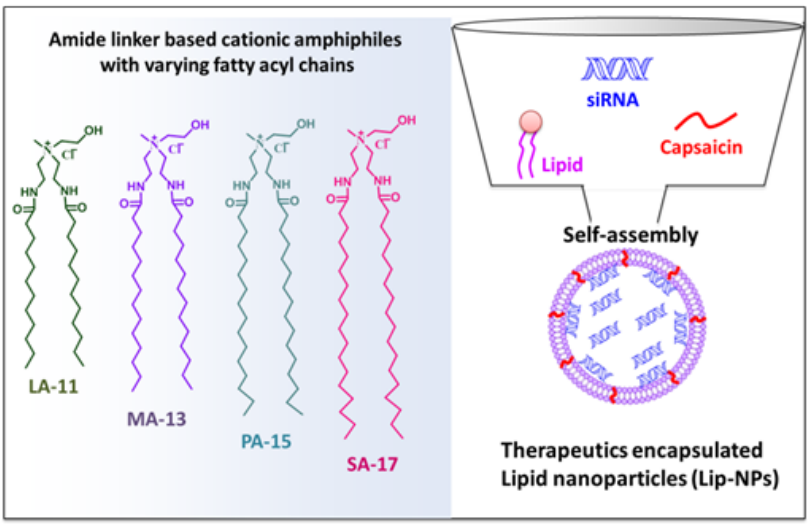


Fig.2.Chemical structures of amide linker based lipids with varying fatty acyl chains. Schematic illustration shows the formulation of Lip-NPs. Lip-NP comprises a fusogenic cationic lipid embedded with capsaicin in hydrophobic core, and siRNAis encapsulated within NPs.

shelf-life. keratinolytic cationic lipids varying acyl chains from palmitoyl to stearyl (Fig-2, Lipids LA-11, MA13, PA-15 and SA-17) are synthesized. Effect of chain length variation in hydrophobic tail on self-assembly will be systematically evaluated. We will optimize the formulations to achieve high payload (siRNA and neuropeptide antagonist) controllable particle size, sustained drug release profile and extended shelf-life.

Aim-2: Evaluate skin permeation of Lip-NPs, and delivery of therapeutic agents - ex vivo study. Efficacy of transdermal delivery of therapeutic agents through Lip-NPs will be assessed in dermatomed human skin using Franz diffusion cells under in vitro conditions. The distribution of Lip-NPs in different skin layers will be studied using cryotome techniques.

Aim-3: Study the effect of co-delivery of siRNA and capsaicin on alteration in secretion profile of cytokines – in vitro study.

The STAT3 plays a role in activation of keratinocytes (KCs) and immunocytes that required for development of psoriasis. Thus, efficacy of siRNA to knock-down STAT3 in KCs and immunocytes will be evaluated through quantification of cytokines such as IL-17, IL-22, IFN-γ, TNF-α using ELISA assays. In addition, sensory substrate P binds to receptors on DCs and KCs in a peptide specific manner to enhance DCs migration and maturation in the skin. The corollary is this leads to T cell infiltration and activation. Inhibition of substrate-P decreases CD11c+and CD4+T cell numbers, which leads to immune suppression. CD11c+and CD4+T cell numbers will be assessed in H&E staining. The pathways influenced with the synergism of immune and neuro-cutaneous modulators will help to refine understanding of the complex interactions that exist in psoriasis.

Aim-4: Determine the therapeutic efficiency of topically applied therapeutic agents loaded Lip-NPs using Imiquimod induced psoriasis like mouse model.Imiquimod induced psoriasis like mouse modelwill be used for evaluating the therapeutic efficacies of Lip-NPs.We willalso probe neuro-inflammatory responses upon the administration of nanoparticles. Efficacy of therapeutic agents loaded Lip-NPs will be assessed using multiple parameters such as quantification of psoriasis area and severity index (PASI), immunohistochemistry and H&E staining.

2. Liver targeted liposomal gene therapy for hemophilia

(in collaboration with Dr. Alok Srivastava, CSCR, Vellore)

Hemophilia A is a hereditary coagulation disorder caused by deficiencies in coagulation factor VIII (FVIII) in one per 5000 males. Although intravenous injection of recombinant or plasma-derived human FVIII is being used as a therapeutic option, the need for frequent intravenous injections is a major concern to patient compliance and affects patient quality of life. Moreover, the development of antibody against the clotting factor FVIII over the period of time complicates the treatment in patients with these developed inhibitors.Higher therapeutic doses for a long period of time (months to years) is needed for the immune tolerance, that makes treatmentsignificantly expensive (>$1,000K), and often have to be stopped due to anaphylactic reactions or nephritic complications. New agents against these inhibitors such as obizur, alb-rFVIIa-FP, theraPEG-FVIIa are in clinical trials. However complexity and expenses of the treatment limits its application to over 80% of the population in the developing world. Hence less

immunogenic and less expensive treatment options are needed for the broader disease population. Gene therapy represents a promising an alternative approach to the current treatment of hemophilia that would ideally reduce the frequent injections and also overcome immune alterations as the clotting factor is naturally produced. Among transfection vectors, viral vectors proved to be efficient and Adeno Associated Virus (AAV) found to be effective for factor IX (F-IX) gene therapy. Major limitations with AAVs include inability to deliver larger gene FVIII and may cause insertional mutagenesis. Cationic lipids hold promiseamong these non-viral transfection vectors fortheir non-immunogenic nature; robust in preparation,more importantly they can deliver genetic payloads to specific body cells by grafting receptor specific ligands.

Asialoglycoprotein receptor (ASGPRs) appears to be of great therapeutic potential since hepatocytes are responsible for the synthesis of Factor VIII (F-VIII) and Factor IX (F-IX) proteins. Galactose is a strong ligand for ASGPRs. Hence it can be a most promising tool to deliver therapeutic genes into hepatocytes to treat hemophilia. Previously, we developed galactosylated lipid based liposomal systemsfor delivering genes to mouse liver (Srujan M. et al., Biomaterials, 2009). In collaboration with Dr. Alok Srivastava, we intend to explore these liposomal systems for the gene therapy to hemophilia. Our current work aims at developing efficient liposomal and lipid emulsion system with increased transfection efficiency for factor VIII gene to liver and less immune responses. Since primary cells such as hepatocytes are hard to transfect, we will study the endosomal escape mechanism to understand the transfection profiles of the galactosylatedlipoplexes. Finally, we will evaluate the therapeutic efficiency with factor VIII gene in hemophilia A mouse model. The parameters for successful therapy are the elevated levels of FVIII, expression, without organ toxicity and zero to minimal immune responses

Grants

1. CSCR start-up grant.
2. Fast Track Grant for young scientists, DST-SERB, Govt. of India.
3. Biotechnology Ignition Grant (BIG), BIRAC, Govt. of India.

Lab member Information

Rasajna Nadella: Post-doctoral Fellow

Priya Dharmalingam: Student trainee (M.Tech Project)

Collaborations:

Dr. Alok Srivastava, CSCR

Dr . R. V. Shaji, CSCR

Dr. Sanjay Kumar, CSCR

Dr. Saravanabhavan Thangavel, CSCR

Dr. Murugan Ramalingam, CSCR

Dr. Mohan Kumar Murugesan, CSCR

Dr. Rajkumar Banerjee, IICT, Hyderabad

Dr. Arabinda Chaudhuri, IICT, Hyderabad

Dr. VGM Naidu, NIPER, Hyderabad

Dr. Praveen Kumar Vemula, inStem

Dr. Dasaradhi Palakodeti, inStem

Poonkuzhali Balasubramanian, PhD.
Adjunct scientist

Broad Research interests: Pharmacogenetics of anticancer agents, Molecular genetics of leukemia, anticancer drug resistance, conditioning regimen in hematopoietic stem cell transplantation.

Brief outline of completed and ongoing research:

Completed projects:

» Pharmacogenetics of cytarabine and daunorubicin resistance in the leukemic stem cell (LSC) compartment in acute myeloid leukemia- INSERM- ICMR; 2011-2014.

» Pharmacogenetics of cytarabine and daunorubicin in Acute Myeloid Leukemia- Department of Biotechnology, 2009-2014.

» Mechanisms of Imatinib resistance in Chronic Myeloid Leukemia- Department of Biotechnology, 2009-2014.

Major findings from the completed research projects:

These were comprehensive studies comparing RNA expression and genetic variants in enzymes and transporters involved in ara-C and daunorubicin metabolism and transport with ex-vivo cytotoxicity to ara-C and daunorubicin in bulk AML as well as leukemic stem cells. We have shown that the RNA expression in the genes involved in the metabolism and transport of ara-c/ Dnr showed wide inter-individual variation in AML patients and there were potential functional polymorphisms in most of the genes which could partly explain variation in treatment outcome. Expression of Ara–C influx transporter hENT1 was identified to be significantly lower while that of the efflux transporters ABCG2, ABCB1 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 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.

Summary of ongoing research projects:

In the Wellcome DBT funded study, the overall aim is to understand the reasons for variability in hematopoietic stem cell transplantation (HSCT) outcome in terms of conditioning regimen and to personalize this regimen for individual patients.

As part of the "Centre of excellence on Evaluation of Biology and Mechanisms of Resistance in Leukemia" there are two individual research grants Residual leukemia cells are a potential source of relapse, and there is considerable interest in identifying additional therapeutic targets to selectively induce apoptosis in leukemic stem and progenitor cells. Nuclear hormone receptors (NHRs) are a family of ligand activated transcription factors and are an attractive and relatively unexploited targets for drug development. Some of the Nuclear receptors are well-characterized drug targets for the treatment of many other human diseases namely Estrogen receptor (ER), Vitamin D Receptor (VDR), peroxisome proliferator activator gamma (PPARg), Androgen receptor (AR), and progesterone receptor (PR)9. However, no study so far had systematically examined the entire family of 49 receptors to determine their role as potential novel drug targets in leukemias- especially myeloid leukemias. This study is proposed to identify novel NHR genes as drug targets to overcome drug resistance in myeloid leukemias. In the research project on CML, by exploring the mechanisms of disease progression, intolerance and resistance to imatinib using various genomic and cellular approaches, we anticipate that this study will eventually aid in personalizing tyrosine kinase inhibitor therapy in this disease.

Publication since last report

» Abraham A, Varatharajan S, Karathedath S, Philip C, Lakshmi KM, Jayavelu AK, Mohanan E, Janet NB, Srivastava VM, Shaji RV, Zhang W, Abraham A, Viswabandya A, George B, Chandy M, Srivastava A, Mathews V, Balasubramanian P. RNA expression of genes involved in cytarabine metabolism and transport predicts cytarabine response in acute myeloid leukemia. Pharmacogenomics. 2015 Jul;16(8):877-90.

