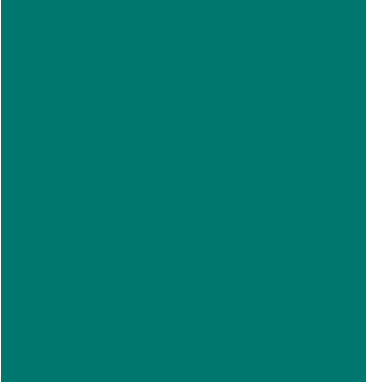
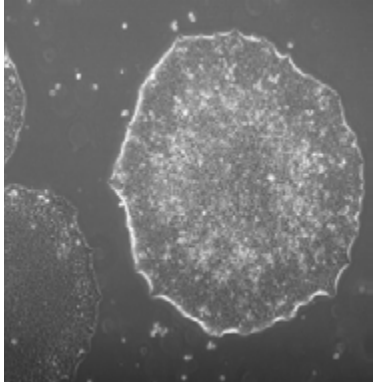
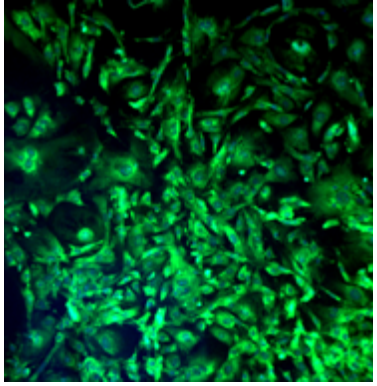
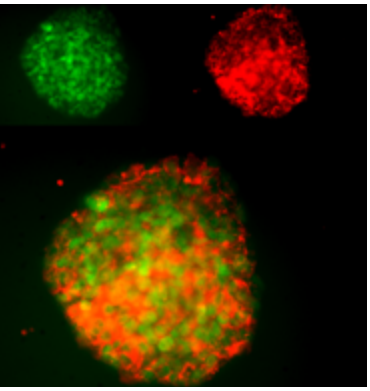
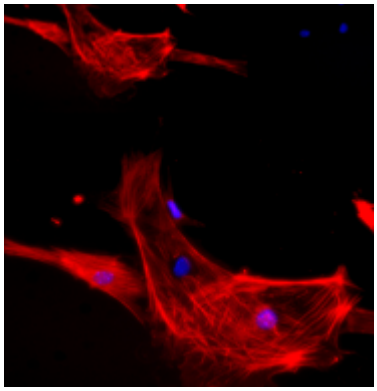
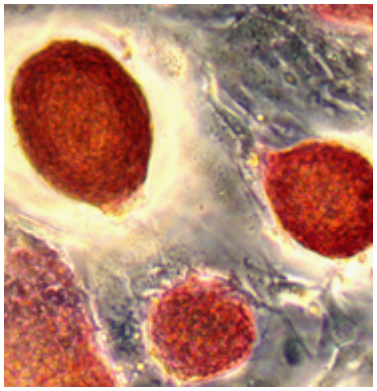
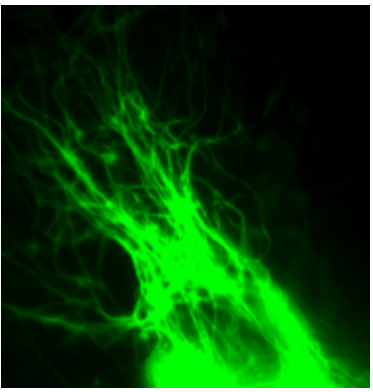
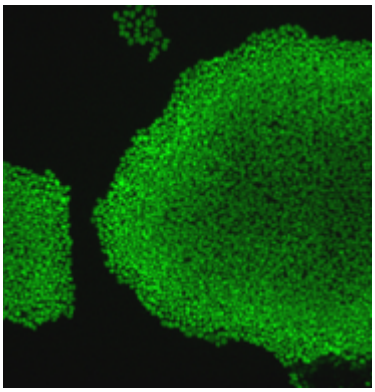
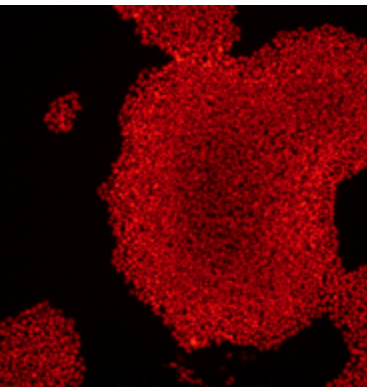
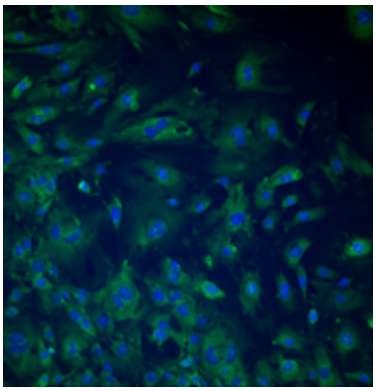











Annual Report

2016-2017

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

CONTENTS

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 - 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 Principal of CMC, Vellore along with the Director and Dean of inStem. It also has a Finance Subcommittee which is part of Finance Committee of inStem both of which report to the inStem Governing Body, chaired by the Secretary, DBT. Given the predominantly translational nature of the research at CSCR, it also has a separate Scientific Advisory Committee.

CORE SCIENTIFIC ACTIVITIES AND INITIATIVES

Thematic Research Programs

1. Gene therapy program

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 erythropoietic 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. Musculoskeletal regeneration program

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 area of cellular reprogramming technology is coordinated 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.

Research Projects

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. 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.

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:

- Dr. Aniket Kumar, Senior Lecturer, Department of Pharmacology & Clinical Pharmacology, CMC Vellore: Study of human keloid fibroblasts in culture and effects of novel drugs.
- Dr. Christhunesa S. Christudass, Associate Professor – Neurochemistry, Department of Neurological Sciences, CMC, Vellore: Isolation of cancer stem cells from primary and secondary high-grade gliomas - their response to microenvironmental cues and Notch signaling blockade.
- Dr. Inian Samarasam, Professor and Head, Upper GI Surgery Unit, Department of Surgery - Unit 3, CMC, Vellore: Tissue regeneration using muscle derived stem cells in the treatment of ventral hernia in a rat model.
- Dr. Ravikar Ralph, Assistant Professor Grade I, Department of Medicine Unit-I, CMC, Vellore: β-chemokine expression and HIV-1 infection in CD34+ haematopoetic stem cells – A pilot study.
- Dr. Jeyanth Rose, Associate Professor, Department of Ophthalmology-Schell Eye Hospital, CMC, Vellore: Efficacy of placenta derived Mesenchymal Stem Cells in reducing corneal scarring in an Ex-vivo organ culture model of post mortem human corneas / A Bioengineered corneal substitute using decellularized human donor cornea rejected for corneal transplant
- Dr. Poonkuzhali Balasubramanian, Professor, Department of Haematology, CMC, Vellore: Exploring the mechanisms of disease progression, tyrosine kinase inhibitor resistance and intolerance in Chronic Myeloid Leukemia / Identification of novel nuclear receptor (NHR) drug targets in myeloid leukemias.
- Dr. Boopalan Ramasamy, Professor, Department of Orthopedics Unit III, CMC Vellore: In vitro characterization and immunogenic profiling of human articular chondroprogenitor cells from normal and osteoarthritic knee joints / Injectable Chondroprogenitor Cells in the Treatment of Osteoarthritis in a Rabbit Knee Model.
- Dr. Aby Abraham, Professor, Department of Haematology, CMC Vellore: Gamma delta T cell-based immunotherapy for blood cancers / Establishing a protocol for expansion of Natural Killer cells

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
Head, CSCR

NOVEL APPROACHES TO HEMATOLOGICAL DISEASES (NAHD) PROGRAM

In 2016, the Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India launched a major project titled '**Accelerating the application of Stem cell technology in Human Disease**' or ASHD program. This program involves leading Indian research institutions engaged in cutting edge research and technology – The Christian Medical College (CMC) with the Centre for Stem Cell Research (CSCR), a unit of inStem, at Vellore, the National Centre for Biological Sciences (NCBS), Institute for Stem Cell and Regenerative Medicine (inStem), and the National Institute for Mental Health and Neurosciences (NIMHANS) from Bangalore – in a massive collaborative effort to use stem cells in research, diagnostics and therapeutics.

In addition, the ASHD program collaborates with the Centre for iPS Cell Research and Application (CiRA), Kyoto University, Japan, under the leadership of Prof. Shinya Yamanaka, a pioneer and Nobel Prize winner in stem cell technology. The program at NCBS, inStem, and NIMHANS - The Accelerator program for Discovery in Brain disorders using Stem cells (ADBS) – encompasses research to unravel complex problems in brain disorders / mental illnesses by exploiting the advances in modern human genetics, stem cell technology and clinical investigations. The program at CSCR / CMC - Novel Approaches to Hematological Disorders (NAHD) aims to enhance current methods / technologies including gene therapy for hereditary blood disorders such as haemophilia, thalassemia and sickle cell disease, all of which are causes of significant morbidity and mortality in India. To ensure maximum impact on hereditary hemoglobin diseases in the population at risk in India, this collaborative initiative blends these efforts with a community outreach program for the control of major haemoglobin disorders.

The major components of this program are:

- Clinical trial for gene therapy of Hemophilia B (see report of Alok Srivastava)
- AAV antibody screening (see report of Asha Abraham)
- Lentiviral (see report of R V Shaji) and gene editing (see reports of Saravanabhavan / Mohankumar) approaches for treatment of major hemoglobin disorders
- Applications of iPSC technology - Haplobanking (see reports of Dolly Daniel / R V Shaji)
- Population-based control program for major hemoglobin disorders (see report of Alok Srivastava)

The components of this program are within the thematic research programs that are ongoing in CSCR. More details of this program are shown in individual reports as mentioned above.

Scientific Research Profile

VRISHA MADHURI, MS (Ortho), MCh (Ortho)
Professor and Head, Department of Pediatric Orthopedics, CMC, Vellore
Adjunct Scientist, CSCR

LABORATORY HIGHLIGHTS

Our lab focuses on treating musculoskeletal regeneration using cell based therapy. We have completed two phase-1 clinical trials for the treatment of large segmental bone defects and physeal arrest in children. Preclinical studies in goats have been conducted for articular cartilage regeneration and osteochondral repair in rabbits using tissue engineered constructs. In addition, we are working on a preliminary rat model for sphincter injury. A phase I/II clinical trial has been initiated for the treatment of osteogenesis imperfecta using fetal derived mesenchymal stem cells. Two preclinical studies are in pipeline for osteochondral and segmental bone repair using functionalized scaffolds.

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 has been completed. A total of ten patients have undergone transplantation. All children had shown radiological evidence of union at 2 -3 months. One graft failed because of infection 1 year after surgery. In addition to assessment of union with radiographs, CT evaluation at 9 months showed integration at both proximal and distal ends.

Articular cartilage:

Goat femoral head articular cartilage regeneration: In this study the bone marrow MSCs were harvested and seeded on an electrospun PCL scaffold and chitosan hyaluronic dialdehyde hydrogel. Cells-seeded constructs were differentiated into chondrocytes prior to transplantation into the goat femur articular cartilage defect. One of the goats received cells labeled with GFP labeled cells. The histology at 2 months post-operation shows evidence of regeneration. The histology of animals sacrificed at 1 year showed good quality of regenerated cartilage in 2/5 and a better regeneration was observed in 1/5 as compared to the controls.

Treatment of osteochondral defects in rabbits: A multilayer hydrogel was fabricated by our collaborator Dr. D. Katti, IIT, Kanpur for in vivo testing. This scaffold mimics the orientation of natural collagen architecture present in the articular cartilage. Allogenic MSCs seeded on this scaffold were differentiated into chondrocytes and transplanted into osteochondral defects in a rabbit model. A scaffold with random fiber alignment served as a control. Gross appearance and histology scoring indicated that both the scaffolds regenerated hyaline like cartilage. Further confirmation using immunohistochemistry and biochemical assays are required to support this outcome.

Cancer stem cells (CSC): Cytotoxicity assay showed higher resistance of cancer stem cells derived from patient samples and cell line for Cisplatin, Doxorubicin and methotrexate and was in accordance to our hypothesis. In vivo tumirogencity was assessed using patient derived CSCs. Tumor from these xenogenic transplants was larger in size and developed at an earlier time point as compared to the group which received cancer cells. We observed enhancement of stemness when tumor cells were cultured in 3D environment. Our results demonstrate that CSCs can be isolated from patient tissue and characterised similar to a cell line. Successful isolation of CSCs and their chemosensitivity and in vivo tumorigenicity may provide a platform to use CSCs to predict the chemo sensitivity for osteosarcoma patients. We are liaising with a group from Netherlands to see if this can be combined with 3D printing technique for drug testing for osteosarcoma.

Growth modulation: This study attempts to evaluate the effect of shockwaves on longitudinal bone growth. Our preliminary in vitro and in vivo outcome suggested changes in the bone length and physis respectively. The results of the long term effect are awaited. In vitro studies on cultured human stromal cell lines suggest dose dependent apoptotic changes. Gene expression studies suggest that increasing energy levels affects cellular proliferation; lower energy shows increased RUNX2 (master transcription for osteogenesis), ALPL (mineralization) COL10A (marker for cellular hypertrophy), and MMP-13 (indicator of cellular differtiation), while high energy levels show increase in VEGF (indicator of vascularity).

Differentiation of mesenchymal stem cells (MSCs) into chondrocytes by sustained delivery of miRNAs using chitosan hydrogel: The current protocol for the differentiation of MSCs into chondrocytes results in hypertrophic cartilage instead of hyaline like cartilage. This upon transplantation leads to endochondral ossification. We hypothesize that the quality of differentiated chondrocytes could be enhanced using miRNAs. In this study, we have selected few miRNA which will aid MSCs to differentiate in to chondrocytes; sustained delivery of specific miRNAs using scaffold will help in the maintenance of chondrocyte phenotype (hyaline), thus preventing hypertrophy. Preliminary results in gene expression suggest that the delivered miRNA could prevent chondrocyte hypertrophy. Further confirmation using immunohistochemistry and biochemical assays are awaited.

Grants

Ongoing studies & funding

Project 1:

- 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 lakhs

Project 2:

- 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

Project 3:

- Title: Musculo-Skeletal Stem Cell Targeting (Indo-Danish Collaborative project)
- Funding agency: Department of Biotechnology
- Budget: Rs. 99.77 lakhs

Project 4:

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

Project 5:

- Title: Tissue regeneration using muscle derived stem cells in the treatment of ventral hernia in a rat model
- Funding agency: CSCR
- Budget: Rs. 5.25 lakhs

Completed studies & funding

Project 1:

- 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

Project 2:

- 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 3:

- 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 4:

- 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 lakhs

Awaiting funding: The following projects have been approved and awaiting funds

Project 1:

- Title: Molecular genetic analysis of Osteogenesis imperfecta in Indian Children
- Funding agency: Indian Council of Medical Research
- Budget: Rs. 74.68 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

Project 3:

- Title: Transplantation of autologous iliac crest physeal chondrocytes cultured in monolayer to treat Physeal bars in children
- Funding agency: Department of Health Research
- Budget: Rs. 74.93 lakhs

Project 4: Status approved

- Title: Boost to Brittle Bones (BOOST2B) (Indo-Swedish)
- Funding agency: Department of Biotechnology
- Budget: Rs. 5.87 crores

Publications:

- David Livingstone, Albert A Kota, Sanjay K Chilbule, Karthikeyan Rajagopal, Sukria Nayak, Vrisha Madhuri Isolation, in-vitro expansion, and characterization of human muscle satellite cells from the rectus abdominis muscle. Paediatric Orthopaedics and Related Sciences. Volume 3. February 2017.
- Vrisha Madhuri, Smitha Elizabeth Mathew, Karthikeyan Rajagopal, Sowmya Ramesh, Antonisamy B. Does pamidronate enhance the osteogenesis in mesenchymal stem cells derived from fibrous hamartoma in congenital pseudarthrosis of the tibia? Bone Reports Volume 5, December 2016, Pages 292-298
- B Balakumar, Rajagopal K, Madhuri V. Bone marrow extract as a growth supplement for human growth plate chondrocyte culture. Indian J Med Res. 2016 Dec;144(6):831-837. doi: 10.4103
- Madhuri V, Santhanam M, Sugumar LK, Rajagopal K. WISP3 mutational analysis in Indian patients diagnosed with progressive pseudorheumatoid dysplasia and report of a novel mutation at p.Y198* Bone Joint Res. 2016 Jul; 5(7): 301–306.
- Radhakrishna VN, Madhuri V Management of pediatric open tibia fractures with supracutaneous locked plates.J Pediatr Orthop B. 2017
- Garge S, Keshava SN, Moses V, Chiramel GK, Ahmed M, Mammen S, Madhuri V Radiofrequency ablation of osteoid osteoma in common and technically challenging locations in pediatric population..Indian J Radiol Imaging. 2017 Jan-Mar;27(1):88-91.

Students trained:

S.No	Student	Qualification	Place
1.	Dr. Banteilang Nonlang	PG Orthopaedics	CMC, Vellore
2.	Mr. Mohammed Abrar Basha	B.Tech Genetic Engineering	Bharath University, Chennai
3.	Ms. Anushna Sen	B.Tech Biotechnology	VIT, Vellore
4.	Ms. Nimmy Vincy	M.Sc Biotechnology	Loyala college, Chennai

Honours & awards

- NRDC Meritorious innovation award 2016 for Dr Vrisha Madhuri and team (Sanjay Chilbule, Dr NS Dinesh et al)
- Dr. Vrisha Madhuri delivered the Mercer Rang Eponymous Lecture on 'Regenerative strategies for physeal and bone defects in children' and was Mercer Rang visiting professor at Hospital for Sick Children Toronto 8th and 9th June 2017
- Mr. Karthikeyan Rajagopal won the first prize for a poster presentation titled "Role of hydroxyapatite and pamidronate on osteogenic differentiation of MSCs derived from fibrous hamartoma of congenital pseudarthrosis of tibia at the Paediatric Orthopaedic Society of India 2017, Nagpur.
- Dr. Sanjay Chilbule received Dr R C Rallan Gold Medal – for Best paper award in the basic Science category at All India Orthopaedic Association annual conference 2016 (IOACON 2016) held at Kochi, December 2016 for the paper titled "Isolation and in vitro and in vivo characterization of cancer stem cells from human osteosarcoma tissue and assessment of chemo sensitivity.
- Ms. Sowmya Ramesh won the second prize for an oral presentation titled "Effect of shockwave on growth plate cartilage" at the Annual Research Day 2016, CMC, Vellore.

Invited lectures

- Indo-German Workshop – Dr. Sanjay Chilbule took part in "Childhood Disorders at Cancer Institute" Chennai, India from 26th to 28th October 2016. Organised by German House (stem cells in osteosarcoma).
- Dr Vrisha Madhuri presented the 'Bone tissue Engineering' as the faculty lecture at POSICON 2017 at Nagpur Jnauary 26th to 28th 2017.

Poster / conference

- Ms. Sowmya Ramesh presented two posters titled "Evaluation of safety and feasibility of novel tissue engineered bone for large segmental bone defects using autologous mesenchymal stem cells" and "Suitability of human muscle-derived stem cells on electrospun PCL scaffolds for skeletal muscle tissue engineering" at the European Chapter Meeting of Tissue Engineering and Regenerative Medicine Tissue Engineering and Regenerative Medicine International Society (26 - 30 June, 2017) in Davos, Switzerland.
- Mr. Karthikeyan Rajagopal presented two posters titled "Chitosan-gelatin nanocomposite scaffold for articular cartilage regeneration" and "Articular cartilage regeneration in osteoarthritis rat model" at the European Chapter Meeting of Tissue Engineering and Regenerative Medicine Tissue Engineering and Regenerative Medicine International Society (26 - 30 June, 2017) in Davos, Switzerland. He was selected for a travel grant from SERB, DST to attend the conference.
- Mr. Karthikeyan Rajagopal presented a paper in 46th Annual Conference of Orthopaedic Surgeon Society of Andhra Pradesh, Nagpur in Feb 2017- "Treatment of growth plate defect using autologous chondrocyte transplantation".

Lab members

- Karthikeyan R., Senior Research Fellow
- Sowmya R., Senior Research Fellow
- Nimmy Vincy, Junior Research Fellow

Collaborations:

External (International):

- Henrik Daa Schrøder, Professor Pathology, and Mustapha Kassem Professor Endocrinology University of Southern Denmark, Denmark
- Jørgen Kjems, Professor Department of Molecular Biology, University of Aarhus, Denmark
- Lars Savendahl, Professor of Pediatric Endocrinology, Karolinska University Hospital, Stockholm, Sweden
- Cecilia Gotherstrom, Associate Professor, Division of Obstetrics and Gynecology, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

External (National):

- Prabha D. Nair, Scientist G, Tissue Engineering and Regeneration Technologies Division, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Trivandrum.
- Harikrishna Varma, Scientist F, Tissue Engineering and Regeneration Technologies Division, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Trivandrum.
- Dhirendra S. Katti, Professor, Department of Biological Sciences & Bioengineering, Indian Institute of Technology, Kanpur

Internal:

- Nihal Thomas, Department of Endocrinology, CMC, Vellore
- Thomas Paul, Department of Endocrinology, CMC, Vellore
- Sumita Danda, Department of Genetics, CMC, Vellore
- Susan Jehangir, Department of Paediatric Surgery, CMC, Vellore
- Sukriya Naik, Department of General Surgery, Unit-4, CMC, Vellore
- Nitin Kekre, Department of Urology, Unit-2, CMC, Vellore
- Inian Samarasam, Department of Surgery, Unit-3, CMC, Vellore
- Dolly Daniel, Transfusion Medicine & Immunohematology, CMC, Vellore

BOOPALAN RAMASAMY, MS
Professor, Department of Orthopedics Unit-3, CMC, Vellore
Adjunct Scientist, CSCR

PROJECT-1

Project title: In vitro characterization and immunogenic profiling of human articular chondroprogenitor cells from normal and osteoarthritic knee joints.

Funding source: AO Trauma Asia Pacific Research Grant 2016

Duration: 2016 - 2017

Brief description: In the present study we aim to isolate, culture, characterize and differentiate chondroprogenitors from articular cartilage of both normal and osteoarthritic knee joints and test their immunogenicity as a capable material for future transplant purposes

Aim:

- Invitro isolation, characterization, differentiation and immunogenic profiling of human articular chondroprogenitors from normal and osteoarthritic knee joints.

Objectives:

- To isolate and culture chondroprogenitor cells from articular cartilage of human knee joints - normal (post trauma) and osteoarthritic knee joints.
- To isolate and culture chondrocytes from the articular cartilage of human knee joints - normal (post trauma) and osteoarthritic knee joints, and study differences between chondrocytes and chondroprogenitor cells.
- To phenotypically characterize chondroprogenitors and chondrocytes at various passages by Fluorescence Assisted Cell Sorting (FACS).
- Proliferative and Senescence assay of passaged chondrocytes and chondroprogenitors.
- Tri-lineage differentiation of the isolated chondroprogenitor cells and immune profiling of the chondroprogenitors cells differentiated to chondrocytes.
- Immunogenic profiling of chondroprogenitors cells by flow cytometry analyses, T cell response in invitro stimulation assays, RT-PCR and ELISA assays.

Work done:
We have acquired 3 sets of osteoarthritic and 2 sets of normal human cartilage which are presently being cultured and are at various passages. Consecutively fluorescence assisted cell sorting, proliferative and senescence assay and RT PCR studies are currently being done.

Support from CSCR: Infrastructure and lab space.

PROJECT-2

Project title: Injectable Chondroprogenitor Cells in the Treatment of Osteoarthritis in a Rabbit Knee Model.

Funding source: AO Trauma Asia Pacific Research Grant 2015

Duration: August 2015 to June 2016

Brief description: This was a project to assess the use of a cell population from the surface of articular cartilage in the healing of an experimentally created osteoarthritis model in rabbits.

Aim:

- To determine the efficacy of injectable chondroprogenitors in the healing of experimentally created osteoarthritis in rabbits.

Objectives:

- To isolate and culture chondroprogenitor cells from the superficial layer of rabbit knee joint articular surface.
- To create osteoarthritis in rabbit knees by injecting monosodium iodoacetate.
- To inject cultured allogenic chondroprogenitor cells suspended in sodium hyaluronate as a delivery vehicle intra-articularly.
- To histologically evaluate the outcome of treatment at 3 and 6 months for hyaline cartilage formation.

Work done:

In this present study, OA was created bilaterally in all the knee joints using MIA. The dosage required to create the required grade of MIA-OA rabbit model was standardized and a time period of one month was allowed for the creation of OA which was further confirmed by ELISA. Concurrently, one rabbit's articular cartilage was harvested and ADCP was isolated and cultured to reach confluence. The ADCP was co-injected with HA intraarticularly into the right osteoarthritic knee joints and HA alone into the left osteoarthritic knee joints which served as control. At 12 and 24 weeks, the rabbits where euthanized and the synovial fluid and joints were harvested for S100A12 protein evaluation and histological scoring respectively.

Specific highlights of the project:

Based on our short arm results the injection of progenitors showed better prognosis as compared to the control arm. The awaited histological reports will confirm our findings and give us a better ground on the use of these cells as treatment for osteoarthritis.

Support from CSCR: Infrastructure and lab space.

INIAN SAMARASAM, MS, FRCS, FRACS
Professor and Head, Upper GI Surgery Unit, Department of Surgery - Unit 3, CMC, Vellore
Adjunct Scientist, CSCR

Project title: Tissue regeneration using muscle derived stem cells in the treatment of ventral hernia in a rat model

Funding source: CSCR

Duration: December 2016 - 2018

Brief description: This study attempts to test the combination of culture expanded autologous muscle derived stem cells over an electrospun poly-caprolactone scaffold in a ventral hernia model. The cell seeded construct along with a polypropylene (PP) mesh was transplanted into the abdominal wall of the rat (n=12) after the creation of a ventral hernia. The animals that received PP mesh alone and PP + PCL served as a control (n=12 each). The outcome at six and ten weeks post transplantation was assessed by adhesions, tensile strength and histology. Our preliminary results suggest gross healing (Fig.1), minimal adhesion and evidence of regenerated muscle towards the peritoneal layer histologically in the cell seeded group. While the PP and PP+PCL group had higher adhesion scores with no significant muscle regeneration. Thus, our study establishes safety and feasibility of using tissue engineered construct for the treatment of ventral hernia.

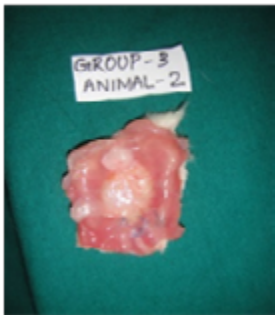


Figure-1

Support from CSCR: Funding, laboratory animal facility, core lab facility

Collaborations:

Internal:

- Vrisha Madhuri, CSCR/CMC, Vellore
- Geeta Chacko, CMC, Vellore

External:

- Amit Kumar, VIT, Vellore

ALOK SRIVASTAVA, MD, FRACP, FRCPA, FRCP
Professor, Department of Haematology, CMC, Vellore
Adjunct Scientist / Head, CSCR

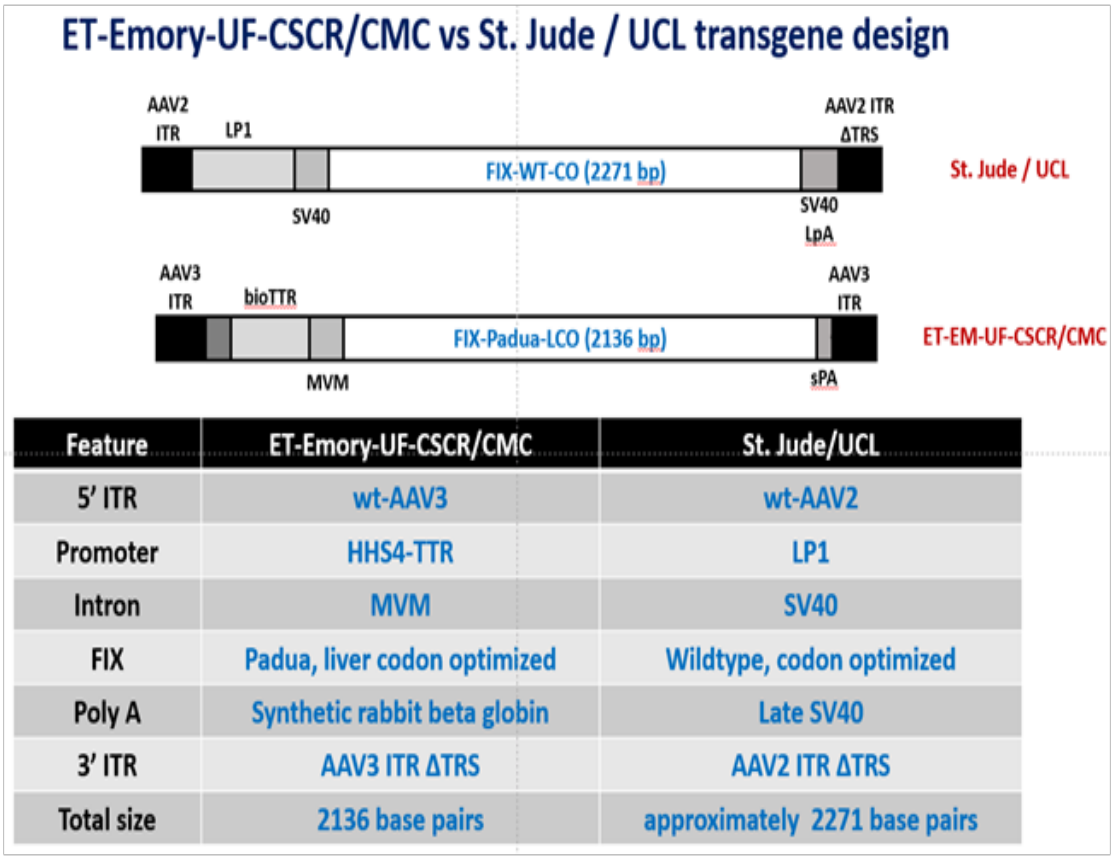
Scientific Aras of Research:

In the past year, there have been major advances in several existing research programs and certain new areas are also being developed. These include the development and coordination of the clinical gene therapy program for hemophilia and also some of the preclinical work for developing gene therapy for hemoglobin disorders as well as the work related to banking of iPSC lines from normal individuals with homozygous HLA haplotypes, as explained below. The new areas being developed are targeted T & natural killer (NK) cell therapy.