Presentations since last report

» Ezhil Pavai Mohanan, John C Panetta, Shareen Stella Backia Royan, Ajay Abraham, Eunice Sindhuvi Edison, Kavitha M Lakshmi, Fouzia Nambiathayil Abubacker, Anu Korula, Aby Abraham, Auro Viswabandya, Biju George, Alok Srivastava, Vikram Mathews, Poonkuzhali Balasubramanian. Population Pharmacokinetics of Fludarabine and Treosulfan in Patients with Thalassemia Undergoing Hematopoietic Stem Cell Transplantation. Blood 2015; 126: 3120.

» Ezhil Pavai Mohanan, Shareen Stella Backia Royan, John C Panetta, Fouzia Nambiathayil Abubacker, Anu Korula, Aby Abraham, Auro Viswabandya, Biju George, Alok Srivastava, Vikram Mathews, DM1, Poonkuzhali Balasubramanian. Generic Intravenous Busulfan in Hematopoietic Stem Cell Transplantation: Relevance of Therapeutic Drug Monitoring. Blood 2015; 126: 4322.

» Sreeja Karathedath, Bharathi M Rajamani, Savitha Varatharajan, Ajay Abraham, Vikram Mathews, Shaji R Velayudhan, and Poonkuzhali Balasubramanian. Role of NF-E2 Related Factor 2 (NRF2) on Chemotherapy Resistance in Acute Myeloid Leukemia (AML) and the Effect of Pharmacological Inhibition of NRF2. Blood 2015; 126: 1272.

Laboratory Highlights

Dr. Poonkuzhali B - Awarded Wellcome DBT Senior fellowship for the year 2015.

Dr. Ajay Abraham

» Successfully defended his Ph.D dissertation entitled: "Mechanisms of Drug Resistance in Leukemia – with special reference to Cytarabine Resistance in Acute Myeloid Leukemia" from Tamil Nadu Dr. MGR Medical University, September 2015.

» Joined as Post-doctoral research fellow under Dr. Ravi Bhatia, University of Alabama in October 2015.

Ms. Sreeja Karathedath

» Abstract Achievement Award Recipient American Society of Haematology (ASH) annual meeting-2015.

» DST travel award to attend American Society for Haematology meeting 2014.

» Best poster award- Fourth prize, Annual Research day CMC, 2015.

» Best poster award- Third prize (Shared) 2nd ISHBT- European Haematology Association Tutorial on Myeloid Malignancies and multiple Myeloma (AML, APL, MDS, CML, MPN and MM), Jan 2016.

Ms. Savitha Varatharajan

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

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

» Best poster award-First Prize -National Conference on Clinical Pharmacology, JIPMER, Pondicherry.

» Best poster award- Third prize (Shared) 2nd ISHBT- European Haematology Association Tutorial on Myeloid Malignancies and multiple Myeloma (AML, APL, MDS, CML, MPN and MM), Jan 2016.

Ms. Ezhilpavai Mohanan

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

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

» Abstract Achievement Award Recipient American Society of Haematology (ASH) annual meeting-2015.

Students trained: Four students are registered for Ph.D. One of them is submitting thesis in April 2016; two new students have joined the lab as JRF and SRF.

Grants Received (Extramural)

S. No.	Title of Project	Funding Agency	Amounts (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.	Personalizing conditioning regimen in hematopoietic stem cell transplantation – Principal Investigator	Wellcome- DBT India Alliance	436.25	5 years (Oct 2015-Sep 30, 2020)
3.	Identification of novel nuclear receptors (NHR) drug targets in myeloid leukemia – Principal Investigator	DBT (part of the Centre of Excellence Scheme "Evaluation of Biology and Mechanisms of Resistance in Leukemia"	75.75	5 years (Nov 11, 2015-Oct 30, 2020)
4.	Exploring the mechanisms of disease progression, tyrosine kinase inhibitor resistance and intolerance in Chronic Myeloid Leukemia – Principal Investigator		83.65	5 years (Nov11, 2015-Oct 30, 2020)
5.	Generation of a novel epigenetic ShRNA library for understanding the roles in stem cell differentiation, disease pathogenesis and drug resistance Co-Investigator	DBT	79.05	3 years (25-03-2015 to 24-03-2018)

ONGOING RESEARCH PROJECTS (Internal- CMC, FLUID grants)

» Establishment of Niche based in vitro culture system for expansion of Acute Myeloid Leukemia Stem cells (Rs. 1 Lakh for 2 years; 2014-2016)

» Evaluating mechanism of cytarabine resistance in AML stem cells using Nische based invitro culture system (Rs. 1 Lakh for 2 years; 2014-2016)

» Mechanism of daunorubicin resistance in AML stem cells using Niche based in vitro culture system (Rs. 1 Lakh for 2 years; 2014-2016)

SANCTIONED RESEARCH GRANTS AWAITING RELASE OF FUNDS

» "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, and Alok Srivastava) Approved by ICMR, 2015

» Modulation of drug resistance in acute myelogenous leukemia: role of Nrf2 and ABCB6- – Principal Investigator. Approved by ICMR, 2014.

Murugan Ramalingam,PhD,FRSC
Associate Professor

RESEARCH PROGRAM:

Area: Tissue Engineering

Our lab focuses on synthesis, design and characterization of biomaterials and scaffolds suitable to control stem cell fate and function, and to engineer human tissues and organs for clinical application.

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 organsby combining patient's own cells with engineered matrices called scaffoldsfor regenerative medicine. The 3D microenvironment is one of the key factors to engineer a physiologically functional tissues and organs, which are investigated in our lab using biomimetics, micro- and nano-technologiesas follows.

1. Combinatorial Designer Platform for High-Throughput Screening of Stem Cells:

This project involves the design of combinatorial platform using nanofiber scaffolding system with composition or modulus gradient libraries that facilitates high-throughput screening of stem cell's response to 3D microenvironmentin terms of cellular 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 3D cellular microenvironment (niche) in order to regulate cellular and biological functions. Screening the effect of scaffold composition and characteristics towards stem cell behavior is the key selection criteria for scaffolding

systems in tissue engineering. Therefore, this project aims to develop gradient nanofiber scaffold libraries, made of poly(caprolactone) (PCL) nanofibers with composition gradients of nano hydroxyapatite (nHA), as a model combinatorial designer platform, suitable for high-throughput screening of human bone marrow-derived mesenchymal stem cells (hBMSCs) (see Figure 1). This kind of gradient libraries could also be used as a model system to study the interface tissue regeneration and underlying mechanisms during soft-to-hard tissue development.

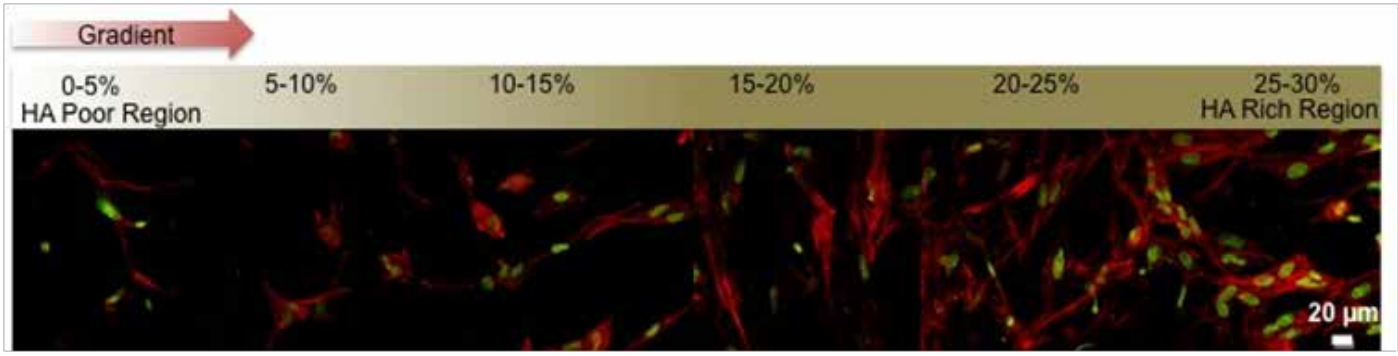


Figure.1. A representative confocal image of hBMSCsresponses to gradient microenvironment. HA denotes hydroxyapatite, a major bone mineral substance.

2. Injectable Gels for Cardiomyogenesis:

The aim of this project is to develop polyethylene glycol (PEG)-based injectable gels with enhanced cellular and biomechanical properties to support the growth and differentiation of adipose-derived stem cells to cardiomyocytes. The use of adult stem cell therapy for cardiac repair has shown enormous potential. 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; a major obstacle is represented by the low efficiency of cell engraftment due to immediate washout and low survival rate. Injectable gels are considered as an efficient delivery system for stem cells. PEG is a promising candidate for culture and delivery of stem cells. However, there is no standard method and measurement tool for generating PEG gels with adequate biomechanical and biochemical cues. The matrix stiffness having been shown to aid in directing stem cell lineage specification. It is thus opportunity to investigate both the optimal gel stiffness and chemical composition required for cardiomyocyte differentiation from MSCs and their ability to enhance cardiogenesis.

3. Shape-Memory Gels for Stem Cell Delivery and Soft Tissue Engineering:

The aim of this project is the development of injectable and shape-memory gels for the use of stem cell delivery and soft tissue engineering. Shape memory is a property of select substances that have the ability to "remember" their original shape after deformation.The injectable, shape-memory biomaterial-based therapies have been gaining much attention, because they are injectable, flexible, elastic in nature, minimally invasive deliverable, macroporous,shape memory ability and can readily fit to any irregular shapes of the tissue defects.

In this study, we have developed a process to design injectable, shape-memory gels based on methacrylated gelatin (GelMA) and studied their injectability and biocompatibility with hBMSCs. The results of this study confirmedthe injectable nature of the gels as well as its ability to regain the initial/original shape after deformation (shape-memory) and biocompatibility with hBMSCs. These findings suggest that GelMA gels may serve as a carrier

system for the delivery of stem cells as well as a cell-responsive platform for tissue engineering applications. In addition, the gels have also been subjected to use as substratum for long-term, scalable culture and differentiation of human induced pluripotent stem cells.

4. Thermogels for Stem Cells, Growth Factor Delivery and Skeletal Tissue Engineering:

The aim of this project is the development of injectable thermogels (also called thermo-sensitive gels) for the use of stem cell delivery, growth factor delivery and skeletal tissue repair. Thermogel is a special form of gel system that is sensitive to temperature, which means that they are in liquid form (sol state) at room temperature (22°C) and gradually change their phase into a semi-solid form (gel state) at physiological temperature(37°C) by sensing their environmental stimuli such as temperature. This kind of gels is preferred in regenerative medicine because of its ability to conform to complex shapes and sizes of the tissues or organs, delivery of bioactive molecules or cells to the defective site under physiological conditions within a short period.

In a pilot study, we have demonstrated the synthesis and characterization of injectable thermogels based on chitosan and nHAp suitable for bone tissue engineering. The prepared thermogels have been characterized for various physicochemical properties such as porosity, injectability, rheology, swelling ratio and biodegradability. The results confirmed thermo-sensitive nature of the gels both at room and physiological temperature (see Figure 2).The gels were also been tested for their efficacy in osteogenic differentiation of hBMSCs. In addition, the gels are also being tested as a carrier system for platelet-rich plasma to enhance the osteogenesis.

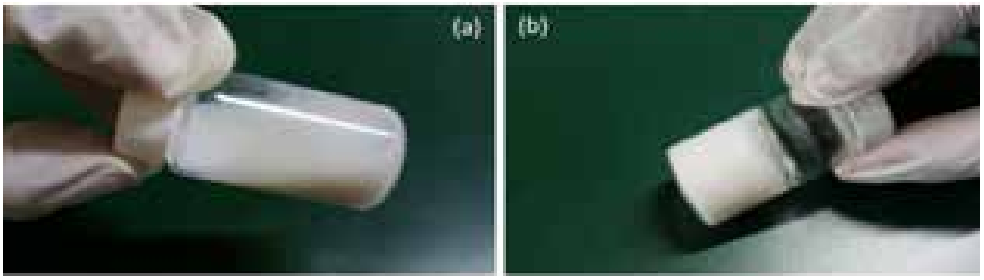


Figure 2. Photographic image of thermogels made of chitosan and nHAp: (a) at room temperature and (b) at physiological temperature.

5. The Bio-Ink: 3D Printing of Organs (Flat, Hollow and Complex Structures):

The aim of this project is the development of bio-ink based on alginate/polyacrylamide for the use of 3D printing of human tissues and organs. Advances in tissue engineering and micro-/nanotechnology have led to new ways to develop a custom-designed, off-the-shelf tissues and organ`s structures in 3D (see Figure 3). In a 3D bioprinting, bio-ink is a key component, which is typically dispensed from an extrusion system and allows 3D printing of replica of damaged tissues and organs by employing a layer-by-layer approach.

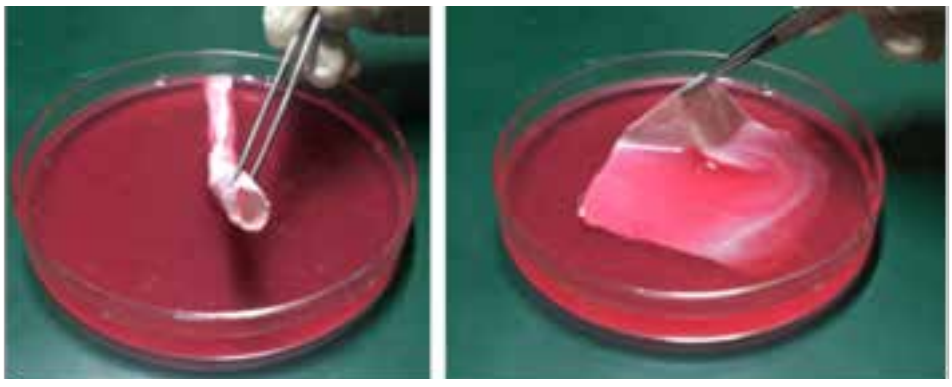


Figure 3. In-house custom made three-dimensional tissue/organ's structures (flat and tubular) made of polymeric biomaterials.

In a pilot study, we have demonstrated the preparation and characterization of hBMSCs encapsulated alginate and acrylamide-based gel-like systems.The preliminary results confirmed that the stem cells cultured in 3D gels show morphology and cellular behavior in resemblance to the native tissue-like microenvironmentas well as adequate mechanical stability. However, further investigations are on-going to improve its printability and structural stability in the encapsulated cell form. This kind of bio-inks might be used in engineering various human tissues and organs.

Funding

» DST and CSCR

Publications (2014-2015)

Journals:

» S. Ahadian, M. Estili, J. Ramón-Azcón, X. Liang, H. Shiku, R. Murugan, H. Bae, K. Nakajima, T. Matsue, Y. Sakka, and A. Khademhosseini. Facile and green production of aqueous graphene dispersions for biomedicalapplications. Nanoscale 7 (2015) 6436-6443.

» D. Rana and R. Murugan. Designer Cell-laden Polyacrylamide-alginate Gels for Stem Cell Delivery. Tissue Engineering Part A 21 (2015) S103.

» T. Ramasamy, J. Kim. S. Yong. D. Rana, J. Campos, R. Murugan and Z.S. Haidar. Novel Core-Shell Nanocapsules for the Tunable Delivery of Bioactive rhEGF: Formulation, Characterization and Cytocompatibility Studies.J. Biomater.Tissue Eng 5(2015) 730.

» S. Ostrovidov, Ramin Sadeghian, Sahar Salehi, T. Fujie, H. Bae, R. Murugan and A. Khademhosseini. Stem cell differentiation toward the myogenic lineage for muscle tissue regeneration: A focus on muscular dystrophy.Stem Cell Rev. Rep. 11 (2015) 866-884.

» S. Ahadian, R. Sadeghian, S. Salehi, S. Ostrovidov, H. Bae, R. Murugan and Ali Khademhosseini. Bioconjugated hydrogels for tissue engineering and regenerative medicine.Bioconjugate Chem 26 (2015) 1984-2001.

» J. Campos, C. Jimenez, C. Trigo, P. Ibarra, Deepti Rana, R.Thiruganesh, R. Murugan and Z.S. Haidar. Quartz Crystal Microbalance with Dissipation Monitoring: A Powerful Tool for BioNanoScience and Drug Discovery.J. Bionanoscience 9 (2015) 249-260.

» Glen Kwon and R. Murugan. Pharm. Nanotech 3 (2015) 2-3.

» R. Murugan and D. Rana. Impact of nanotechnology in induced pluripotent stem cells-driven tissue engineering and regenerative medicine. J. Bionanoscience 9 (2015) 13-21.

» 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

Tissue Engineering 20 (2014) 403-436.

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

» J. R. 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, 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. 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.

» K. Sampathkumar, S. Arulkumar and R. Murugan. Advances in stimuli responsive nanobiomaterials for cancer therapy. J. Biomed. Nanotech. 10 (2014) 367-382.

» 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.

» N. Varadarajan, R. Balu, D. Rana, R. Murugan, 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.

» D. Rana, A. Tabasum and R. Murugan. Cell-laden alginate/polyacrylamide beads as carrier for stem cell delivery: preparation and characterization. RSC Advances (in press).

» D. Rana, H. Zreiqat, N. Benkirane-Jessel and S. Ramakrishna and R. Murugan. Development of decellularized scaffolds for stem cell-driven tissue engineering. J. Tissue Eng. Reg. Med. (in press).

» S. Ahadian, R. Obregón, J. R. Azcón, R. Murugan, T. Matsue. Carbon-basednanomaterials for stem cell differentiation and tissue regeneration. J. Nanosci. Nanotech (in press)

» 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. (in press).

» Tomasz Trzeciak, Jakub Rybka, Magdalena Richter, J. Kaczmarczyk, R. Murugan, Michael Giersig.

Cells and nanomaterial-based tissue engineering techniques in the treatment of bone and cartilage injuries. J. Nanosci. Nanotech. (in press)

» D. Rana, M. Leena, M. Nithyananth, R. Pasricha, G. Manivasagam and R. Murugan. Control of stem cell fate and function by polymer nanofibers. J. Nanosci. Nanotech. (in press)

» R. Murugan, Alicia El Haj, Thomas Webster and S. Ramakrishna. The role of nanotechnology in stem cell research. J. Nanosci. Nanotech. (in press)

» V. Sabapathy, M. Hurakadli, D. Rana, R. Murugan, and S. Kumar. Decellularized amniotic membrane scaffold compared to synthetic PLGA and hybrid scaffolds exhibit superlative biomechanical properties for tissue engineering applications. J. Biomater. Tiss Eng (in press).

» L. Maria, D. Rana and R. Murugan. Accelerated synthesis of nanophase hydroxyapatite using stimulated body fluids (in preparation).

» D. Rana and R. Murugan. Tough gels made of alginate, polyacrylamide and human bone marrow-derived mesenchymal stem cells as bio-ink for 3D printing (in preparation).

» A. Barade, D. Rana, R. Keerthana, and R. Murugan. Injectable shape memory GelMA cryogels for stem cell delivery (in preparation).

» L. Maria and R. Murugan. Development of PCLnanofiber scaffolds with gradientin mineral content and random to aligned morphology for interfacial tissue engineering (in preparation).

» C.P. Thulya, D. Ranaand R. Murugan. Injectable thermogels made of chitosan and nano hydroxyapatite for bone tissue engineering (in preparation).

» R. Keerthana, A. Barade and R. Murugan. Methacrylated gelatin as a tissue scaffold: Synthesis and characterization (in preparation).

» R. Keerthanaand R. Murugan. Stiffness gradient of PEGDA hydrogels for 3D culture and screening of stem cells (in preparation).

Text Books:

» A. 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 (in process)

» R. Murugan. Hydroxyapatite. Springer Publications, USA (in process).

» Z. Haidar and R. Murugan. Bioceramics: Principles and Applications. Wiley-Scrivener Publication, USA (in process).

» X. Wang, R. Murugan, X. Kong and L. Zhao. Nanobiomaterials: Classification, Fabrication and Biomedical Applications. Wiley Publication, USA (in process).

Book Chapters:

» Deepti Rana, Shylaja Arulkumar and R. Murugan. Nanocarriers for Breast Cancer Therapeutics.In Biological and Pharaceutical Applications of Nanomaterials. Polina Prokopovich (Ed.), CRC Press, USA (2015) 101-130.

» Deepti Rana, Shylaja Arulkumar, Ajaykumar Vishwakarma and R. Murugan. Considerations on designing scaffold for tissue engineering. In Stem Cell Biology and Tissue Engineering in Dental Science, A. Vishwakarma, P. Sharpe, S. Shi, X. Wang and M. Ramalingam (Eds.), Elsevier, USA (2014) 133-148.