Highlights from different research programs

A. The gene therapy program

1. Significant advances have been made in the work towards initiating a clinical trial for gene therapy for hemophilia in India supported by the major grant provided by the Department of Biotechnology under the Novel Approaches to Hematological Disease (NAHD) program. In collaboration with Dr. Trent Spencer, Chief of the division of Gene and Cellular Therapies at the Emory University, Atlanta, Georgia, USA, and his team, a suitable IP free transgene has been developed for FIX gene replacement therapy (see figure & table below).



Feature	ET-Emory-UF-CSCR/CMC	St. Jude/UCL
5' ITR	wt-AAV3	wt-AAV2
Promoter	HHS4-TTR	LP1
Intron	MVM	SV40
FIX	Padua, liver codon optimized	Wildtype, codon optimized
Poly A	Synthetic rabbit beta globin	Late SV40
3' ITR	AAV3 ITR ΔTRS	AAV2 ITR ΔTRS
Total size	2136 base pairs	approximately 2271 base pairs

Development of a novel and efficient transgene

While conceptually similar, the ET-Emory-UF-CSCR/CMC transgene shares NO components with the St. Jude/UCL transgene

Based on collaborative research arrangements / agreement with Dr. Arun Srivastava for the Division of Cell and Gene Therapy and Dr. Barry Byrne of the Powell Gene Therapy Center at the University of Florida, USA, this construct was first evaluated in human hepatocytes and in haemophilia mice as naked plasmid and compared with the St. Jude/UCL construct. Each of the changes made have shown improved efficiency of expression. It is now being tested in an AAV3 vector in humanized mouse models after which it will be tested in IND enabling non-human primate studies for dose finding, safety, toxicity and biodistribution. In parallel, methods for its culture and expansion under GMP conditions are also being developed at the Powell Gene Therapy Center. AAV3 has been shown to have the highest degree of tropism for the human hepatocyte among all AAV serotypes. This will be the first time AAV3 vector will be used for liver directed gene therapy in humans. Apart from this, the clinical gene therapy program is also being developed with screening of patients for AAV antibodies (see report from Dr. Asha M. Abraham) and development of the clinical protocol with appointment of the necessary clinical research team. The regulatory aspects of the clinical trial have also progressed. A pre-IND proposal submitted to the CDSCO and the Joint ICMR/DBT Working Group for Gene Therapy was reviewed recently. The overall research development plan was appreciated and thought to adequate. Final reports from these reviews are expected soon which will help tune the pre-clinical development of this product as recommended. This is expected to be completed in next ~6 months following which an IND proposal will be filed with the CDSCO.

2. Apart from this effort for developing gene therapy for hemophilia, a parallel gene therapy program for haemophilia B is also being pursued with industry collaboration. An agreement has been made for pre-clinical development and clinical studies with one of the major pharmaceutical groups in India that has made significant investment in this area of research and product development over the last two years. Here the goal is to proceed with a known transgene and an AAV vector which has been tested in published clinical studies already but for which there is freedom to operate in India. Preclinical studies in mice are ongoing with this therapeutic product. A corollary to this collaboration is that the technology for AAV vector production will also get established in India which will be useful for other AAV based clinical trials as needed.

3. Within the gene therapy program at CSCR, several other components exist. An extensive program is also directed at the major haemoglobin disorders such as thalassemia major and sickle cell disease under the NAHD project. In this area, two approaches are being taken. A lentiviral vector based gene therapy approach to replicate the broad design on the current clinical trials. This is led by Dr. R.V. Shaji (see report) and is being done in collaboration with Dr. Trent Spencer and his group at Emory University, USA. Several transgene designs have been tested and more new transgenes are being developed for evaluation in cellular models of erythropoiesis followed by testing in animal models. Also being pursued for this area of gene therapy are the genome editing based approaches through the work of Dr. Saravanabhavan Thangavel (ST) and Dr. Mohankumar Murugesan (MM) along with Dr. Srujan Marepally. (see reports). Finally, ST and MM are also working on genome editing approach to gene correction for Wiskott Aldrich syndrome (an immune deficiency disorder) and hemophilia, respectively. The details are described in their reports.

Given together, we have established a major gene therapy program in India with several international collaborations aimed at addressing some of the major unmet health needs in the country.

B. Haplobanking

This is a novel project aimed at creating a bank of induced pluripotent stem cell lines from normal individuals with homozygous HLA haplotypes. These cell lines could then serve as a source for cell therapy for many individuals with different organ dysfunctions. I work with Dr. Dolly Daniel and Dr. R.V. Shaji for developing this program in collaboration with DATRI stem cell donor registry. Details are in the reports by Dr. R. V. Shaji and Dr. Dolly Daniel. An area to consider in the coming years is to see how to differentiate these cells towards possible clinical applications keeping in mind the developments in the world in this field.

C. Population-based control program for major hemoglobin disorders

This program is coordinated by Dr. Kuryan George and Dr. Shantidani Minz from the department of Community Medicine in CMC, Vellore along with Dr. Alok Srivastava. Apart from attempting to develop novel options for the treatment of major haemoglobin disorders, the NAHD program also includes support for a program for the control of thalassemia and sickle cell disease in the community. Even though screening for carriers of these diseases has been done in India in different states for more than two decades, there is as yet no effective model for the control of these diseases beyond these initial steps as has been so effectively implemented in several countries around the world. The aim of this project is therefore to create a model where after systematic and accurate screening, the program will look at implementing a strategy for prevention and early effective treatment of these conditions. The emphasis will be on

providing information about these conditions along with easily accessible and good options for prenatal diagnosis as well as effective therapies. With greater awareness and access to both preventive and treatment options, we hope to reduce the stigmatization that is a major barrier in effective implementing of these programs. We have established a partnership with the Government of Odisha for this program and are now working out a detailed program for implementation.

D. Developing newer areas of research

One of the major areas of innovation that has completely changed the way certain diseases particularly certain cancers can be treated are is with the use of different kinds of T cell therapies. There is very little if any work being done in this area by any group in India. Over the last one year we have attempted to develop this area of translational research with a multidisciplinary team to develop gamma delta T cell and NK cell therapy. We are also establishing international collaborations in this area. Current focus is on standardizing culture and expansion methods for these cells for transfer to the GMP facility to initiate early phase clinical studies. (see report of Drs. Aby Abraham / Aniket Kumar) We are exploring options of scientists with specific laboratory skills in this area joining CSCR.

Other areas of work related to stem cell research

Apart from the work described above, I also continue to be involved with clinical hematopoietic stem cell transplantation and research related with it. I also coordinate the Indian Stem Cell Transplant Group and the Indian Stem Cell Transplant Registry for all hematopoietic stem cell transplantations done in India.

Grants

Ongoing project

- Project Title: Novel Approaches to Hematological Disorders (NAHD)
- Funding Agency: Department of Biotechnology, Ministry of Science and Technology, Govt. of India.
- Duration & Total Grant: 5 years (2015-2020); ~Rs. 60 crores.

Completed project

- Project Title: Establishing a Centre for Stem Cell Research
- Funding Agency: Department of Biotechnology, Ministry of Science and Technology, Govt. of India.
- Duration & Total Grant: 6 years (2005-2011); ~Rs. 28 crores.

Selected publications

- 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.
- 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. 2016Jan;18(1):120-3.
- Bharathan SP, Manian KV, Aalam SM, Palani D, Deshpande PA, Pratheesh MD, Srivastava A, Velayudhan SR. Systematic evaluation of markers used for the identification of human induced pluripotent stem cells. Biol Open. 2017 Jan 15;6(1):100-108. Srivastava A, Shaji RV. Cure for thalassemia major - from allogeneic hematopoietic stem cell transplantation to gene therapy. Haematologica. 2017 Feb;102(2):214-223.
- Bharathan SP, Nandy K, Palani D, Janet A NB, Natarajan K, George B, Srivastava A, Velayudhan SR. Generation of an induced pluripotent stem cell line that mimics the disease phenotypes from a patient with Fanconi anemia by conditional complementation. Stem Cell Res. 2017 Apr; 20:54-57.
- Mohanan E, Panetta JC, Lakshmi KM, Edison ES, Korula A, Fouzia NA, Abraham A, Viswabandya A, Mathews V, George B, Srivastava A, Balasubramanian P. Population pharmacokinetics of fludarabine in patients with aplastic anemia and Fanconi anemia undergoing allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant. 2017 May 8.

Invited talks

- Gene therapy futures - Bleeding disorders & Hemoglobinopathies: Blue Ribbon Art and Film Festival 2017 and Rare Diseases Symposium, Bengaluru (India); March 2017
- Cure for the major hemoglobin disorders in India: 3rd Global Congress on Sickle Cell Disease, Bhubaneswar (India): February 2017

- Cure for Thalassemia major - From allogeneic stem cells to autologous gene correction: New Trends in Hematology, Histiocytic Disorders and Transfusion Medicine Symposium and Workshop, Riyadh (Saudi Arabia); November 2016
- Cure for Thalassemia major - From allogeneic stem cells to autologous gene correction: Thalassemia and Sickle Cell Disease Bilateral Workshop (USA-India), Chandigarh (India); November 2016
- Allogeneic Stem Cell Transplantation to Gene Therapy for Thalassemia Major: National Thalassemia Welfare Society (India); November 2016
- The Malti Sathe Oration-2016 - Cure for major hemoglobin disorders in India - The journey so far & the way forward: 57th Annual Conference of Indian Society of Hematology and Blood Transfusion-Haematcon 2016, Jaipur (India); November 2016Curing Thalassemia major - From allogeneic stem cells to autologous gene correction: 21st Annual Congress of Asia Pacific Blood and Marrow Transplantation Group, Singapore; October 2016
- Healthcare innovations to address unmet needs - Stem Cells & Gene Therapy: Rotary Club of Madras (India); August, 2016
- The science behind gene therapy in hemophilia: World Federation of Hemophila, 2016 World Congress, Orlando (USA); July 2016
- What can we do together to achieve prompt transplant turnarounds for patients undergoing MUD HSCT?: 11th International Donor Registry Conference and WMDA Working Group Meetings, Singapore; May-June, 2016
- Gene Therapy – 'Definitions' of Outcomes: Safety and Efficacy (in the context of hemophilia): International Society on Thrombosis and Haemostasis (ISTH) Factor VIII / IX Subcommittee of the Scientific & Standardization Committee, Montpellier (France); May 2016

Students trained

PhD completed

Nishanth Gabriel

- PhD Thesis Title: Effect of notch signalling on human mesenchymal stromal cells homeostasis and differentiation
- Date of Completion (viva voce): 08th February 2016

Sangeetha H

- PhD Thesis Title: Strategies to evade immune response against adeno-associated virus vectors during gene therapy
- Date of Completion (viva voce): 09th June 2016

Nancy Beryl Janet

- PhD Thesis Title: Molecular basis of Fanconi anemia in Indian population
- Date of Completion (viva voce): 22nd March 2017.

PhD thesis submitted / awaiting evaluation & viva voce

Salar Abbas

- PhD Thesis Title: Evaluating the bone marrow hematopoietic stem cell niche in bone marrow failure syndromes

Honors & Awards

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

I completed 6 years as chair the National Apex Committee for Stem Cell Research and Therapy, Department of Health Research, Ministry of Health and Family Welfare (http://bic.icmr.org.in/nacscrt/), in late 2016. During this period the current guidelines for stem cell research in India was formulated completely updated from its first version from 2007. I continue to be the vice-chair of the Asia-Pacific Blood and Marrow Transplantation group coordinating some of their academic and research activities (http://www.apbmt.org/organization/).

Other Academic Activities

In attempting to develop different areas of stem cell research and cell therapies, I work with different departments in CMC, Vellore and faculty colleagues from both clinical and basic science backgrounds to mentor research programs. In 2016-17, these have included helping Dr. Jeyant Rose from the department of Ophthalmology set up corneal stromal cell research, Dr. Aniket Kumar from the department of Pharmacology to work on cellular mechanisms of hypertrophic scars and possible pharmacological interventions and working with Dr. Aby Abraham of the department of Haematology to develop Gamma Delta T cell and NK cell culture expansions for clinical therapies. As recommended by the SAC last year, I am also involved with enhancing the work being done in the cGMP facility at CSCR by inculcating new faculty to be responsible for it as well establishing external collaborations in that area. (see report on cGMP facility).