» S. Ostrovidov, A. Seidi, Deepti Rana, Kaarunya Sampathkumar, Queeny Dasgupta, A. Srivastava, A. Khademhosseini and R. Murugan. Introduction to nanobioscience: A tissue engineering perspective.In Encyclopedia of Life Support Systems, UNESCO Project, EOLSS Publications, France (2014) 6.152.34.

» Ajaykumar Vishwakarma, Paul Sharpe, Songtao Shi, Xiu-Ping Wang and R. Murugan. An introduction to Stem Cell Biology and Tissue Engineering. In Stem Cell Biology and Tissue Engineering in Dental Science, A. Vishwakarma, P. Sharpe, S. Shi, X. Wang and M. Ramalingam (Eds.), Elsevier, USA (2014) 1-16.

» 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, A. Vishwakarma, P. Sharpe, S. Shi, X. Wang and M. Ramalingam (Eds.), Elsevier, USA (2014) 207-220.

» 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, A. Vishwakarma, P. Sharpe, S. Shi, X. Wang and M. Ramalingam (Eds.), Elsevier, USA (2014) 175-186.

» Maria Leena, Aruna Barade, Deepti Rana, Chetna Dhand, S. Ramakrishna and R. Murugan Nanofiber Composites in Biomolecular Delivery. In Nanofiber Composite Materials for Biomedical Applications, M. Ramalingam and S. Ramakrishna (Eds.), Elsevier Publication, UK (in press).

» Maria Leena, Deepti Rana, C. P. Thulya, S. Ramakrishna and R. Murugan. Nanofiber. Composites in Gene Delivery. In Nanofiber Composite Materials for Biomedical Applications, M. Ramalingam and S. Ramakrishna (Eds.), Elsevier, UK (in press).

» R. Murugan and S. Ramakrishna. Introduction to Nanofiber Composite Materials. In Nanofiber Composite Materials for Biomedical Applications, M. Ramalingam and S. Ramakrishna (Eds.), Elsevier Publication, UK (in press).

» Chetna Dhand, Neeraj Dwivedi, Harini Sriram, Samiran Bairagi, Deepti Rana, Lakshminarayanan Rajamani, R. Murugan, and S. Ramakrishna. Nanofiber Composites in Drug Delivery. In Nanofiber

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» Amit Jaiswal, Geetha Manivasagam and R. Murugan. Mechanical characterization of nanofiber composites. In Nanofiber Composite Materials for Biomedical Applications,M. Ramalingam and S. Ramakrishna (Eds.), Elsevier, UK (in press).

» Samad Ahadian, Raquel Obregonb, Javier Ramon-Azconc, Georgina Salazard and R. Murugan. Clinical/pre-clinical study of nanofiber composite materials. In Nanofiber Composite Materials for Biomedical Applications, M. Ramalingam and S. Ramakrishna (Eds.), Elsevier Publication, UK (in press).

» Deepti Rana, Minal Thacker, Maria Leena and R. Murugan. Induced Pluripotent Stem Cells in Scaffold-based Tissue Engineering. In Tissue Engineering for Artificial Organs, Anwarul Hasan (Ed.), John-Wiley Publication, USA (in press).

» Deepti Rana, R. Keerthana, Maria Leena, Renu Pasricha, Geetha Manivasagam and R. Murugan. Surface Functionalization of Biomaterials. In Stem Cell Niche Biology and Engineering, Ajaykumar Vishwakarma and Jeff Karp (Eds.), Elsevier, USA (in press).

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, USA

» Regional Editor (Asia), Pharmaceutical Nanotechnology, USA

» Associate Editor, 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

» Member, Editorial Board of Translational Medicine, USA

» Member, Editorial board of International Journal of Stem Cell Therapy, USA

» Scientific Committee Member of ICTE, Portugal

» Advisory Board Member, Stem Cell Research, WebmedCentral, UK

» Advisory Board Member, The European Society for Biomaterials

» Executive Member, Asian Council of Science Editors, UAE

» JBT Best Paper Award from the American Scientific Publisher, USA

» Scientific Committee, Ministry of Business, Innovation and Employment, New Zealand

Invited talks

» High-throughput screening of stem cells using gradient biomaterials. Cardio Vascular Unit, University of Cape Town, South Africa.

» Control of stem cell fate and function using biomaterials. Advanced Institute for Materials Research, Tohoku University, Japan.

» Biomaterials and stem cells for tissue engineering, VIT University, India.

Course Taught

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

» Scaffolds for Cell Therapy, Gene Therapy Module, PhD Programme at CSCR.

» Scaffolds for Cell Culture, Hands on Workshop on Isolation, Culture and Characterization of Adult Stem Cells at CSCR.

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

Deepti Rana, JRF
Keerthana R, JRF
Minal Thacker, Short-term Trainee
Maria Leena, Short-term Trainee
Thulya. C.P, Short-term Trainee
Aruna Barade, Short-term Trainee

Collaborators

Alok Srivastava, CSCR/CMC, Vellore
Sanjay Kumar, CSCR, Vellore
R. V. Shaji, CSCR/CMC, Vellore
Manasseh Nithyananth, CMC, Vellore
Ashish Gupta, CMC, Vellore

Kingsly Paul, CMC, Vellore
Geetha Manivasakam, VIT University, Vellore
Raunak Das, VIT University, Vellore
Amit Jaiswal, VIT University, Vellore
Sampath Kumar, Indian Institute of Technology, Chennai
Renu Pasricha, National Centre for Biological Sciences, Bangaluru
Ali Khademhosseini, Harvard University and MIT, USA
Thomas Webster, Northeastern University, USA
Seeram Ramakrishna, National University of Singapore, Singapore
Serge Ostrovido, Tohoku University, Japan
Tomokazu Matsue, Tohoku University, Japan
Ziyad Haidar, Universidad de los Andes, Chile
Nadia Benkirane-Jessel, University of Strasbourg, France
Tomasz Trzeciak, Poznan University of Medical Sciences, Poland
Hala Zreiqat, University of Sydney, Australia
Xiumei Wang Professor, Tsinghua University, China
Neil Davies, University of Cape Town, South Africa
Thomas Franz, University of Cape Town, South Africa

Rekha Samuel, MBBS, MD,
Professor

Laboratory Highlights

Diabetes mellitus is one of the most widespread diseases afflicting the Indian population. It is a condition where blood sugar levels are abnormally high over extended periods of time, mainly due to a lack of the hormone insulin (Type 1 diabetes) or other causes that make cells resistant to the action of insulin (Type 2 diabetes). A third type of diabetes, known as gestational diabetes (GDM), occurs temporarily in pregnant women who have had no previous history of diabetes. Despite the prevalence of this condition, much is unknown about the unique characteristics presented by Indian diabetic patients, especially those involving the blood vessels – the veins, arteries and capillaries – of the human body.

Indian diabetics often have major problems in blood vessels that can lead to complications such as retinopathy (impaired retinal function leading to vision loss). In our efforts to understand how diabetes affects blood vessels, as a primary focus of the lab, we have turned to using the placenta as a model system – a 9 month old, relatively easily available organ with a high density of blood vessels. Currently, we are focused on detecting and understanding the changes to the smallest components of blood vessels – the capillaries – whose walls are made of two types of cells known as endothelial cells and pericytes.

Our findings so far indicate that women who experienced gestational diabetes, have placentas with blood vessels that look distinctly different from normal placentas. These diabetic placentas have a unique 'GDM phenotype', which includes the presence of angiomatous lesions not commonly seen in healthy placentas. Striking ultrastructural examination reveals abnormalities of pericytes such as pericyte ghosts that are remarkably similar to those seen in diabetic retinopathies. Since pericytes are essential for the development and maintenance of blood vessels (by providing a perivascular support to endothelial cells and promoting their maturation), their loss is the likely cause of the lesions observed.

Growth experiments on foetal progenitor cells from GDM placentas also showed that such cells had higher death rates, and formed fewer pericytes in blood vessels that were engineered from the GDM placental cells. The results indicate that GDM is very likely to affect blood vessel growth in the developing foetus and could influence the susceptibility of an unborn child to diabetes-related conditions in the future. We hope that this research would also lead to the generation of a model system within which diabetic retinopathy can be studied.

Our work thus far has been centred primarily on histological, ultrastructural and in vivo murine implantation studies to examine abnormalities of blood vessels formed by GDM vascular progenitor cells. This year we will extend our investigations to include longitudinal in vivo imaging studies and molecular analyses to examine possible mechanisms for the abnormal GDM phenotype.

Our work was selected as one of the posters at the 2015 Guided Audio Tour at the American Diabetes Association Conference, Boston, USA, an award that showcases recent developments in the field of investigative Diabetes research.

In September 2015, we were awarded the European Foundation for the Study of Diabetes/ Sanofi Programme for Collaborative Clinical Diabetes Research between European and Non-European countries 2015. This project will examine the transcriptome of Gestational Diabetic placental capillaries using Laser Capture Microdissection (LCM) to look at molecular signatures that determine the abnormal GDM phenotype in our Indian population. Our collaborator in Austria, Professor Gernot Desoye's team at the University of Graz, Austria will similarly examine the transcriptome of Austrian placental GDM vessels. This is the first study that will use LCM on vessels to examine two geographically distinct GDM populations.

Other collaborations involve examining scleroderma affected capillaries (with Colin Jamora at InStem, Bangalore).

Ongoing studies and funding:

» A transcriptome analysis of Gestational Diabetic placental capillaries using Laser Capture Microdissection. European Foundation for the Study of Diabetes/ Sanofi Programme for Collaborative Clinical Diabetes Research between European and Non-European countries 2015, award of 100,000 Euros. (Rs. 70 lakhs).

» Research Society for the Study of Diabetes, India. SNAIL associated microvascular defects in hyperglycemia of pregnancy. Rs. 500,000.

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

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

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

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

» Isolation and expansion of human endothelial progenitor cells (epcs) from peripheral blood using human platelet lysate (hPL) as a substitute for foetal bovine serum. Fluid Research Grant, Christian Medical College, IRB Min 7846, 2012-2014. Rs.80, 00.

Completed studies and funding:

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

» Isolation of placental perivascular cells and endothelial progenitor cells from Gestational Diabetes to explore early microvascular functional abnormalities. Fluid Research Grant, Christian Medical College, IRB Min 7737, 2012-2014.

Publications since last report:

» The matrix protein Fibulin-5 is at the interface of tissue stiffness and inflammation in fibrosis. Nakasaki M, Hwang Y, Xie Y, Kataria S, Gund R, Hajam EY, Samuel R, George R, Danda D, M J P, Nakamura T, Shen Z, Briggs S, Varghese S, Jamora C. Nat Commun. 2015 Oct 15;6:8574. doi: 10.1038/ncomms9574.

» Vascular diseases await translation of blood vessels engineered from stem cells. Samuel R, Duda DG, Fukumura D, Jain RK. Sci Transl Med. 2015 Oct 14;7(309):309rv6. doi: 10.1126/scitranslmed.aaa1805. Review.

» An orthotopic mouse model of hepatocellular carcinoma with underlying liver cirrhosis. Reiberger T, Chen Y, Ramjiawan RR, Hato T, Fan C, Samuel R, Roberge S, Huang P, Lauwers GY, Zhu AX, Bardeesy N, Jain RK, Duda DG. Nat Protoc. 2015 Aug;10(8):1264-74. doi: 10.1038/nprot.2015.080. Epub 2015 Jul 23.

» Targeted delivery of AAV-transduced mesenchymal stromal cells to hepatic tissue for ex vivo gene therapy. Gabriel N, Samuel R, Jayandharan GR. J Tissue Eng Regen Med. 2015 Jun 5. doi: 10.1002/term.2034. [Epub ahead of print].

Honors and awards:

» 2015 Guided Audio Tour for poster entitled "Dysfunctional Placental Fetal Gestational Diabetic Vascular Progenitors resemble Type 2 Diabetic Retinopathy" at the American Diabetes Association Conference, Boston, USA. June 5th-9th. (to RS).

» 2015 International Travel Scheme, Science and Engineering Research Board (DST), travel for airfare to attend the American Diabetes Association Conference, Boston, USA. June 5th-9th .(to RS).

» 2015 Partial Travel Assistance, Council of Scientific and Industrial Research, for European Association for the study of Diabetes (Eye Complication Study Group), Turin, Italy. June 26th-28th. (to RS).

» 2015. Chorangiosis in Gestational Diabetes Mellitus Image on cover of Diabetes Journal March 2015, Volume 64, No.3.

Invited talks

» 2016 "Placental Pericyte and Endothelial Cell Cross-talk in Gestational Diabetes Mellitus". IFOM-inStem Conference on Inflammation and Tissue Homeostasis", Bangalore, India. Feb 3rd-5th.

» 2015 "Vascular biology of Blood vessels in diabetes - from bench to bedside".14th Annual conference of Uttar Pradesh Diabetes Association(UPDA), Nainital , Uttarakhand, India. Oct 10th- 11th.

» 2015 "Placental studies in Gestational Diabetes Mellitus". International Update in Gestational Diabetes Mellitus, Chennai, India. September 26th-27th.

» 2015 "Vascular Biology in Diabetes- Clinical Implications of Basic Research". Research Society for Study of Diabetes in India, Maharashtra Chapter, Pune, India. May 1st-3rd.

» 2015 "Engineering functional durable blood vessels from human induced pluripotent stem cells". Clinical Applications of Stem Cells, Singapore Bioimaging Consortium and Select Biosciences South East Asia, Singapore. February 26th-27th.

» 2015. 73rd Annual Conference of All India Ophthalmological Society. Delhi: 1. Invited keynote lecture, 2. Co-instructor, Pre- conference workshop on "Pathophysiology of Diabetic Retinopathy" and 3. Convenor of the Ophthalmic Pathology session. February 5th-8th.

Other Academic Activities

» Faculty in charge of Core Histopathology and Core Imaging facilities at the Centre for Stem Cell Research.

» Board of adjudicator, Dr. Maneesha Inamdar's student at Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore.

Lab Members

» Chitra Premkumar, Staff III Graduate Technician. BSc Zoology.

» Saranya Rajendran, Staff III Graduate Technician. BSc Microbiology.

Collaborators

» Dr. Jiji Elizabeth Mathews, DGO, MD. Professor and Head, V, Obstetrics and Gynecology, CMC, Vellore.

» Dr. Gernot Desoye, PhD, Research Director, Department of Obstetrics and Gynecology, Medical University of Graz, Austria.

» Santhosh Benjamin, MS. Assistant Professor, Obstetrics and Gynecology, V, CMC, Vellore.

» MS Seshadri, MD, PhD, FRCP. Professor & Retired Head, Endocrinology, Diabetes and Metabolism, CMC, Vellore.

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

» Praveen Vemula, PhD. Laboratory of Self-Assembled Biomaterials, inStem, Bangalore.

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

» Sanjay Kumar, PhD. CSCR, Vellore.

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

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

» Renu George, MD. Professor and Head Dermatology Unit I, CMC, Vellore.

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

RV Shaji, PhD
Prof and Adjunct Scientist

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.

Using a robust ex-vivo erythropoiesis system to obtain cultured erythroid cells our laboratory is trying to understand the transcriptional regulation of human erythropoiesis. We carried out the comprehensive screening of microRNAs by small RNA sequencing in the cultured erythroid cells and found several miRNAs and miRNA clusters that are regulated by erythroid transcription factors, including novel miRNAs. We have evaluated their regulation by erythroid specific transcription factors and have initiated knock out experiments using CRISPR/Cas9 to determine their role in human erythropoiesis. In another experiment, we have studied the role of transcription cofactors, CBP and P300, for identification of erythroid specific transcription enhancers. Our data showed distinct regulatory DNA elements where these two proteins bind in haematopoietic stem cells and the differentiated erythroid cells. We have performed comprehensive bioinformatics analysis to compare the co-occupancy of erythroid specific transcription factors, KLF1, GATA1 and TAL1, with CBP and P300 and determined the transcriptional regulatory networks involving these proteins.

For understanding the epigenetic factors involved in human erythropoiesis we have identified the most efficient lentiviral back bone and the promoter for shRNA expression in stem cells and progenitors. After validation of shRNAs for 31 epigenetic factors for their knock down efficiency, we have generated a shRNA library for 500 epigenetic factors and the experiments are being carried out for the comprehensive screening for the positive and negative epigenetic regulators of human erythropoiesis.

To identify the genetic elements that cause phenotypic diversity in beta thalassaemia and sickle cell disease we sequenced the entire 100KB region in 10 patients with different phenotypes using next generation sequencing based technologies. The putative regulatory sequences identified in this project are being evaluated by CRISPR/Cas9 method to determine their role in globin gene regulation.

II. Somatic Cell Reprogramming: Mechanisms and disease modelling

We have successfully generated human induced pluripotent stem cells (hiPSCs) from adult skin fibroblasts and peripheral blood mononuclear cells using retroviral vectors, lentiviral vectors, episomal plasmids and Sendai virus for expression of OSKM. We found a reprogramming barrier from OCT4+NANOG- state to OCT4+NANOG+ state in the late stages of reprogramming, which is overcome by treatment with small molecules. Further analysis on the factors involved reprogramming showed that grainy head like factors-1, 2 and 3, which are involved in mesenchymal epithelial transition, and protein arginine methyl transferases (PRMTs) are potential players in reprogramming. Currently, we are performing CRISPR/cas9 experiments to validate these results. For comprehensive screening of signalling pathways involved in reprogramming we used a lentiviral shRNA library for 2500 signalling pathway genes and the reprogrammed cells were sorted by FACS and the enrichment and depletion of shRNAs was evaluated by next generation sequencing of integrated shRNAs. For identification of epigenetic factors involved in reprogramming, we are studying the enrichment and depletion of shRNAs using the lentiviral shRNA library explained in the erythropoiesis project.

For disease modelling, we have successfully generated hiPSCs from fibroblasts from patients with Fanconi anaemia (FA), a disease caused by the mutations that affect FA pathway. In a large survey of patients with this disease, we recruited 100 patients and they were studied by chromosome breakage, FANCD2 ubiquitination, complementation and mutation analyses. Using an inducible lentiviral vector to express the complementation group proteins, we could generate of FA-hiPSC lines with morphology and gene expression similar to hiPSCs generated from normal individuals. The clones have been tested for their differentiation potential and chromosome stability. Current experiments are focussed on differentiation of these clones to haematopoietic cells to generate models to study the mechanisms of bone marrow failure in FA.

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.

Biju George, Department of Haematology, CMC: Clinical evaluation of Fanconi anaemia patients.
Eunice Sindhuvi, Department of Haematology, CMC: Globin gene regulation

Sanjay Kumar, CSCR: Role of epigenetic factors in stem cell differentiation by RNAi
Saravanabhavan Thangavel, CSCR: Gene editing

LABORATORY MEMBERS:

PhD students:

Completed:

- Nancy Beril Jannette (In collaboration with Dr. Alok Srivastava)
- Thiyagaraj Mayuranathan
- Janakiram Rayabram

Current students:

- Kannan VM
- Syed Mohammed Musheer Aalam
- Sumitha P Bharathan
- Aneesha Nath

Junior Research Fellows:

- Abhirup Bagchi (In collaboration with Dr. Alok Srivastava)
- Smitha I

Senior Reseach Fellow (Project):

- Kasthuri N

Technician:

- Dhavapriya Palani

Ongoing Research Projects:

- Generation of human induced pluripotent stem cells for studying the mechanisms of haematological diseases (ICMR, 2015-2018).
- Generation of an epigenetic factor shRNA library for studying the mechanisms of stem cell differentiation, disease pathogenesis and drug resistance (DBT, 2015-2018).

Publications (Recent):

» Manian KV, Aalam SM, Bharathan SP, Srivastava A, Shaji RV. Understanding the Molecular Basis of Heterogeneity in Induced Pluripotent Stem Cells. Cell Reprogram. 2015 Dec;17(6):427-40.

» Jayasree D, Shaji RV, George B, Mathews V, Srivastava A, Edison ES. Clinical, Hematological and Molecular Analysis of Homozygous Hb E (HBB: c.79G>A) in the Indian Population. Hemoglobin. 2016;40(1):16-9.

» Deshpande P, Kamalanathan N, Sampath E, George B, Shaji RV, Edison ES. Characterization of Clinical and Laboratory Profiles of the Deletional α2-Globin Gene Polyadenylation Signal Sequence (AATAAA>AATA--) in an Indian Population. Hemoglobin. 2015;39(6):415-8.

» RNA expression of genes involved in cytarabine metabolism and transport predicts cytarabine response in acute myeloid leukemia. Abraham A, Varatharajan S, Karathedath S, Philip C, Lakshmi KM, Jayavelu AK, Mohanan E, Janet NB, Srivastava VM, Shaji RV, Zhang W, Abraham A, Viswabandya A, George B, Chandy M, Srivastava A, Mathews V, Balasubramanian P. Pharmacogenomics. 2015 Jul;16(8):877-90.

Alok Srivastava, MD, FRACP, FRCPA, FRCP
Scientist

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 (HSCT) as a service for patients with hematological diseases and includes several clinical studies evaluating mobilization of stem cells from the bone marrow as well as new protocols for transplantation using new drugs or post-transplant management of these patients. These include several investigator initiated studies addressing different aspects of clinical HSCT.