Collaborations

Internal:

- Anu Korula, Dept. of Haematology, CMC, Vellore
- Aby Abraham, Dept of Haematology, CMC, Vellore
- Eunice Sindhuvi, Dept. of Haematology, CMC, Vellore
- Biju George, Dept of Haematology, CMC, Vellore
- Vikram Mathews, Dept of Haematology, CMC, Vellore
- Sukesh Nair, Dept. of Transfusion Medicine & Immunohematology, CMC, Vellore
- Asha Mary Abraham, Department of Clinical Virology, CMC, Vellore
- Hubert Daniel, Department of Clinical Virology, CMC, Vellore
- Rajesh Kannangai, Department of Clinical Virology, CMC,Vellore
- Saravanabhavan Thangavel, CSCR, Vellore
- Mohankumar Murugesan, CSCR, Vellore
- R V Shaji, CSCR / CMC, Vellore
- Dolly Daniel, Dept. of Transfusion Medicine & Immunohematology, CMC, Vellore
- Kuryan George, Department of Community Health, CMC, Vellore
- Shantidani Minz, Department of Community Health, CMC, Vellore
- J. P. Muliyil, Retd. Professor, CMC, Vellore
- Vrisha Madhuri, Department of Pediatric Orthopedics, CMC, Vellore
- Mahendra Rao, inStem, Bengaluru

External:

- External collaboration for selection of the AAV vector for gene therapy of hemophilia B and its production in cGMP is with Arun Srivastava, Department of Genetics, University of Florida and Barry Byrnes, Powell Gene Therapy Center, University of Florida; INTAS Pharmaceuticals, Ahmedabad, will be the industry collaborator from India for production of AAV vectors.
- Mavis Agbandje-McKenna, Director, Center for Structural Biology, University of Florida and Arun Srivastava, Chief, Center for Cellular and Molecular therapy, University of Florida, Gainesville, Florida, USA.
- External collaboration for development of lenti vectors with Trent Spencer, Director, Gene Therapy Program, Aflac Children's Cancer Center, Emory University, Atlanta, USA, Chris Doering and John Lollar from the Emory University, USA.
- Nezih Cereb, Chief Scientific Officer, & Raghu Rajagopal, CEO, DATRI, Chennai.

R. V. SHAJI, PhD
Professor, Department of Haematology, CMC, Vellore
Adjunct Scientist, CSCR

LABORATORY HIGHLIGHTS

Area of research: Molecular mechanisms of human erythropoiesis, somatic cell reprogramming and preclinical gene therapy

I. Molecular mechanisms of human erythropoiesis

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 are currently investigating the role of small RNAs and epigenetic factors in human erythropoiesis. Previously, we carried out 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 established a robust method to knock out the significant miRNAs by CRISPR/Cas9 to determine their role in human erythropoiesis. 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 the knock down efficiency of the lentiviral backbone and the shRNA design algorithm, using a shRNA library for 500 epigenetic factors we are performing a highthroughput RNAi screen to identify the positive and negative epigenetic regulators of human erythropoiesis.

II. Somatic Cell Reprogramming: Disease modelling and iPSC banking

With the expertise in generating human induced pluripotent stem cells (iPSCs) using these cells we have initiated projects on disease modelling and haplobanking. For disease modelling, we have generated iPSCs from patients with Fanconi anaemia (FA) by inducible complementation of FA-pathway. The iPSCs exhibited all the FA cellular phenotypes in the absence of an active FA pathway. With the protocol established for differentiation of iPSCs to haematopoietic progenitors we are carrying out the experiments to understand the molecular basis of bone marrow failure in the FA patients. We have also initiated a project on generating mutant iPSCs for disease modelling. For this, we are creating mutations that cause congenital diserythropoietic anaemia and Diamond Blackfan Anaemia. This will allow isogenic wildtype and mutant lines for disease modelling. For haplobanking, we have established a xeno-free protocol for generation of iPSCs from cultured erythroid cells generated from donors homozygous for HLA haplotypes. In Collaboration with Dr. Dolly Daniel, Department of Transfusion Medicine, CMC and DATRI Blood Stem Cell Donors Registry, we have cryopreserved peripheral blood mononuclear cells from 113 donors homozygous for HLA haplotypes and the cells from more donors are being collected. We have generated iPSCs from 6 donors so far.

III. Late pre-clinical gene therapy (Lentiviral approach)

Four lentiviral vectors have been generated for the expression of β-globin gene for testing the expression of transgene in human erythroid cells. The vectors differed in the regulatory sequences, locus control region sequences, untranslated regions (UTRs) and promoters. For testing these vectors, an ex-vivo erythropoiesis system, in which haematopoietic stem cells are differentiated to erythroid cells in culture was developed. The investigators generated high titre lentiviral particles and transduced the purified haematopoietic stem cells from normal donors. Subsequently, the transduced haematopoietic stem cells were differentiated to erythroid cells in the presence of specific cytokines. To differentiate the expression from endogenous beta globin gene and the exogenous beta globin gene, a previously reported mutation was introduced in the β-globin gene. Real time PCRs and HPLCs were carried out to measure the transgene expression. The expression of the transgene was analyzed by digesting the RT-PCR product of beta globin with DraIII restriction enzyme which cuts the PCR product from the endogenous beta globin gene and does not cut the transgene due to the mutation. The results showed that the ratio of endogenous beta globin gene mRNA and the transgene beta globin mRNA was: 21:4. One of the lentiviral vectors showed 16% expression of β-globin gene mRNA in the ex-vivo differentiated erythroid cells. All the laboratory methods required for testing the lentiviral vectors have been established and a research fellow has been trained in the methods required for carrying out this project-generation of lentiviruses, purification of CD34+ cells and ex-vivo erythropoiesis. The vector copy number calculation showed that there were 1.5 copies of the transgene integrated into the genome. We have modified these vectors to express shRNAs against BCL11A gene to increase gamma globin expression along with the expression of beta globin gene. We have made strategies to further modify these vectors for increased expression of β-globin gene and they will be tested in the ex-vivo system.

PhD students

Completed:

- Nancy Beril Janet *(In collaboration with Dr. Alok Srivastava)*
- Thiyagaraj Mayuranathan
- Syed Mohammed Musheer Aalam
- Sumitha P Bharathan

Thesis submitted:

- Janakiram Rayabaram
- Kannan VM

Current students:

- Aneesha Nath
- Abhirup Bagchi *(In collaboration with Dr. Alok Srivastava)*
- Smitha Ijee
- Krittika Nandy
- Thulaj D Meharwade
- Sonam Rani

Technician:

- Dhavapriya Palani

Lab members - Haplobanking (NAHD)

- Kannan VM
- Chitra P
- Praveena

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).
- Preclinical model using lentiviral vectors for gene therapy for Thalassemia and Sickle Cell Disease (SCD) (under NAHD program - DBT, 2015-2020)
- iPSC based disease modelling for erythroid disorders (under NAHD program - DBT, 2015-2020)

Invited talks:

- Disease modelling using induced pluripotent stem cells: Indian Academy of Biomedical Sciences 2017, Bhavnagar, Gujarat, 6-8th January 2017
- Applications of induced pluripotent stem cells: National Conference of Young Researchers 2017, Goa University, Goa, 16-17th March 2017
- Stem Cells for Haematological Diseases- Disease modelling and Banking for Clinical Applications: GIAN Stem Cell Workshop, University of Hyderabad, Hyderabad, 21st July 2017

Publications:

- Karathedath S, Rajamani BM, Musheer Aalam SM, Abraham A, Shaji RV, Krishnamurthy P, Mathews V, Velayudhan SR, Balasubramanian P. Role of NF-E2 related factor 2 (Nrf2) on chemotherapy resistance in acute myeloid leukemia (AML) and the effect of pharmacological inhibition of Nrf2. PLoS One. 2017 May 15;12(5):e0177227.

- Sumitha Prameela Bharathana, Krittika Nandy, Dhavapriya Palani, Nancy Beryl Janet A, Kasthuri Natarajan, Biju George, Alok Srivastava, Shaji RV. Generation of an induced pluripotent stem cell line that mimics the disease phenotypes from a patient with Fanconi anemia by conditional complementation. Stem Cell Research Volume 20, April 2017, Pages 54–57.
- Sumitha Prameela Bharathan, Kannan Vrindavan Manian, Syed Mohammed Musheer Aalam, Dhavapriya Palani, Prashant Ajit Deshpande, Mankuzhy Damodaran Pratheesh, Alok Srivastava, Shaji RV. Systematic evaluation of markers used for the identification of human induced pluripotent stem cells. Biology Open 2017 6: 100-108; doi: 10.1242/bio.022111.
- Srivastava A,Shaji RV. Cure for thalassemia major: from allogeneic hematopoietic stem cell transplantation to gene therapy. Haematologica 2017 Feb;102(2):214-223.
- Aalam SM, Manian KV, Bharathan SP, Mayuranathan T, Shaji RV. Identification of Stable OCT4+NANOG- State in Somatic Cell Reprogramming. Cell Reprogram. 2016 Nov;18(6):367-368.
- Deshpande P, Kathirvel K, Alex AA, Korula A, George B, Shaji RV, Mathews V. Leukocyte Adhesion Deficiency-I: Clinical and Molecular Characterization in an Indian Population. Indian J Pediatr. 2016 Aug;83(8):799-804.
- Kamath MS, Pradhan S, Edison ES, Shaji RV, Antonisamy B, Karthikeyan M, Mangalaraj AM, Kunjummen A, George K. Chorionic villous sampling through transvaginal ultrasound approach: A retrospective analysis of 1138 cases. J Obstet Gynaecol Res. 2016 Oct;42(10):1229-1235.
- Pal R, Mariappan I, Shaji RV. Editorial: Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells: Ushering of a New Era in Personalized Cell Therapies. Curr Stem Cell Res Ther. 2016;11(2):97-8.

Collaborations:

- Alok Srivastava, CSCR/CMC, Vellore: Lentiviral pre-clinical gene therapy
- Biju George, CMC, Vellore: Clinical evaluation of Fanconi anaemia patients.
- Sanjay Kumar, CSCR: Role of epigenetic factors in stem cell differentiation by RNAi
- Saravanabhavan Thangavel, CSCR: Gene editing
- Dolly Daniel, CMC, Vellore: Haplobanking
- B Poonkuzhali, CMC, Vellore: Role of epigenetic factors in leukemia resistance

SARAVANABHAVAN THANGAVEL, PhD
Assistant Investigator, CSCR

LABORATORY HIGHLIGHTS

Our lab was started in September 2015. Our main goal is to setup genome editing as a therapeutic option for patients with genetic disorders. Recent pre-clinical studies used genome editing to successfully modulate the disease-causing mutation in haematopoietic stem cells of the patients. This ex vivo editing process raises the hope for therapeutic genome editing in the clinics. Our research goal involves three broad areas:

1. Genome editing mediated gene therapy for Wiskott-Aldrich Syndrome (WAS)

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. Allogeneic stem cell transplantation is the existing treatment for WAS. We are building genome editing mediated targeted expression of WASP to potentially reverse the WAS phenotype. Towards achieving it, we have constructed the transgene, identified the targets for integrating it in the genome and also validated our CRISPR/Cas9 tools for targeted cleavage and specificity. Currently, we are generating human cellular models for WAS. These models will be used for testing our transgene and genome editing strategy in rescuing cellular defects of WAS.

2. Genome editing mediated gene therapy for haemoglobin disorders

ß-thalassemia, one of the most common genetic diseases in India, is caused by mutations in the human hemoglobin beta (HBB) gene. These mutations lead to reduced/absent synthesis of the beta globin chains of the hemoglobin tetramer. Sickle Cell Anemia is caused by a single point mutation in the seventh codon of the beta-globin gene and characterized by sickle shaped red blood cells which are deficient in transporting oxygen. To correct both β-thalassemia and Sickle Cell Anemia we are inducing the activation of fetal haemoglobin. We use CRISPR/Cas9 genome editing tools to introduce Non homologous End joining repair mediated nucleotide deletions. These deletions are targeted to modulate the transcriptional repressors of fetal haemoglobin. We have identified the targets for fetal haemoglobin activation and performing proof-of-concept studies validating the targets.

We are also aiming for a site-specific correction of the sickle mutation in hematopoietic stem cells for a permanent production of normal red blood cells. We have developed CRISPR/Cas9 tools and Homology directed repair donor to correct the sickle mutation.

3. Technology development for genome editing applications

- The CRISPR/Cas9 is becoming a valuable tool for gene therapy applications. The efficiency of genome editing is partly linked with the delivery mode. In collaboration with Dr.Srujan Marepally, we are developing lipid based delivery system for delivering genome editing tools for the efficient and safe gene therapy applications.

- The application of genome editing for HSC gene therapy is hampered by the limited availability of patient HSCs. We are working on modulating the genetic factors to ex vivo culture and expand HSCs for gene therapy applications.

- It's essential to achieve a high frequency of genome editing to be useful therapeutically. We have initiated the work with various small molecules targeting DNA repair system to improve the efficiency of genome editing.

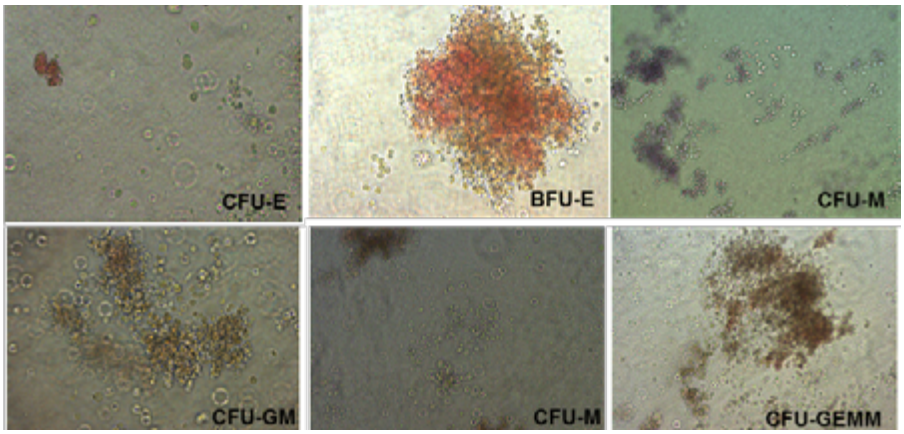


Fig: Characterisation of ex vivo expanded mPB-HSCs by MethoCult analysis. Representative images showing the formation of growth of erythroid progenitor cells (BFU-E and CFU-E), granulocyte-macrophage progenitor cells (CFU-GM, and CFU-M), and multipotential granulocyte, erythroid, macrophage and megakaryocyte progenitor cells (CFU-GEMM).

Honors and awards

- Early Carrier Research award by SERB-DST, India

Grants

- CSCR start-up grant.
- Pre-clinical studies for gene therapy of Wiskott-Aldrich Syndrome (WAS): SERB-Early Carrier Research Award, DST, Govt. of India.
- Novel Approaches to Hematological Diseases (NAHD); DBT, Govt. of India (Co-Investigator).

Lab members

- Abisha Crystal, JRF
- Harish N Kumar, JRF
- Delvin Pauley, JRF
- Shalini Ramasamy, Short-term project trainee
- Swathi Radhakrishnan, Short-term project trainee

Academic activities

- Co-organiser: 1st Annual Cell and Gene Therapy Symposium, Vellore
- In-Charge: Imaging facility, Work-in-progress presentation, JRF review process.
- Stem cell gene therapy class to JRFs

Collaborators

- R. V. Shaji, CSCR/CMC, Vellore
- Mohankumar Murugesan, CSCR
- Srujan Marepally, CSCR
- Sanjay Kumar, CSCR
- Alok Srivastava, CSCR/CMC, Vellore

MOHANKUMAR MURUGESAN, PhD
Assistant Investigator, CSCR

LABORATORY HIGHLIGHTS

The current focus of my lab is to develop a novel genome editing approach for the treatment of β- hemoglobinopathies and Haemophilia A.