I coordinate the Indian Stem Cell Transplant Registry, which is responsible for collecting all data pertaining to hematopoietic stem cell transplantation (HSCT) for blood diseases in India, with a team of colleagues from different institutions in India. I am also the Vice Chair of the Asia Pacific Blood and Marrow Transplant Group executive board. These positions also require involvement with various educational and research activities of these groups.

2. Translational stem cell research and novel therapies –

This involves the following 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. Based on the successful clinical trial with the scAAV8 vector for gene therapy of FIX deficiency, several more clinical trials have been initiated in the last 2 years. There is also more recent data from Dr. Arun Srivastava's laboratory that compared to all other AAV serotypes, it is AAV3 that has the highest tropism for the human liver. This then becomes one of the best options for gene transfer to the human hepatocyte. In collaboration with Dr. Trent Spencer and his group at Emory University, Dr. Arun Srivastava and his group along with Dr. Barry Byrne, Director of the Powell Gene Therapy Center both at the University of Florida, Gainesville, USA we are now developing a complete package for a clinical trial for gene therapy for hemophilia B in India. Preclinical data has shown the advantage of this serotype over the others in terms of transduction efficiency. We are now working rapidly to identify the best transgene construct in different models which will then be finally tested in both humanized mice as well the non-human primate models to decide on efficacy and safety parameters before

non-human primate models to decide on efficacy and safety parameters before taking it to a clinical trial in India. Discussion with regulatory agencies (ICMR & DBT) in India is also being done in parallel to ensure that those aspects are also resolved in the next 6-9 months as the preclinical work reaches completion. The recent grant obtained from the DBT towards Novel Applications in Hematological Diseases (NAHD), the efforts for this trial has got a very major boost. We are now in a position to put together a timeline.

B. The second gene therapy program is towards the major hemoglobin disorders. There has been much progress in this area of work also in the last 1-2 years. The initial results of the lentiviral vector based gene therapy sponsored by Bluebird Bio are encouraging with 4-6g/dl increase in transgene Hb over 3-6 months after autologous gene modified hematopoietic stem cell (HSC) transplantation. Several new studies have been initiated using a similar approach with novel transgene and vector constructs both for beta thalassemia major and sickle cell disease. Continuing our collaboration with Dr. Trent Spencer of Emory University, USA and his group this work will now be developed further in pre-clinical models using a marked transgene in human HSC systems. This work will be done in CSCR in collaboration with Dr. R V Shaji. In other related work being developed at CSCR, two new scientists (Dr. Saravanabhavan Thangavel and Dr. MohankumarMurugesan) who have joined the gene therapy team will work also work on hemoglobin disorders using the genome editing techniques. Details of these work will be shown in their reports. This is also part of the recently sanctioned NAHD project.

II. Translational stem cell research:

1. We are engaged with the creation of an induced pluripotent stem cell "haplobank". The concept here is to identify individuals who are homozygous for a HLA haplotype. The profile of HLA haplotypes in any population has shown that a relatively small number of HLA haplotype homozygous individuals can provide histocompatible cells for transplantation 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 ~50%,the next 361 ~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 >120,000 donors. Several homozygous individuals have already been identified. Two samples have already been collected and making of iPSC has been initiated with them. With the recently granted NAHD project grant, we are now able to initiate a filed program in collaboration with DATRI to collect samples from such individuals. Dr. Dolly Daniel is responsible for this part of the work. We have used the data that exists in the literature to decide on the priorities for haplotypes with higher frequencies. Once the samples are in place, Dr. R. V. Shaji will coordinate the generation of iPSCs with the help of scientific staff selected on the NAHD project for this goal.

2. There are a few other projects related to specific clinical problems that are to be addressed through stem cell based research towards new therapeutic possibilities. These include a project on keloids with the Departments of Pharmacology and Plastic Surgery, another of corneal stroma damage treated with mesenchymal stromal cells with the Department of Ophthalmology and the third on genome editing approaches with HSC to prevent HIV infections with the department of Infectious Diseases. My role in these projects is to support young physicians interested in these areas by helping them think through their projects and write them up in a way that is practical to execute.

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

I continue to chair the National Apex Committee for Stem Cell Research and Therapy, Department of Health Research, Ministry of Health (http://bic.icmr.org.in/nacscrt/), which has the mandate to oversee all aspects of human stem cell research and therapy in India. These include formulating policies and guidelines (http://bic.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. One of the major challenges to prevent exploitation of vulnerable patients by clinics offering unproven stem cell therapies for huge costs. A revision of the national guidelines for stem cell research has also been initiated.

PhD Students:

» Salar Abbas works on the BM niche (collaboration with Dr. Aparna Venkatraman and Dr. Sanjay Kumar). – Ready to submit thesis in the next 6 months.

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

» Nishant Gabriel works on AAV vector modifications (in collaboration with Dr. G. Jayandharan) – thesis successfully defended in Feb 2016.

» Nancy Beryl Janet A - Fanconi anemia (in collaboration with Dr. R.V. Shaji) – Thesis submitted – evaluation awaited.

Selected publications:

1. Weiss DJ, Rasko JE, Cuende N, Ruiz MA, Ho HN, Nordon R, Wilton S, Dominici M, Srivastava A. Part 2: Making the "unproven" "proven". Cytotherapy. 2016 Jan;18(1):120-3.

2. Srivastava A, Mason C, Wagena E, Cuende N, Weiss DJ, Horwitz EM, Dominici M. Part 1: Defining unproven cellular therapies. Cytotherapy. 2016 Jan;18(1):117-9.

3. Thangakunam B, Christopher DJ, Mathews V, Srivastava A. Mesenchymal stromal stem cell therapy in advanced interstitial lung disease - Anaphylaxis and short-term follow-up. Lung India. 2015 Sep-Oct;32(5):486-8.

4. Dominici M, Nichols K, Srivastava A, Weiss DJ, Eldridge P, Cuende N, Deans RJ, Rasko JE, Levine AD, Turner L, Griffin DL, O'Donnell L, Forte M, Mason C, Wagena E, Janssen W, Nordon R, Wall D, Ho HN, Ruiz MA, Wilton S, Horwitz EM, Gunter KC; 2013–2015 ISCT Presidential Task Force on Unproven Cellular Therapy. Positioning a Scientific Community on Unproven Cellular Therapies: The 2015 InternationalSociety for Cellular Therapy Perspective. Cytotherapy. 2015 Dec;17(12):1663-6.

5. Manian KV, Aalam SMM, Bharathan SP, Srivastava A, Velayudhan SR. Understanding the Molecular Basis of Heterogeneity in Induced Pluripotent Stem Cells. Cellular Reprogramming 2015;17(6):427-40.

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

Current studies and grants

1. Pharmacokinetic study of Treosulfan in patients eligible for treosulfan based conditioning regimen prior to HSCT (Hematopoietic stem cell transplantation) – Principal Investigator

2. A study of the kinetics of CMV viremia in patients undergoing bone marrow transplantation and identification of cutoffs for initiation of ganciclovir therapy in patients who show reactivation of CMV infection (Co-investigator)

3. Phase II trial to study if the addition of fludarabine to the standard regimen of anti-thymocyte globulin (ATG) and cyclosporine will improve remission rates in patients with severe and very severe aplastic anemia (SAA/VSAA) (Co-investigator)

4. Phase II trial to study if the addition of meloxicam to the standard regimen of mobilization with colony stimulating factor (G-CSF) will improve mobilization rates in patients undergoing mobilization for autologous stem cell transplantation. (Co-investigator)

5. Novel Approches to Hematological Diseases (NAHD Program coordinator) – part of a multi-institutional grant with three major components:

   a. Gene therapy program
   b. Applications of iPSC technology – Haplobanking
   c. Population based thalassemia and sickle cell disease control program funded by the Department of Biotechnology, MoS & T, GOI

Invited Presentations (2015)

» "Cure for thalassemia major – From stem cell transplant to gene therapy" - at the annual congress of the European Hematology Association in Vienna, Austria in June, 2015.

» "Future of hematopoietic stem cell transplantation – Asia pacific perspective" at the annual congress of the Asia Pacific Blood and Marrow Transplant group meeting in Okinawa, Japan in October, 2015.

Collaborators:

Internal Collaborators:

» Dr. R. V. Shaji, PhD, CSCR / CMC, Vellore

» Dr. Mohankumar Murugesan, PhD, CSCR, Vellore

» Dr. Saravanabhavan Thangavel, PhD, CSCR, Vellore

» Dr. Anu Korula, MD, Dept. of Haematology, CMC, Vellore

» Dr. Aby Abraham, DM, Dept of Haematology, CMC, Vellore

» Dr. Eunice Sindhuvi, PhD, Dept. of Haematology, CMC, Vellore

» Dr. Biju George, DM, Dept of Haematology, CMC, Vellore

» Dr. Vikram Mathews, DM, Dept of Haematology, CMC, Vellore

» Dr. Sukesh Nair, MD, Dept. of Immunohematology, CMC, Vellore

» Dr. Asha Mary Abraham, Dept. of Clinical Virology, CMC, Vellore

» Dr. Hubert Daniel, PhD, Department of Clinical Virology, CMC, Vellore

» Dr. Rajesh Kannangai, MD, Dept. of Clinical Virology, CMC,Vellore

» Dr. Dolly Daniel, MD,Dept. of Immunohematology, CMC, Vellore

» Dr. Kuryan George, MD, Department of Comm. Health, CMC, Vellore

» Dr. Shantidani Minz, MD, Department of Comm. Health, CMC, Vellore

» Dr. J. P. Muliyil, MD, DrPH, Retd. Professor, CMC, Vellore

» Dr. Vrisha Madhuri, Department of Pediatric Orthopedics, CMC, Vellore

External Collaborators:

1. External collaboration for selection of the AAV vector for gene therapy of hemophilia B and its production in cGMPiswithDr. Arun Srivastava, Department of Genetics, University of Florida and Dr. Barry Byrnes, Powell Gene Therapy Center, University of Florida; INTAS Pharmaceuticals, Ahmedabad, will be the industry collaborator from India for production of AAV vectors.

2. Dr. Mavis Agbandje-McKenna, Director, Center for Structural Biology, University of Florida and Dr.Arun Srivastava, Chief, Center for Cellular and Molecular therapy, University of Florida, Gainesville, Florida, USA.

3. External collaboration for development of lenti vectors with Dr. Trent Spencer, Director, Gene Therapy Program, Aflac Children's Cancer Center, Emory University, Atlanta, USA, Dr. Chris Doering and Dr. John Lollar from the Emory University, USA.

4. Dr. Nezih Cereb, PhD, Chief Scientific Officer & Mr. Raghu Rajagopal, CEO, DATRI, Chennai.

Saravanabhavan Thangavel, PhD,
Scientist

Our lab started in September 2015. Our main goal is to setup genome editing as a therapeutic option for patients with genetic disorders. Our research goal involves three broad areas:

1. Targeted Gene therapy for primary immunodeficiency disorders.

Primary immunodeficiency disorders (PID) are a diverse group of more than 300 rare, incurable diseases that occur as a result of genetic mutations in genes involved in the immune response cascade. The affected individuals are susceptible to infections that can be fatal. Allogeneic hematopoietic stem cell (HSC) transplantation is the existing treatment for the most severe forms of PID. Ex vivo genetic correction of autologous HSCs by gene therapy is an emerging option for those who do not have an available HLA- matched donor. Recent gene therapy clinical trials using viral vectors for delivering the functional gene into HSCs treats the immune deficiency, but also poses associated oncogenic risks.

As an alternative to virus-mediated gene therapy for PID, we propose a safer, non-viral gene therapy by combining the new generation clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) based gene-editing tools with a target specific expression of a functional gene. We aim to test this gene editing approach using Wiskott-Aldrich syndrome (WAS) as a candidate for PID. Wiskott-Aldrich syndrome is a life-threatening X-linked recessive disorder characterized by thrombocytopenia, eczema and immunodeficiency. WAS is caused by mutations in the WAS gene, which leads to compromised expression of the Wiskott-Aldrich Syndrome Protein (WASP). WASP plays a key role in hematopoietic actin cytoskeleton reorganization, and the deregulation of this process is responsible for the pathophysiology of WAS. We hypothesize that genome editing mediated targeted expression of WASP would potentially reverse WAS phenotype. Currently, we are setting up preclinical trials to test our hypothesis.

2. Targeted Gene therapy for Blood disorders.

β-thalassemia, one of the most common genetic diseases in India, is caused by mutations in the human hemoglobin beta (HBB) gene. The mutations lead to reduced/absent synthesis of the beta globin chains of the hemoglobin tetramer. We plan to treat this disease by inserting a functional copy of β -globin gene at genome safe harbor locus to complement the deficient production beta globin chains.

Sickle Cell Anemia is characterized by sickle shaped red blood cells which are deficient in transporting oxygen. Sickle diseases are caused by a single point mutation in the seventh codon of the beta-globin gene. We aim for a site-specific correction of the sickle mutation in hematopoietic stem cells for a permanent production of normal red blood cells.

3. Development of technologies for improving the efficiency of targeted therapeutics.

It's essential to achieve a high frequency of genome editing to be useful therapeutically. We plan to employ various small molecules that might help to improve the efficiency of genome editing and also to expand the genome edited cells.

Grants

- CSCR start-up grant.
- "Accelerating the application of stem cell technology in human diseases" CSCR core grant funded by DBT, India.
- "Pre-clinical studies for gene therapy of Wiskott-Aldrich Syndrome (WAS)" SERB-Early Carrier Research award funded by-DST, India.

Lab member Information

Abisha Crystal: JRF

Collaborators

Dr. Alok Srivastava, CSCR
Dr. R.V. Shaji, CSCR
Dr. Srujan Marepally, CSCR

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.

a. 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.

b. 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.

c. 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 FACS Aria III cell sorter with a 5 laser 11 colour setup, BD FACS Aria I SORP cell sorter with a 3 laser 9 colour setup for sorting applications and a BD FACS Calibur cell analyzer for analysis. The BD FACS Aria III system has a throughput of 70,000 events per second and can do 4-way sorting and single cell sorting. The BD FACS Calibur has a 2 laser 4 colour system and is routinely used for intracellular and surface marker analyses by scientists both within CSCR and CMC.

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.

d. Imaging Core Facility:

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

» Technical Officer: Mr. Vaidyanathan. S.

I. Leica DMI6000B Inverted Fluorescence Microscope

The Leica DMI6000B is an inverted fluorescence microscope comprising of 6 interchangeable filters for detecting various fluorochromes. It has two independent cameras – 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.

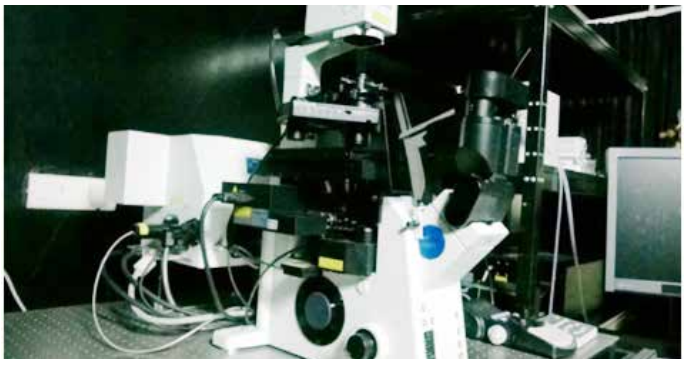
II. Leica Light Microscopes

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

III. Zeiss Inverted Fluorescence Microscope

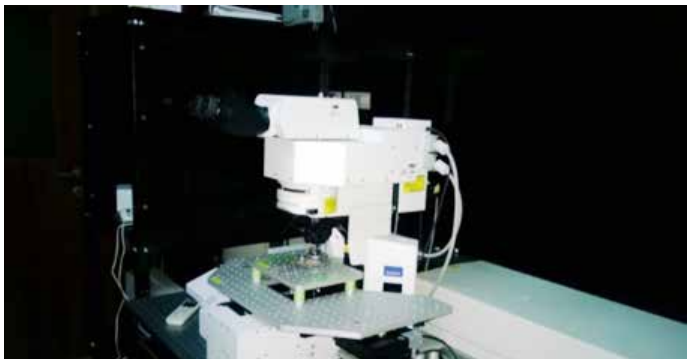
A Carl Ziess Axiovert 40 CFL equipped with 3 filters (DAPI, FITC and TRITC) for routine fluorescence imaging is available, along with a ProgRes C3 camera module for image acquisition.

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



The Olympus FV1000 confocal system comprises a motorized microscope with z focus drift compensation facility for bright field, differential interference contrast and fluorescence imaging with motorized XY scanning stage and CO2 incubation facility 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.

V. 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.

e. Histopathology Core Facility:

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

» Technicians: Mrs. Esther Rani, DMLT and Mr. Ashok Kumar, DMLT.

Special stains standardized:

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, Verhoeff's elastic stain and Sirus Red stain.

2. Cytology:

Cell block preparation.

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

» Faculty In-Charge : Dr. Sanjay Kumar, Ph.D.

» Scientific Officer : Dr. V. Arunprabhakaran M.V.Sc

The IVIS Spectrum CT supports low dose micro CT 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: Dr. Sanjay Kumar, Ph.D.

» Veterinary Officer: Dr. V. Arunprabhakaran M.V.Sc

» Technical Staff: R. Pavithra M.Sc., J. Esther Rani, and S. Ashok Kumar.

The aim of the laboratory animal facility at CSCR is to ensure humane and ethical treatment of animals, while facilitating legitimate scientific research involving experiments on animals.

Objective

The goal of the CSCR-Laboratory Animal Facility is to promote the humane care and use of laboratory animals by providing information that will enhance animal wellbeing, the quality of research, and the advancement of scientific knowledge that is relevant to both humans and animals 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. 88/PO/RcBi/SL/1999/CPCSEA. 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

Quality animal management and human comfort and health protection require separation of animal facilities from personnel areas. For that reason 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 micro-isolator caging (IVC) system for breeding, holding and experimentation. The IVC-systems in which the animals are kept ensures that lab animals are breathing HEPA-filtered air (High Efficiency-Particulate Air) that defends them from most of airborn micro-organisms. The cages are constructed and designed in a specific way to ensure an absolute microparticle-free inner environment. It is also designed to allow maximum comfort for the animals and to provide a secure, chew proof environment. An external ventilation unit supplies the cages with fresh HEPA-filtered air which passes through the filtered cage lids. The ventilation-system mostly consists of two tubes for inlet and outgoing air.

Temperature, Humidity, and Ventilation

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.

Veterinary Care

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 autoclavable vegetable 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 Veterinary and technical staffs of the CSCR-LAF are also supporting and facilitating all animal facility users (including PhD students and Project Assistants) on mouse and rat bio-methodologies, principles of three R's, ethics, IAEC 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, Pseu-dopregnancy	Charles River, UK
5	B6.129S4-F8tm1Kaz/J	Mutant Stock; Tar-geted 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 Thrombo¬-cytic Purpura	Jax Lab, US
9	B6.CB17-Prkdcscid/SzJ	SCID	Transplantation stud-ies	Jax Lab, US
10	C.B-17/Icr-Prkdc<Scid>Ic-rIcoCrl	SCID	Xeno Graft Research	Charles River, UK
11	Sprague Dawley	Rat- Outbred strain	Orthopedic surgery	Jax Lab, US

Quality control (QC)

A quality control program for environmental microbiology, clinical pathology, genetic analysis is being implemented for monitoring of the laboratory rodents and animal feed. Reporting of the QC tests is done in standard formats and QC reports are maintained in the Animal Facility.

Routine/ Conventional Microbiology

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.

ELISA based Microbiology

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).

PCR based Monitoring

Blood samples of sentinel animals are checked for Mycoplasma pulmonis by PCR method.

Genetic Monitoring

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
and Timed pregnancy were established.

C. Current Good Manufacturing Practice Facility (cGMP Facility)

Personnel

» Faculty In-Charge: Dr. Vikram Mathews, MD, DM

» Technical Officer: Mr. Augustine Thambaiah, MSc, P.G. Diploma

» Technician: Ms. Aleya Tabasum, BSc

Clinical grade cells manufactured

» Bone Marrow derived Mesenchymal Stromal Cells (MSC)

» No. of Samples Processed: 58

» Total Cell yield: 6663.29 x 10^6 MSC

» Placenta derived MSC

» No. of Samples Processed: 7

» Total Cell yield: 2458 x 10^6 MSC

Description

The facility is designed to develop and manufacture cellular and tissue engineered products for clinical applications. It provides the infrastructure to conduct Phase I/II clinical trials by supporting translational medicine in the fields of cell therapy and regenerative medicine. The trained staff, directly interact with investigators and help in process development and manufacture of clinical grade products for use in early phase clinical trials of cell therapy and regenerative medicine. The trained staff, directly interact with investigators and help in process development and manufacture of clinical grade products for use in early phase clinical trials.