Genome editing of fetal globin repressors in patient derived hematopoietic stem cells for treatment of β-hemoglobinopathies

The switch from fetal to adult hemoglobin is a very important developmental event that occurs in erythroid cells during the months following birth. Reversing the fetal to adult hemoglobin switch is substantial therapeutic interest, since persistence high levels of HbF ameliorates clinical symptoms of sickle cell disease and thalassemia. Recent studies have identified some of the key transcriptional factors involved in the repression of fetal globin gene and activation of adult globin expression. In this study, we plan to utilize targeted genome engineering platform based on CRISPR/CAS9 system to reactivate gamma globin by editing the potent gamma globin repressor in hematopoietic stem cells for the treatment of SCD and thalasemmia. In this regard, the design and synthesis of guide RNAs for specific cleavage of targets are completed. The validation of these guide RNAs was achieved by T7- endonuclease based screening in human embryonic kidney cells.

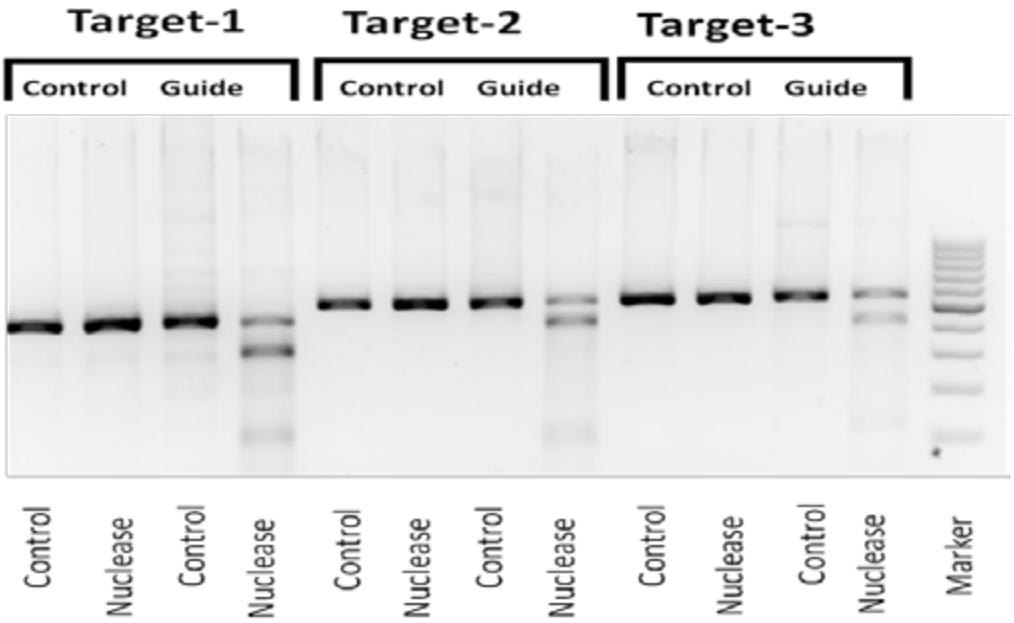


Figure: Genome editing of fetal globin repressors: T7 endonuclease assay showing the cleavage of different fetal globin repressor genes.

Novel therapeutic genome editing approach for targeted lineage specific expression of FVIII for Hemophilia A

Hemophilia A is an X-linked monogenic congenital bleeding disorder caused by the absence of functional FVIII in the bloodstream. The severity of the disease varies according to FVIII residual activity and ranges from a mild phenotype (5-30% of normal FVIII activity), moderate phenotype (1-5% of normal FVIII activity) or severe phenotype (< 1%). Even a small increase in FVIII levels (above 1%) can significantly ameliorate disease phenotype and patients' quality of life, making this disease an ideal candidate for gene therapy approaches. In recent years sustained correction of Hemophilia B was achieved in selected human subjects by systemic administration of an AAV8 containing FIX cDNA

under the control of a liver specific promoter. Despite these promising outcomes for Hemophilia B, gene therapy for Hemophilia A is still an open challenge. To improve the current approaches of gene therapy for Hemophilia A, we are working on a novel ex vivo gene therapy approach for targeted expression of FVIII in specific progeny of hematopoietic stem cells for the treatment Hemophilia A.

Grants

- Novel Approaches to Hematological Diseases (NAHD) - (Co-Investigator)
- C11orf95-RELA fusions in supratentorial ependymomas: Relevance in prognostication: SERB-DST, Govt. of India (Co-PI).
- CSCR start-up grant

Lab members

- Bhanu Prasad Bandlamudi, JRF
- Rachel Anand Nethala, JRF
- Bhargav Sanketi, Short-term project trainee
- Sharada Gopal, Short-term project trainee

Academic activities

- Co-organiser: 1st Annual Cell and Gene Therapy Symposium, Vellore
- In-Charge: JRF review process.
- Stem cell gene therapy class to JRFs

Collaborators

- R . V. Shaji, CSCR/CMC, Vellore
- Saravanabhavan Thangavel, CSCR
- Srujan Marepally, CSCR
- Alok Srivastava, CSCR/CMC, Vellore
- Geeta Chacko, CMC, Vellore

SRUJAN K MAREPALLY, PhD
Scientist, CSCR

LABORATORY HIGHLIGHTS

My current research focus involves developing bio-inspired cationic amphiphiles for gene therapy and genome editing applications, in particular, to treat blood disorders including β-thalassemia, sickle cell anemia and hemophilia. To overcome translational hurdles such as poor transfection efficiency and cytotoxicity, we are probing the mechanisms involved in transfections.

Improving safety and efficacy of cationic lipid mediated transfections

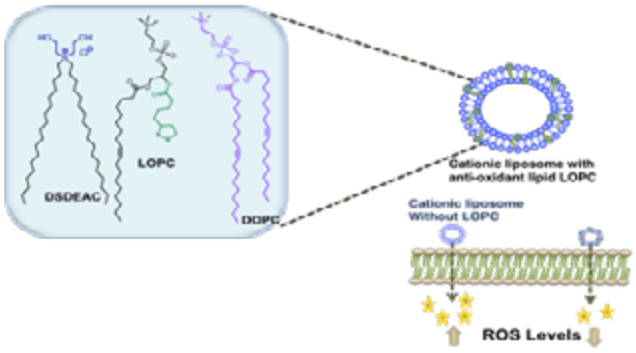
Cationic lipid guided nucleic acid delivery holds a great promise in gene therapy and genome editing applications for treating genetic diseases. However, major challenge lies in achieving the therapeutically relevant efficiency. Several attempts have been made previously to understand the relation between the lipid structure and its transfection efficiency. Transfection profiles of cationic amphiphiles are highly sensitive to even subtle changes such as orientation of the linker functionalities between hydrophilic and hydrophobic domains, and asymmetry in the hydrophobic region, which determines their cytotoxicity as well. More importantly, prior studies demonstrated that cationic lipids induce reactive oxygen species (ROS) generation that in turn activates apoptotic and inflammatory pathways.

To improve the transfections and safety profiles, we took two intriguing approaches:

- Developing an antioxidant lipid and doing it in cationic lipid formulations to improve the safety of transfections
- Developing cationic lipids from natural vegetable source to enhance the transfections

A) An anti-oxidant, α-lipoic acid conjugated oleoyl-sn-phosphatidylcholine as a helper lipid in cationic liposomal formulations

To overcome, cytotoxicity issues associated with cationic liposomes, we developed an antioxidant lipid named α-lipoyl, oleoyl-sn-phosphatidylcholine (LOPC) and doped into cationic liposomal formulations with varying concentrations. Transfection results suggested that doping LOPC to certain extent (~25%) possibly increased liposomal membrane fluidity and also contributed to early endosomal escape.



(Fig.1) Since, LOPC has ROS quenching properties that rescues cells from necroptosis. Our findings demonstrated that LOPC could be a potential helper lipid in cationic lipid formulations to overcome toxicity related issues of liposomal transfections.

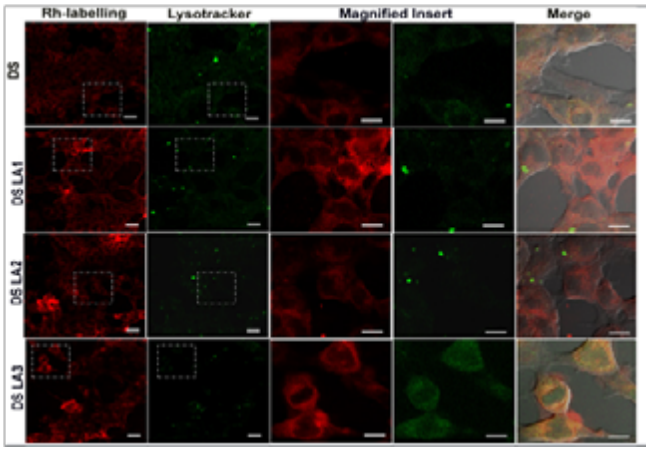


Fig. 1. Co-localization studies of lysosome with Rhodamine labelled liposomal formulations in HEK293 cells.

Highlights of the project:

- Synthesized LOPC as an antioxidant helper lipid for liposomal formulations
- Developed an efficient delivery system for enhanced transfection with quenching of ROS
- LOPC doped liposomal formulation improves the safety profiles of cationic liposomes
- 25% LOPC in DSDEAC:DOPC liposomes enhance transfection with improved safety profiles

Further studies: We are using LOPC in skin permeating nanocarriers system for delivering nucleic acids into the skin with an intrinsic Anti-psoriasis activity.

B. Green Transfection: Cationic lipid nanocarriers system derivatised from vegetable fat, Palmstearin enhances nucleic acid transfections

Prior findings, including our own demonstrated that asymmetry in the hydrophobic core imparted superior transfection efficiencies. To this end, we developed a lipid nanocarrier system with asymmetric hydrophobic core (PS-Lips) derived from mixture of fatty acids of food grade Palmstearin and compared its efficiency with symmetric Palmitic acid based nanocarrier system (P-Lip).(Fig.2) PS-Lips exhibited superior transfection efficiencies with both pDNA and mRNA in multiple cultured cells than control P-Lip. More importantly, PS-Lips exhibited two fold superior transfections with linear nucleic acid, GFP mRNA in hematopoietic cells, when compared with commercial control Lipofectamine RNAi Max.

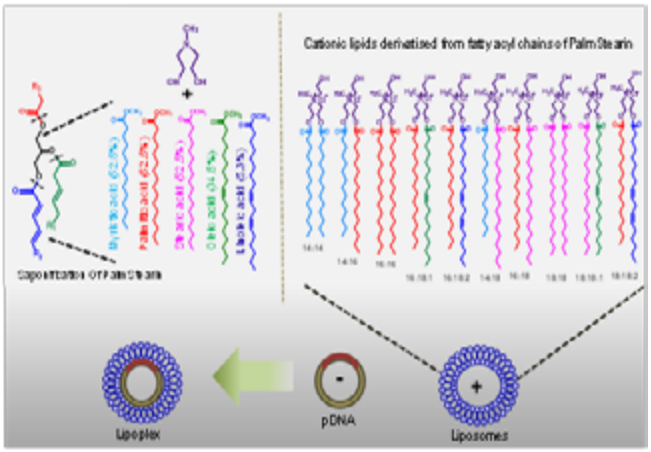


Fig.2 Chemical structures cationic amphiphiles synthesized from Palmstearin. Schematic illustration shows the complex formation between PS-Lips and pDNA.

(Fig. 3) PS-Lips also found to be effective in delivering genome editing tools, (CRISPR/Cas9, sgRNA encoded pDNA with a reporter GFP construct) than P-Lip in HEK-293 cells. In this study, we demonstrated that cationic liposomes derivatised from natural food grade fat Palmstearin with natural hydrophobic core asymmetry are efficient in delivering both linear and circular nucleic acids. In particular, PS-Lips are efficient in delivering mRNA to hematopoietic cells. These findings can be further exploited in genome editing approach for treating β-globinopathies.

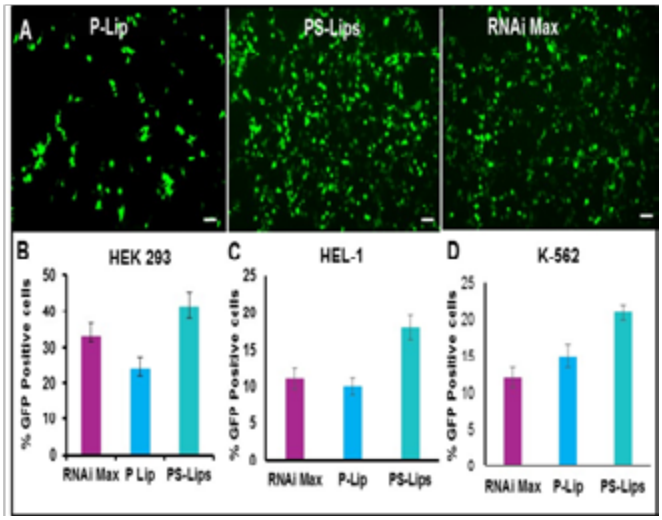


Fig.3 in vitro mRNA transfection efficiencies of PS-Lips & P-Lip in adherent cell line, HEK 293 (A&B); and suspension cell lines, HEL-1 (C); K-562 (D). Lipofectamine RNAi Max was used as positive control for mRNA transfections

Key findings of the project:

- Synthesized cationic lipid pool from vegetable fat palmstearin
- Developed an efficient delivery system for enhanced transfection than their symmetric palmstearin
- Cantionic lipid pool has efficiently delivered mRNA into suspension hematopoietic cells.

Further studies: To improve the transfection efficiencies further, we are planning to use other vegetable source that has more unsaturated fatty acids.

Project 2: Developing lipid based nanocarriers to deliver CRISPR/Cas9 tools for efficient genome editing

In recent times, CRISPR/Cas9 system has become a revolutionary technology in genome editing for correcting genetic disorders and understanding the biological processes. This technology emphasized the importance of developing safer and effective delivery systems of nucleic acids. Non-viral vectors found to be potential alternative delivery system to overcome the limitations associated with viral vectors and offer to deliver wide range of nucleic acids such as pDNA, siRNA, shRNA and miRNA. However suffer with low efficiency and high cytoxicity.

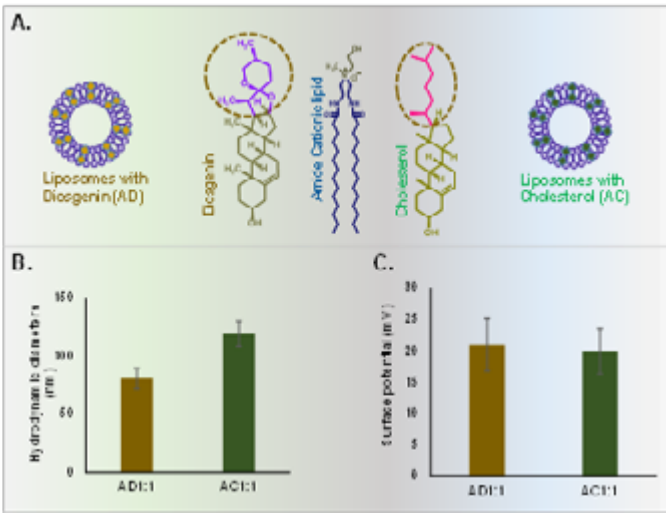


Fig. 1: Schematic representation of nanocarriers system (A), their particle sizes (B) and surface potentials (C)

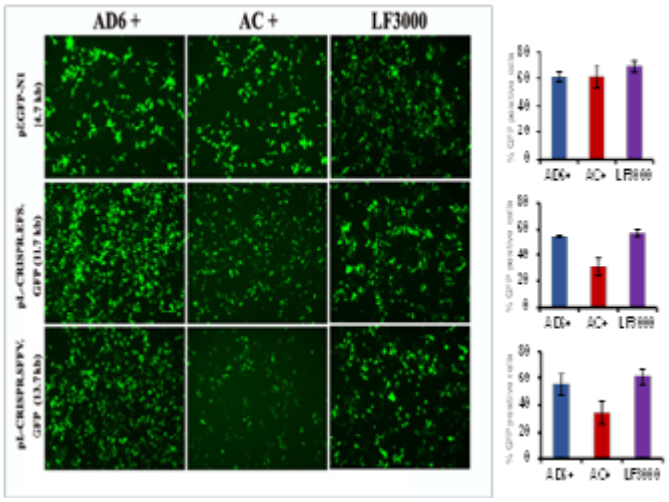


Fig. 2: Transfection experiments in HEK 293 with multiple pDNAs including CRISPR plasmids

Cholesterol has been being used as a most common helper lipid in cationic lipid formulations. In this study, demonstrated that a steroidal saponin, diosgenin as a best alternative to cholesterol in cationic lipid formulations to further improve nucleic acid delivery efficacy. Uniqueness of the liposomal system lies in delivering wide range of bio-cargos that includes from larger pDNAs encoding Cas9 protein and gudie RNAs to shorter sgRNA and Cas9 protein for efficient genome editing. Owing to its surfactant properties, diosgenin significantly reduced liposomal size to 50nm when compared to cholesterol as co-lipid and delivered CRISPR/Cas9 encoded plasmids 11.7kb and 13.7kb respectively with equal efficiency of LipofectAmine 3000. It demonstrated better efficiency that CRISPR Max in delivering ribonucleoprotein complexes (RNP).

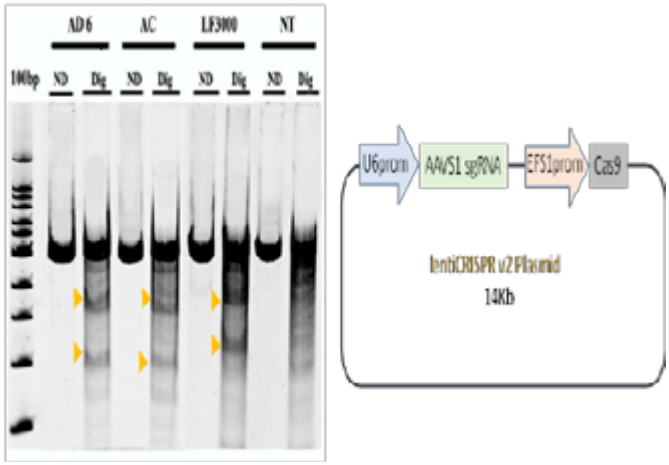


Fig. 3: Delivery of AAVS1 targeted plasmid and cleavage efficiency was analyzed with CEL-1 assay

These findings demonstrated that AD1:1 can deliver larger plasmids including CRISPR plasmids as well. Further we demonstrated their functional ability to deliver CRISPR plasmid to AAVS-1 locus and analyzed their cleavage efficiency and compared with LF3000 (Fig. 3).

Safe harbor sites are regions in the genome that do not show a deleterious phenotype when disrupted, and have been used for the targeted insertion of transgenes. AAVS1 (also known as the PPP1R12C gene) is a relatively well-characterized safe harbor locus with an open chromatin state and no detectable adverse effects are associated with inserting exogenous DNA at this locus. Therapeutic transgenes inserted at this locus by homology directed repair produce functional proteins. As AAVS1 is a most targeted region for transgene expression, we used CRISPR/Cas9 ribonucleoproteins targeting the AAVS1 locus. Both AD1:1 alone and in combination with albumin nanoparticles could able to deliver Cas9 and sgRNA complex efficiently. The cleavage efficiency was analyzed with CEL-1 assay (Fig. 4). Most intriguing observation is that the formulation demonstrated activity where commercial CRISPR max failed to deliver RNP complexes. To the best of our knowledge no nanocarriers system is available to deliver RNP complexes without any physical modification on them. This would be first in the world in its class to deliver RNPs. However, we have evaluated their efficiency only in HEK 293. We are further evaluating their efficiencies in suspension cells and primary cells including HUDEEP and CD34+ cells.

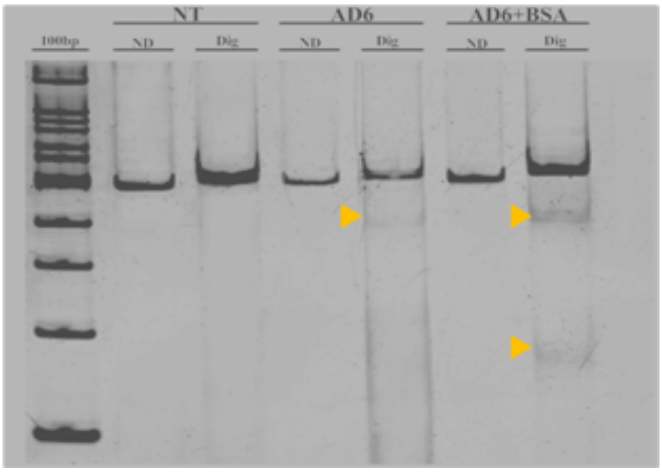


Fig. 4: Delivery of AAVS1 targeted RNP complex and cleavage efficiency was analyzed

Key findings of the project:

- Developed a unique first in its class nanocarriers system that can deliver 150b to 14.7kb nucleic acids and RNP complexes
- pDNA delivery efficiency of the nanocarrier system is as efficient as commercial LF3000
- Nanoparticle system can deliver CRISPR Cas9 RNP complexes without any further modifications on them.

Current lab members:

- Santhosh Kumar Maddila, Post-Doctoral Fellow
- Priya Dharmalingam, Junior Research Fellow
- Brijesh Lochania, Student trainee from Sarda University, New Delhi

Lab alumni:

- Rasajna Nadella, Post-Doctoral Fellow
- Vignesh K. Rangasami, Junior Research Fellow
- Divyashree HB, Junior Research Fellow
- Dinesh Vemuri, Student Trainee from VIT University, Vellore
- Deepika Singh, Student Trainee from VIT University, Vellore

Publications:

- Ankita Hiwale, Chandrashekhar Voshavar, Priya Dharmalingam, Ashish Dhayani, Rajesh Mukthavaram, Rasajna Nadella, Omprakash Sunnapu, Sivaraman Gandhi, VGM Naidu, Arabinda Chaudhuri, Srujan Marepally, Praveen Kumar Vemula. Scaling the effect of hydrophobic chain asymmetry on gene transfer properties of cationic amphiphiles (Just accepted in RSC Advances) (Co-corresponding author) IF: 3.28
- Priya Dharmalingam, Balakrishna Marrapu, Chandrashekhar Voshavar, Rasagna Nadella, Vignesh Kumar Rangasami, R.V. Shaji, Salar Abbas, R.B.N. Prasad, Shiva Shanker Kaki and Srujan Marepally. An anti-oxidant, α-lipoic acid conjugated oleoyl-sn-phosphatidylcholineas a helper lipid in cationic liposomal formulations. Colloids and Surfaces B: Biointerfaces Volume 152, 1 April 2017, Pages 133–142. (Corresponding author) IF: 3.92
- Ambika S Kurbet, Samarth Hegde, Oindrila Bhattacharjee, Srujan Marepally, Praveen K Vemula, Srikala Raghavan. Sterile inflammation enhances ECM degradation in integrin β1 KO embryonic skin. Cell Reports 2016, 16(12):3334-47 IF: 7.87
- Rakeshchandra R. Meka, Sudhakar Godeshala, Srujan Marepally, Ketan Thorat, Hari Krishna Reddy Rachamalla, Ashish Dhayani, Rajkumar Banerjee, Arabinda Chaudhuri, Praveen Kumar Vemula. Asymmetric cationic lipids based non-viral vectors for an efficient nucleic acid delivery. RSC Advances 2016 6, 77841-77848 IF: 3.28

» Shrivastava S, Jeengar MK, Thummuri D, Koval A, Katanaev VL, Marepally S, V G M N. A chalcone, inhibits human triple negative breast cancer cell invasiveness by downregulation of Wnt/β-catenin signaling cascades and reversal of epithelial–mesenchymal transition. Biofactors 2016 Sep 1. doi: 10.1002/biof.1315. [Epub ahead of print] IF: 4.5

Manuscripts under revision:

» Hari Krishna Reddy R, Priya Dharmalingam, Bhanuprakash B, Saravanabhavan Thangavel, Mohan Kumar KM, Rajkumar Banerjee, Arabinda Chaudhuri, Chandrashekhar Voshavar* and Srujan Mareaplly*, Green transfection: Cationic lipid pool derived from vegetable fat palm stearin enhances nucleic acid transfections. (Manuscript under review in Bioconjugate Chemistry) (* Corresponding authors)

» Sasidharan Vidyanand#, Srujan Marepally#, Sarah Elliot, Vairavan Laxman, Dhiru Bansal, Alejandro Alvarado Sanchez, Praveen Kumar Vemula*, Dasaradhi Palakodeti* MicroRNA, miR-124c regulate axon guidance cues and planar cell polarity pathway essential for cephalic ganglion and photoreceptor organization during anterior regeneration in planarian Schmidtea mediterranea. (Manuscript under development) (# Equal contribution)

Invited talks:

» Delivered a talk on cationic lipid mediated gene therapy at NIPER-Hyderabad

Academic activities:

» Organizing committee member of 1st Annual Symposium on Cell and Gene Therapy, August 5-6 2016, CSCR, Vellore

» Taught nanotechnology in stem cells applications for doctoral students

Collaborations:

Internal:

» Saravanabhavan Thangavel, CSCR

» Mohankumar Murugesan, CSCR

» R V Shaji, CSCR/CMC, Vellore

» Sanjay Kumar, CSCR

» Poonkuzhali Balasubramanian, CMC, Vellore

» Alok Srivastava, CSCR/CMC, Vellore

External:

» Rajkumar Banerjee, CSIR-IICT, Hyderabad

» Shivashankar Kaki, CSIR-IICT, Hyderabad

» VGM Naidu, NIPER, Guwahati

ASHA MARY ABRAHAM, MD
Professor, Department of Clinical Virology, CMC, Vellore
Adjunct Scientist, CSCR

Project title: Establishing methods for screening for AAV antibodies to different serotypes in humans.

Funding source: Department of Biotechnology, Govt. of India

Duration: 2015-2020

Brief description: Adeno-associated virus (AAV) is a small non-enveloped virus which requires a helper virus for active replication. In the absence of a helper virus AAV establishes a latent infection either by integrating into the host genome or remain as episomal form. AAV is classified into 12 serotypes and over 100 isolates. AAV is used as a gene therapy vector because they are not pathogenic, they persistently express the transgene in the transduced cells, and they can transduct into both dividing and non-dividing cells. However, the major obstacle to gene therapy is the generation of immune response against AAV capsid antigens. Humoral immune response against AAV vector is classified into neutralizing and binding antibodies. Studies have shown that neutralizing antibodies bind against a serotype of AAV vector can neutralize the vector and reduce the efficiency of the gene delivery of the vector. Reports show that pre-existing neutralizing antibodies against a serotype of AAV do not neutralize other serotypes. Hence serotype prevalence and detection of neutralizing antibodies of AAV is very important for the effective use of AAV as a vector for gene therapy. Several methods have been used for the detection of antibodies against AAV. Some of the methods detect total antibodies while others detect neutralizing antibodies by in vivo or in vitro methods. Total antibodies have been detected mostly by ELISA and Western blot. The presence of different serotypes and the vast advancement in gene therapy requires tests which can detect neutralizing antibodies against specific serotypes. Screening of AAV serotype specific antibodies is done mainly by transduction inhibition assay (TIA) thus far. However, TIA is expensive, cumbersome and has longer assay duration than ELISA. ELISA is being used for screening of total antibodies against AAV serotypes. However, this format (using VLP) does not give information on serotype-specific detection of AAV antibodies. AAV Peptides which can detect neutralizing antibodies against different AAV serotypes will be cheaper, easier to perform and give serotype specific information for screening individuals before gene therapy.

Aim: The aim is to establish an efficient method of screening for AAV antibodies to different serotypes in individuals for potential gene therapy.

Specific Objectives:

- To establish an in-house peptide ELISA for the serotype specific detection of AAV serotypes 3, 5 and 8 and compare with TIA.
- To establish a transduction inhibition assay (TIA) for the detection of neutralizing antibodies against AAV serotypes 3, 5 and 8.
- To screen for AAV antibodies in healthy volunteers and hemophilia A or B patients before gene therapy using the standardized in-house peptide ELISA and TIA.

Work Done:

- Plasmids with mCherry and luciferase transgene were made in bulk and the expression of transgene confirmed by flow cytometry and luciferase assay respectively.
- Processes for virus packaging and AAV transduction for in vitro transduction inhibition assay have been established and optimized for AAV serotype 3 and 5.
- Antigenic peptides were designed for AAV serotypes 3, 5 and 8.
- An in-house peptide ELISA has been established for the detection of AAV serotypes 3 and 8.
- An in-house whole capsid ELISA has been established for the detection of AAV3.
- Transduction inhibition assay (TIA) for AAV serotype 3 has been standardized.

Specific highlights of the project:

- Design peptides for the serotype specific detection of AAV.
- Establish an in-house peptide ELISA for the serotype specific detection of antibodies against AAV.
- Establishing transduction inhibition assay (TIA) for quantitation of antibodies against different serotypes of AAV.