Facility Layout

Approximately 1200 square feet, the clean room area is divided into four independent manufacturing suites and one common staging room which are all ISO Class 7 (Class 10,000). The manufacturing rooms have positive pressure to adjacent areas. Each suite is equipped with biological safety cabinet, CO_2 Incubators, refrigerated high speed centrifuge and inverted phase contrast microscope. Also a one pass-through both sides is fitted to each room. The facility maintains unidirectional traffic flow for personnel and materials.

Services

There are four independent production suites capable of handling four different projects at a time. The following are the services provided by the GMP facility for users:

» Provides clean-room suites for manufacture of clinical grade products under cGMP conditions for clinical applications.

» Cryopreservation and storage of cell therapy products.

» Bacterial Endotoxin testing using the Charles River EndosafePTS system.

» Mycoplasma testing using ATCC universal mycoplasma detection kit.

» Provides support in the regulatory approval process - Evaluate and interpret regulations and standards for cell based therapy from relevant agencies to determine its applicability to a PI's clinical trial or study.

Facility Resources

» Manufacturing Suites cleaned weekly (includes ceiling, wall, floor).

» Change over cleaning between each manufacturing step.

» Environmental Monitoring Program for both viable & non-viable contaminates- monthly.

» Daily QC checks for door pressure, temperature, etc.

Current scientific activities

The cGMP facility is currently involved in the culture and expansion of autologous MSC for a clinical trial headed by Dr. Vrisha Madhuri (Department of Paediatric Orthopaedics, CMC, Vellore), titled "Treatment of large segmental bone defects with custom made tri-phasichydroxyapatite scaffolds loaded with mesenchymal stem cells in children". Autologous clinical grade MSC is seeded on a synthetic scaffold and is allowed to differentiate into osteogenic cells. The scaffold with differentiated bone cells is transplanted to large segment defects and is monitored for natural bone formation. They have successfully transplanted the cell/scaffold product in 5 patients with no report of any adverse reaction.

GMP facility is also involved with the following research projects:

» A study of human keloid fibroblasts in culture conditions and analyzing the effects of novel drugs on its progression. Department of Pharmacology, Clinical Pharmacology and Plastic Surgery unit, CMC and Centre for Stem Cell Research, CMC Campus.

- To establish the protocol for culture and characterization of Human skin derived fibroblasts.

» Efficacy of Placenta derived Mesenchymal stem cells in reducing corneal scarring, in an ex-*vivo* organ culture model of post mortem human corneas. Department of Opthalmology, CMC and Centre for Stem Cell Research, CMC Campus.

- To culture and characterize human placenta derived Mesenchymal Stromal Cells and develop an ex-vivo organ culture model to maintain human corneas in culture.

Access

Access to the facility is limited onlyto GMP trained staff. The services are available for investigators from Christian Medical College, Vellore and other non-profit organizations. For any service related queries please contact Augustine Thambaiah at +91-416-307-5168 or e-mail cscrcpf@cmcvellore.ac.in

Training

I. Ph.D Program

CSCR has an active PhD programme and the students can register for PhD under Sree Chitra Thirunal Institute of Medical Sciences and Technology (SCTIMST), Thiruvananthapuram, or Thiruvalluvar University, Vellore.
one student has registered for PhD in 2014-2015.

II. Thesis Submitted

- Ms. Sangeetha. H
- Mr. Vikram Sabapathy

III. Other training programs:

- Short term student projects (Bi-annual)

S.No	Name	Duration	Qualification	University	Project title	PI /Lab
1.	Ms. Latha Priyadarshini	Jan 15 - Jun 15	B.Tech - Biotech	Sree Sastha institute of engineering & technology	Phenotypic characterization and stable expression of firefly luciferase in human adipose derived Mesenchymal Stem Cells (hAD-MSc) by second generation lentivirus.	Dr. Sanjay/ Lab- 3
2.	Ms. Priyadarshini.G	Jan 15 - Jun 15	B.Tech - Biotech	Sree Sastha institute of engineering & technology	Phenotypic characterization and stable expression of firefly luciferase in human adipose derived mesenchymal stem cells (had-msc) by second generation lentivirus.	Dr. Sanjay/ Lab- 3
3.	Ms. Aruna. T	Jan 15 - Jun 15	M.Sc - Biotech	Thiruvalluvar University	Phenotypic characterization and stable expression of firefly luciferase in human bone marrow derived Mesenchymal Stem Cells (hAD-MSc) by second generation lentivirus.	Dr. Sanjay/ Lab- 3
4.	Mr. Naveen Kumar	Jan 15 - Jun 15	M.Sc - Biotech	Thiruvalluvar University	Phenotypic characterization and stable expression of firefly luciferase in human adipose tissue derived Mesenchymal Stem Cells (hAD-MSc) by second generation lentivirus.	Dr. Sanjay/ Lab- 3

8	Ms. Jaya Preethi. K	Jan 15 - Jun 15	M.Tech (Integrated) Biotech	Bharathidasan University	Construction of mirna (486, 191, 92a2, 181, 148a, 30e, 16, 10a, 21, let 7a-1, let 7a-2, let 7a-3) vectors and access the knockdown efficiency of shrna (hdac-1, prmt8, ubiq-uitin ligase-1,5,11) in fibroblast stem cells	Dr. Shaji/ Lab- 2
9	Ms. Nivedita. S.R	Jan 15 - Jun 15	B.Tech - Bio-tech	SRM University	Gene Transfer in Stem Cells	Dr. Shaji/ Lab- 2
10	Ms. Aruna Barade	Jul 15 - Dec 15	M.Tech Bio-tech	VIT University	Hydrogels for stem cell delivery	Dr. Murugan/ Lab- 8
11	Ms. Thulya. C.P	Jul 15 - Dec 15	M.Tech Mate-rials Science (Integrated)	University of Mysore	Gradient biomaterials for interfacial tissue engineering	Dr. Murugan/ Lab- 8

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

Secretary, DBT, New Delhi	Chairman
Director, inStem Bengaluru	Member
Deans, inStem, Bengaluru	Member
NII, New Delhi	Member
Director, NII, New Delhi	Member
IISc, Bengaluru	Member
Director, KEM Hospital, Pune	Member
Director, CMC, Vellore	Member
Head, CSCR, CMC, Vellore	Member
JS & FA, DBT, New Delhi	Member
Adviser, DBT, New Delhi	Member
Joint Director, DBT, New Delhi	Member
Head, A & F, inStem, Bengaluru	Non Member Secretary

b. CSCR Committee

Director, Christian Medical College, Vellore	Chairperson
Director inStem, Bengaluru	Member
Deans, inStem, Bengaluru	Member
Principal, Christian Medical College, Vellore	Member
Joint Director, Department of Biotechnology, Govt. of India, New Delhi	Member
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.

Director, CMC	Chairperson
Principal, CMC Vellore	Member
Associate Dir. - Finance	Member
Associate Dir. - General Admin	Member
Associate Dir. - HR	Member
Medical Superintendent	Member
Treasurer	Member
General Superintendent (Ag)	Member
Addi. Vice Principal (Research)	Member
Members of core committee of CSCR	Member
Head, CSCR	Member Secretary

Academic Staff's

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. Srujan Kumar Marepally	-	Scientist
4.	Dr. Murugan Ramalingam	Associate Professor, Centre for Stem Cell Research	Scientist
5.	Dr. Rekha Samuel	Professor, Centre for Stem Cell Research	Adjunct Scientist
6.	Dr. Alok Srivastava	Professor and Head, Centre for Stem Cell Research	Adjunct Scientist
7.	Dr. R.V. Shaji	Professor, Department of Haematology	Adjunct Scientist
8.	Dr. Saravanabhavan T	Asst. Investigator, Centre for Stem Cell Research	Scientist
9.	Dr. B. Poonkuzhali	Professor, Department of Haematology	Adjunct Scientist
10.	Mrs. Samrajyam Nara	Technical Officer	-
11.	Mr. Vaidyanathan Subramaniam	Technical Officer	-
12.	Mr. Augustine Thambiah	Technical Officer	-
13.	Dr. Arunprabhakaran V	Veterinary Officer	-
14.	Mr. Syed Mohammad Musheer Aalam	SRF	-
15.	Mr. Salar Abbas	SRF	-
16.	Mr. Thiyagaraj M	SRF	-

17	Ms. Kasthuri N	SRF	-
18	Mr. Vikram Sabapathy	SRF	-
19	Mr. Balasubramanian S	SRF	-
20	Mr. Kannan V.M	SRF	-
21	Mr. Abhirup Bagchi	JRF	-
22	Ms. Abisha Crystal	JRF	-
23	Ms. Aneesha Nath	JRF	-
24	Ms. Remi Treasa Eugine	JRF	-
25	Mr. Franklin Jebaraj Herbert	JRF	-
26	Mr. David Livingstone I	JRF	-
27	Mrs. Deepti Rana	JRF	-
28	Ms. Keerthana R	JRF	-
29	Ms. Sumithra Yasaswini	JRF	-
30	Ms. Aleya Tabasum	Graduate Tech.	-
31	Mrs. Dhavapriya B	Graduate Tech.	-
32	Mrs. Kalaivani G	Graduate Tech.	-
33	Mrs. Esther Rani J	Technician	-
34	Ms. Saranya J	Graduate Tech.	-
35	Mr. Sathish P	Technician	Till August 2015
36	Mrs. Chitra P	Graduate Tech.	-
37	Ms. Saranya R	Graduate Tech.	-
38	Mrs. Pavithra R	Graduate Tech.	-
39	Ms. Saranya R	Graduate Tech.	-
40	Mr. Ashok Kumar S	Technician	-

Non-Academic staffs

S. No.	Name	Designation
1.	Mrs. Anupama Nambiar	Office Admin
2.	Mrs. Shirley Anandanathan	Secretary
3.	Mrs. Selvi P	Clerk Typist
4.	Mrs. Geetha R	Accountant
5.	Mr. Muthu Krishnan J	Artisan
6.	Mr. Tamil Vanan J	Asst. Librarian
7.	Mr. Silambarasan. R	Driver
8.	Mr. Ramraj M	Hospital Attendant
9.	Mr. Nithyanand S	Hospital Attendant
10.	Mr. Arun Kumar J	Hospital Attendant
11.	Mr. Shankar S	Hospital Attendant
12.	Mr. Augustin Vasanthakumar	Hospital Attendant
13.	Mr. Sakthivel S	Housekeeping Attendant
14.	Mrs. Renuka Devi G	Housekeeping Attendant

Other Activities

Ministers visit to CSCR 2015

SAC 2015

Christmas celebration 2015

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- 416-3075103
Email: cscr@cmcvellore.ac.in | website: www.cscr.in