Support from CSCR: Infrastructure and lab space

Collaborations:

Internal:

- Hubert Daniel, CMC, Vellore
- Rajesh Kannagai, CMC, Vellore
- Sanjay Kumar, CSCR
- Alok Srivastava, CSCR/CMC, Vellore

External:

- Mavis Agbandie-Mckenna, Director, Center for Structural Biology, University of Florida, Gainesville, Florida, USA
- Arun Srivastava, University of Florida, Gainesville, Florida, USA

DOLLY DANIEL, MD
Professor, Department of Transfusion Medicine and Immunohaematology, CMC, Vellore
Adjunct Scientist, CSCR

Project title: Creating a bank of cells homozygous for HLA haplotypes

Funding source: Department of Biotechnology, Government of India

Duration: December 2015 - 2020

Brief description: A major limitation to the use of Stem cell therapeutics is the immunological barrier, contributed largely by the diversity of the HLA system. Developing individual personalized cell lines is prohibitive in terms of cost and labour. Identifying individuals who are most "immune compatible" with the largest number of potential recipients, and creating a bank of iPSC lines from these individuals, thus creating a haplobank is a model being trialled worldwide. However, considering the diversity and uniqueness of the Indian population, it is important that we identify individuals homozygous for the most common haplotypes in the Indian population

The Haplobanking project primarily involves four stages:

- Identifying the most common haplotypes from India / Asia using published and unpublished data available;
- Collaboration with DATRI – the stem cell registry in Chennai and identifying individuals with those haplotypes;
- Donor Recruitment - counselling and sample collection followed by;
- Banking of peripheral blood mononuclear cells (PBMNC) for generation of iPSC lines from the cultured donor cells in the laboratory, through Good Manufacturing Practice (GMP).

Peripheral blood samples collected from donors after consent are screened for infectious diseases at the CMC, Vellore blood bank, in a manner identical to blood donors. An efficient protocol has been established at CSCR to generate iPSC lines from cultured erythroid cells derived from PBMNCs. So far 113 samples have been collected through DATRI from donors residing in Tamil Nadu covering Chennai, Thiruvannamalai, Coimbatore and Thiruppur. There are plans to initiate sample collection from neighbouring states in the future. Generation and banking of iPSCs is shown in report of R V Shaji. PBMNC banks for all the samples collected have been established and six (6) iPSC lines have been generated. These lines will be characterized by immunofluorescence, real-time PCR and teratoma formation in mice. For the haplobanking program, the Centre for Stem Cell Research has been part of the International consortium through the Department of Biotechnology, Government of India aiming to establish harmonized approaches to generate iPSCs for regenerative medicine applications.

Collaborations:

- Raghu Rajagopal, CEO, DATRI, Chennai
- Nezih Cereb, PhD, Chief Scientific Officer, DATRI, Chennai
- R. V. Shaji, PhD, CMC/CSCR, Vellore
- Alok Srivastava, MD, CSCR / CMC, Vellore

ABY ABRAHAM, MD
Professor, Department of Haematology, CMC, Vellore
Adjunct Scientist, CSCR

PROJECT-1

Project title: Gamma delta T cell-based immunotherapy for blood cancers

Funding source: CSCR

Duration: July 2017 to June 2019 (2 years)

Objectives:

A. Cell expansion

- To establish a protocol for the culture and expansion of γδ T cells from human peripheral blood mononuclear cells using serum-free medium and serum-rich medium and to compare fold expansion with IL-2 and Zoledroinc acid as supplements.
- To compare fold expansion of cells with and without IL-15 along with IL-2 and Zoledronic acid in serum free medium.
- To check if the expansion of αβ T cells can be minimized by delayed addition of IL-2 and IL-15 in culture.
- To assess the stability and functionality of the cells post-cryopreservation, cells expanded in three different conditions will be compared i.e. fresh cells, cells post cryopreservation and cells cultured post cryopreservation

B. Preclinical models

- To investigate the antitumor activity of selectively expanded γδ2 T lymphocyte from patients with human low grade lymphoma and myeloma both in vitro and in vivo.
- Analyse the molecular integrity of γδ2 T cells during different stages of expansion including cryopreserved cells to address suitability for its preclinical applications.
- Establishing in vitro non-radioactive and in vivo novel bioluminescent assay to assess the cytolytic potential of γδ2 T Cells Determine the effect of cryopreservation, different culture conditions and expansion methods on γδ2 T cell cytolyic function.
- Investigate the cytotoxic potential of autologous compared with allogeneic γδ2 T cells against human low grade lymphoma and myeloma cells.
- Evaluate the efficacy of γδ2 T cells on the growth of human low grade lymphoma and myeloma xenografts in SCID mice

Support from CSCR: CSCR has provided lab infrastructure, funding and scientific inputs for this project.

Collaborations:

- Aniket Kumar, CSCR/CMC, Vellore
- Augustine Thambaiah, CSCR
- Mohankumar Murugesan, CSCR
- Alok Srivastava, CSCR/CMC, Vellore
- Vikram Mathews, CMC, Vellore
- Biju George, CMC, Vellore

PROJECT-2

Project title: Establishing a protocol for expansion of Natural Killer cells

Funding source: CSCR

Duration: July 2017 to June 2018 (1 year)

Brief description: The project has two components, the first one is the development of the cGMP compliant protocol for the expansion of NK cells and the second component is the cytotoxicity of these expanded NK cells.

In the first part, methods for culture and expansion of NK cells suitable for clinical use will be established. NK Cells will be expanded in vitro from mononuclear cells isolated from human peripheral blood obtained from healthy volunteers. At the end, the expanded NK cells will be analysed by flow cytomery for its phenotypic characters.

The next part of the proposal, which will run in parallel, tests the ability of these expanded NK cells to cause cytotoxicity in K562 cells. This will also be assessed in-vitro. The overall results obtained from phenotypic analysis and cytotoxicity assays will be compared against NK cells obtained by a standard method of expansion.

Objectives:

- To establish a protocol for the culture and expansion of NK Cells from peripheral blood mononuclear cells using ionomycin and IL-2.
- To compare the difference in cytotoxicity profile between the expanded NK cells which will not be preactivated with IL-12, 15 and 18 with the ones which will be pre-activated.
- To compare fold expansion of NK cells and its cytotoxicity with NK cells which are expanded with a regular method of feeder layer using modified K562 cells.

Current limitations to be addressed in this project:

- Higher number of NK cells with a modified protocol.
- To find a cost effective method which does not require the involvement of CliniMACS for isolation of pure population of NK cells.
- To make the NK cells more cytotoxic in nature by converting them into memory like NK cells against malignant cells.

Expected utcome

- Sufficient number of NK cells with maximum purity in the final cell culture and with enhanced cytotoxic activity against K562 cells

Support from CSCR: CSCR has provided lab infrastructure, funding and scientific inputs for this project.

Collaborations:

- Aniket Kumar, CSCR/CMC, Vellore
- Augustine Thambaiah, CSCR
- Mohankumar Murugesan, CSCR
- Alok Srivastava, CSCR/CMC, Vellore

POONKUZHALI BALASUBRAMANIAN, PhD
Professor, Department of Haematology, CMC, Vellore
Adjunct Scientist, CSCR

PROJECT-1

Project title: Identification of novel nuclear receptor (NHR) drug targets in myeloid leukemias

Funding source: Department of Biotechnology (Part of Centre of Excellence Grant)

Duration: November 2015 - October 2020

Brief description: Acute myeloid leukemia (AML) is a biologically, clinically and genetically heterogeneous disease characterized by increased proliferation and defective maturation affecting the cells of the myeloid lineage. The backbone of AML treatment for the last 30 years has been the combination of daunorubicin and cytosine arabinoside (Ara-C). The BCR/ABL tyrosine kinase Imatinib mesylate (IM) is the mainstay of treatment for CML but does not eliminate primitive leukemia stem and progenitor cells. 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. We attempt to extend the understanding of novel biology of leukemias with an effort to understand potential novel targets in AML, as well as to better understand mechanisms of resistance and intolerance to therapy in Chronic myeloid leukemia (CML). Our research is proposed to identify novel NHR genes as drug targets to overcome drug resistance in myeloid leukemias.

Objectives:

- To screen for basal RNA expression of NHR genes in AML and CML cell lines, normal CD34+ cells and primary human CML/AML CD34+ cells.
- To assess the effect of shRNA mediated knock down of NHRs in AML cell lines with different mutations on cell proliferation, apoptosis and RNA expression after treatment with ara-C/Dnr/both.
- To assess the effect of shRNA mediated knock down of NHRs in CML cell lines representing different disease stages on cell proliferation, apoptosis and RNA expression after imatinib treatment.
- Validation of the findings by overexpression / knock down experiments / AML mouse models.

Work done:

We are in the second year of this project. We have standardized crispr/cas mediated silencing of selected NHR targets with help from Dr. Shaji. NHR ligand treatment improved chemosensitivity in drug resistant AML and CML cell lines and primary cells. We have obtained animal ethics approval for carrying out the ligand treatment in AML mouse model to see if this can improve survival in AML mice.

Specific highlights of the project:

- Analysis of NHR expression in drug sensitive and resistant AML cell lines showed targets such as PPARg, RXR downregulated and AR, NR1P1 upregulated in resistant AML cell lines.
- Treatment with PPARg ligands improved IC50 to arsenic trioxide and moderately to daunorubicin but not to ara-C in resistant AML cell lines.
- PPARg ligand treatment improved chemosensitivity by inhibition of phosphorylation of STAT5 and brings about differentiation in resistant AML cell lines.
- Many novel NHR targets were identified in CML cell lines resistant to TKIs; AHR, AR, ESR1, ESRRG, PPARG, RXRA, RXRB and THRA were found to be up-regulated in the sensitive cell lines compared to the resistant CML cell lines.
- Treatment of specific NHR ligands significantly improved TKI sensitivity in CML cell lines as well as primary CML cell lines.
- The NHR ligands improve TKI IC-50 by inhibiting phosphorylation of BCR-ABL downstream targets crkl and stat5; this also resulted in increased hOCT1 expression.

Support from CSCR: Lentiviral based knockdown experiments are done in Dr. Shaji's lab CSCR, under his help and guidance.

Collaborations:

» R V Shaji, CSCR/CMC, Vellore

PROJECT-2

Project title: Exploring the mechanisms of disease progression, tyrosine kinase inhibitor resistance and intolerance in Chronic Myeloid Leukemia

Funding source: Department of Biotechnology (Part of Centre of Excellence Grant)

Duration: November 2015 - October 2020

Brief description: Targeted therapy with BCR-ABL tyrosine kinase inhibitors has tremendously improved the outcome of chronic myeloid leukemia (CML). However, about 15-20% patients treated with imatinib develop resistance or intolerance to the drug resulting in disease progression. Hence adequate molecular tools are required to diagnose, prognosticate and monitor minimal residual disease to make appropriate decisions on continuing therapy or changing. This decision process is compounded by the high cost of these second generation drugs in a predominantly self-paying system as exists in our country. In this project, we plan to work towards an integrated model with a combination of the existing and novel parameters to more accurately prognosticate response to therapy in these patients. We believe that such a model working towards a cost-effective therapy in patients with CML is critical for patients in our country where the cost of second generation TKI is prohibitive for most. Using a multidisciplinary approach, we anticipate that this study will have sufficient strength to arrive at genetic/prognostic signatures to identify poor responders and personalize therapy which could result in better treatment outcome. 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.

Support from CSCR: Knock down experiments are done in Dr. Shaji's lab, CSCR, under his help and guidance

Collaborations:

» R V Shaji, CSCR/CMC, Vellore

CHRISTHUNESA S. CHRISTUDASS, PhD
Associate Professor – Neurochemistry, Department of Neurological Sciences, CMC, Vellore
Adjunct Scientist, CSCR

Project title: Isolation of Cancer Stem Cells from primary and secondary high grade gliomas - their response to microenvironmental cues and Notch signaling blockade.

Funding source: Department of Biotechnology, Government of India.

Duration: August 2016 to August 2019.

Brief description: Cancer Stem Cells (CSCs) are considered as the driving force of cancer formation and are more resistant to treatment. Gliomas are the most common tumors of the Central Nervous System and glioblastoma multiforme (GBM) are the most malignant tumors of the brain. The prognosis for patients with GBM remains dismal, largely due to the highly invasive nature of this disease and inadequate treatment strategies. On the basis of clinical presentation, GBMs have been further subdivided into primary or secondary GBMs and there is also evidence that CSCs in primary and secondary glioblastomas may also be different. Brain CSCs are characterized by their ability to form neurospheres, undergo self-renewal and differentiate into other cell lineages.

Objectives:

- Identify and characterize CSCs in high grade gliomas using neurosphere formation and expression of cell markers CD133, A2B5 and/or Nestin
- Establish primary or secondary nature of gliomas based on age, mutation(s) in IDH1 and overexpression EGFR
- Study CSCs response to microenvironmental cues by measuring VEGF, HIF-1α,HIF-2α, MMP-9 and CCL-3 levels before and after pretreatment with HIF & VEGF inhibitors
- Study CSCs capacity to differentiate into endothelial cells (ECs)
- Study the role of Notch signaling pathway in both primary and secondary CSCs by Notch pathway blockade. Till date we have successfully standardized isolation and characterization of GBM derived CSCs by neurosphere culture and by CSC markers A2B5 (21-24% positive) and nestin (70-90%). Now we are culturing the sorted cells for further experiments.

Any specific highlights of the project:
Identified and characterised CSCs from primary high grade glioma tissues.

Support from CSCR: CSCR culture facility and core facility for FACS analysis.

Collaborations:

- George Chacko, CMC, Vellore
- Geeta Chacko, CMC, Vellore

ANIKET KUMAR, PhD
Senior Lecturer, Department of Pharmacology & Clinical Pharmacology, CMC Vellore
Adjunct Scientist, CSCR

Project title: Study of human keloid fibroblasts in culture and effects of novel drugs

Funding source: Fluid grant / CSCR

Duration: April 2016 – March 2018 (2 years)

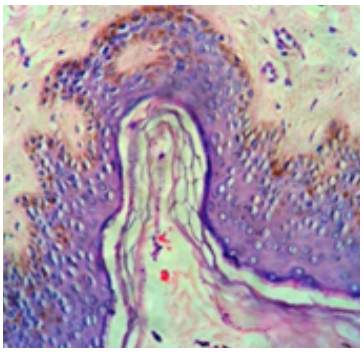
Brief description: Samples of normal skin and keloid will be taken from the patients, after getting their consent in the department of plastic surgery. One portion of the obtained specimen of keloid and normal skin will be utilized for morphological studies, by histopathology and immunohistochemistry for identification of keloid specific structures and proteins. From the other part of normal skin and keloid sample, fibroblasts will be isolated and cultured. Once the cultures are ready, it will be further utilized for understanding their in-vitro characteristics by different techniques. After the characterization studies, cultured fibroblasts (normal and keloid) will be treated with log doses for 24 and 48 hours, with novel drugs of interests, for dose optimization, and its effect on the proliferation and expression of various biomarkers in cultured fibroblasts will be analyzed.

Objectives:

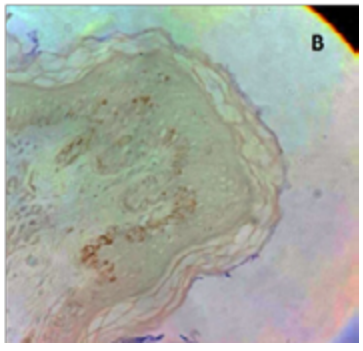
- To isolate and culture keloid fibroblasts obtained from keloids of patients.
- To identify the expression of specific biomarkers for keloid in the cultured keloid fibroblasts.
- To evaluate the effect of various pharmacological therapies on proliferation and differentiation of keloid fibroblasts in-vitro.

Work Done:

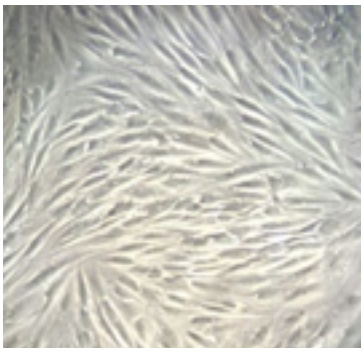
All the samples have been collected from department of plastic surgery and the protocol for the culture, expansion and cryopreservation of keloid as well as normal fibroblasts have been established. Master cell bank and working cell banks are also prepared. To differentiate keloid fibroblasts and normal fibroblast morphologically, we have already done histopathological analysis with the help of a trained pathologist. The base line expression of biomarkers, in normal and keloid fibroblasts, such as TGFβ, PAI-2, COL-I and III has already been done using qRTPCR. Currently the fibroblast cells are undergoing various drug treatments in batches to evaluate the role these drugs in the treatment of keloid disease in-vitro.



A.H&E stained section of keloid tissue.



B. Van Gieson stained section of keloid tissue.



C. Confluent culture of keloidal fibroblasts.

Support from CSCR:

CSCR has provided lab infrastructure, funding and scientific inputs for this project.

Other projects:

In collaboration with other scientists at CSCR / CMC, I am also working on the following projects:

- Gamma delta T cell-based immunotherapy for blood cancers
- Establishing a protocol for expansion of Natural Killer cells

Details of these projects are shown in Dr. Aby Abraham's report.

Collaborations:

- Aby Abraham, CMC, Vellore
- Mohankumar Murugesan, CSCR
- Sanjay Kumar, CSCR
- Alok Srivastava, CSCR/CMC, Vellore
- Ashish Kumar Gupta, CMC, Vellore
- Meera Thomas, CMC, Vellore

SANJAY KUMAR, PhD
Scientist, CSCR

LABORATORY HIGHLIGHTS

Ongoing studies:

- 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 (2017-2020). Funding: 34.94 Lakhs.
- Generation of a novel epigenetic factor shRNA library for studying the mechanisms of stem cell differentiation, disease pathogenesis and drug resistance (2015-2018). (Co-PI: Shaji RV) DBT Grant No. BT/PR8742/AGR/36/773/2013. Total Funding: 79.04 Lakhs.
- A novel multifaceted approach to widen the therapeutic window of spinal cord injury in SCID mice model using hPD-MSC/neuro-progenitors and/or PTEN modulation in axons by inducible shRNA (2014-2017). DBT Grant # BT/PR8527/MED/31/234/2013. Total Funding: 36.19 Lakhs.
- Foot-print free iPSC technology (2010-2016) Ramalingaswami fellowhip project. DBT Grant # BT/HRD/35/02/14/2009. Total Funding: 91.95 Lakhs.

Grants preparations for submission process:

- Genetically-engineered human umbilical cord-derived mesenchymal stem cells (UC-MSC) / engineered UC-MSC derived exosomes as therapeutic delivery vehicles for tumor-targeted therapy or maintaining tissue-homeostasis. (To DBT)
- Evaluating 3D cultured perinatal tissue derived-mesenchymal stem cells under physiological oxygen conditions and comparison with 2D MSC characteristics and therapeutic applications. (To DBT)
- Biological studies on mesenchymal stem cells differentiation commitments with reference to MSCs osteogenic fate choices in metabolic bone diseases. (To DST)

Completed studies:

- 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

Publications:

Book Chapters:

- Human Mesenchymal Stem Cells (hMSCs) Derived Exosomes/Exosome Mimetics as a Potential Novel Therapeutic Tool for Regenerative Medicine. (Springer Publishing Press, 2017) Sundaram B, Herbert FJ, Kumar S*.
- Therapeutic Application of Placental Mesenchymal Stem Cells Reprogrammed Neurospheres in Spinal Cord Injury. (Springer Publishing Press, 2017) 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, 2017).

Journals:

- hiPSCs derived iMSCs: NextGen MSCs as an advanced therapeutically active cell resource for regenerative medicine. (J Cell Mol Med. 2016 Aug;20(8):1571-88) Sabapathy V Kumar S*. [Impact Factor:- 5.818]
- 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. (J of Biomaterials and Tissue Engineering, 2016; 6 (7), 549-562) Sabapathy V, Hurakadli M, Rana D, Ramalingam M, Kumar S*. [Impact Factor:- 2.07]

- Human Mesenchymal Stem Cells (hMSCs) Derived Exosomes/Exosome Mimetics as a Potential Novel Therapeutic Tool for Regenerative Medicine. Sundaram B, Herbert FJ, Kumar S*. Methods Mol Biol. In Press, 2017. [Impact Factor:- 1.290]
- Therapeutic Application of Placental Mesenchymal Stem Cells Reprogrammed Neurospheres in Spinal Cord Injury. (Methods Mol Biol. 2017;1553:91-113) Sabapathy V, Herbert FJ, Kumar S*.[Impact Factor:- 1.290]
- Therapeutic application of human Wharton Jelly Mesenchymal Stem Cells in skin injury. (Methods Mol Biol. 2017;1553:115-132) Sabapathy V, Sundaram B, Kumar S*.[Impact Factor:- 1.290]

Manuscripts submitted for peer review / in preparation:

- hMSCs or neurospheres derived from hiPSCs Augment functional recovery following spinal cord injury in SCID (Submitted for peer review in Plos One) 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, Boston, MA-2017.
- American Society of Hematology (ASH-2016).
- International Society of Stem Cell Research (ISSCR) Annual Meeting, 2016.
- World Stem Cell Summit, Regenerative Medicine Capital, Atlanta, GA, 2016.

Seminars Given:

- Therapeutic applications of hMSCs in mice models. Anna University, Chennai.
- Therapeutic applications of MSCs in regenerative medicine and in vivo imaging. Translational Research Platform for Veterinary Biologicals (TRPVB), Chennai.
- 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
- Human Mesenchymal stem cells (hMSCs) and approaches to utilise them in regenerative medicine using mice models. Manipal Institute of Regenerative Medicine, Bangalore.
- Therapeutic use of human mesenchymal stem cells (hMSCs) in various mice models. Madhav Institute of Technology & Sciences (MITS) Gwalior, India.

Patents:

- Publication Date: 10/06/2016 Journal No. 24/2016. 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:

- M.Sc. Biotechnology, Stem Cell Biology course at Department of Biotechnology, Thiruvalluvar University Serkadu, Tamil Nadu.
- Stem cell biology module courses for PhD students
- Cell & molecular biology courses for PhD students
- Gene therapy courses for PhD students

Students trained:

- Trained 21 students for partial fulfillment of their M. Tech., B.Tech., M. Sc.(Biotech) and B. Sc. (Biotech) degree from across the country.

- Conducted 7 Stem cell workshops/courses and demonstrated mouse bone marrow mesenchymal stem cell isolation, in vitro characterization and immunophenotypic assays.

Other Academic Activities:

Providing support to following core facilities:

- 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)
- International Society for Stem Cell Research (ISSCR)
- American association for advancement of Science

Preclinical work & ongoing studies:

Biological Question: "During in vitro expansion of human Mesenchymal Stem cells (hMSCs), Are we providing every opportunity (in terms of total growth conditions) to Primary Mesenchymal Stem Cells following their isolation from 3D tissue architecture?

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, 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 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 do 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 mechanism 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 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 regenerative conditions:

Finding 1 for the questions “Can human perinatal tissue-derived MSCs (Placenta-derived MSCs or Wharton jelly-derived MSCs) augment therapeutic outcome in mice models?” & “Do perinatal tissue-derived hMSCs maintain their phenotypic attributes and retain intrinsic characteristics during long-term in vitro cultures?”

Publications

- hiPSCs derived iMSCs: NextGen MSCs as an advanced therapeutically active cell resource for regenerative medicine. (J Cell Mol Med. 2016 Aug;20(8):1571-88) Sabapathy V Kumar S.
- 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, Kumar S.
- Quest for the alternate personalized clinical source of MSCs: Advancing towards hiPSCs derived iMSCs. Current Stem Cells Research and Therapy, Vol. 11, No. 2, 2016. Sabapathy V, Kumar S.
- 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.
- Decellularized amniotic membrane scaffold compared to synthetic PLGA and hybrid scaffolds exhibit superlative biomechanical properties for tissue engineering applications. (J of Biomaterials and Tissue Engineering, 2016; 6 (7), 549-562) Sabapathy V, Hurakadli M, Rana D, Ramalingam M, Kumar S.
- Therapeutic Application of Placental Mesenchymal Stem Cells Reprogrammed Neurospheres in Spinal Cord Injury. (Methods Mol Biol. 2017;1553:91-113) Sabapathy V, Herbert FJ, Kumar S.
- Therapeutic application of human Wharton Jelly Mesenchymal Stem Cells in skin injury. (Methods Mol Biol. 2017;1553:115-132) Sabapathy V, Sundaram B, Kumar S*.
- Bone defect repair in mice by mesenchymal stem cells. Methods Mol Biol. 2014; 1213: 193-207. Kumar S.

Book Chapters

- Human Mesenchymal Stem Cells Derived Exosomes/Exosome Mimetics as a Potential Novel Therapeutic Tool for Regenerative Medicine. (Springer Publishing Press, 2017) Sundaram B, Herbert FJ, Kumar S.
- Therapeutic Application of Placental Mesenchymal Stem Cells Reprogrammed Neurospheres in Spinal Cord Injury. (Springer Publishing Press, 2017) Sabapathy V, Herbert FJ, Kumar S.

- Therapeutic application of human Wharton Jelly Mesenchymal Stem Cells in skin injury. (Springer Publishing Press, 2017) Sabapathy V, Sundaram B, Kumar S.
- Bone defect repair in mice by mesenchymal stem cells. Springer Publishing Press 2014; 1213: 193-207. 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)

Publications

- 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
- hMSCs / neurospheres derived from hiPSCs Augment functional recovery following spinal cord injury in SCID. Manuscript submitted for peer 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 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) will augment functional recovery following spinal cord injury in small animal models. Furthermore, we will assess if these regenerating CST axons have the ability to reform synapses in spinal segments distal to the injury. Also, test if modulating injured neuronal intrinsic PTEN/mTOR activity for a transient period by small molecule inhibitors or PTEN gene specific 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 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: Can we evaluate human Mesenchymal Stem Cells (hMSCs)-derived Exosomes/Exosome Mimetics as a potential novel therapeutic tool for regenerative purposes? & "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-MSCs) / engineered UC-MSCs-derived exosomes as therapeutic delivery vehicles for tumor-targeted therapy or maintaining tissue homeostasis.

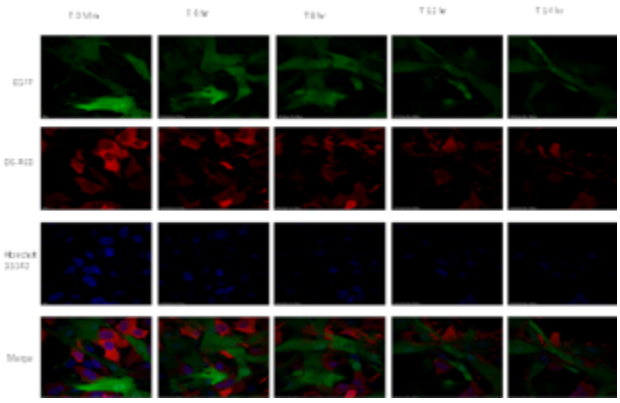


Figure 1: Confocal live cell time-lapse imaging of hWJ-MSC is expressing EGFP, suicide gene and co-cultured with cancer cells MCF-7 expressing dsRed Express. Figure depicting apoptotic cell death of cancer cells by a reduction in red fluorescence with time mainly due to loss of dsRed labeled MCF-7 cells.

Finding 4 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 (3D ECM & 3% Oxygen) concentrations by comparative transcriptome analysis (by small RNA sequencing) and in vivo transplantation experiments in mice models?"

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, and 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 5 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?"

Publications

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

Collaborations:

International:

- Selvarangan Ponnazhagan, Department of Pathology, University of Alabama at Birmingham (UAB), Birmingham, Alabama, USA.

Internal:

- RV Shaji, CSCR/CMC, Vellore
- George Tharion, CMC, Vellore
- Antony Devasia, CMC, Vellore
- Vrisha Madhuri, CSCR/CMC, Vellore
- Alok Srivastava, CSCR/CMC, Vellore
- Suresh Devhsayam, CMC, Vellore
- Paul MJ, CMC, Vellore
- Asha Mary Abraham, CMC, Vellore
- Ashish Gupta, CMC, Vellore
- Margaret Shanthi, CMC, Vellore

RAVIKAR RALPH, MD
Assistant Professor, Department of Medicine Unit-I, CMC, Vellore
Adjunct Scientist, CSCR

Project title: β-chemokine expression and HIV-1 infection in CD34+ haematopoetic stem cells – A pilot study

Funding source: Fluid grant

Duration: 2016-2018

Brief description: Despite the advent of highly active anti-retroviral therapy, a functional cure for HIV has been impossible to achieve due to the existence of cellular reservoirs with latent infection. These latently infected cells are all derived from CD34+ haematopoetic progenitor cells. Engineered CD34+ cells could potentially result in mature cells resistant to HIV infection thereby resulting in a functional cure. In this context, CD34+ cellular susceptibility to HIV-1 infection is an important question. CD34+ cells are largely resistant to HIV-1 infection in-vivo despite a subset expressing the CD4 and CCR5 co-receptors. Autocrine binding of cellular β chemokines to the CCR5 co-receptor has been suggested as a putative hypothesis to explain this phenomenon.

This study aims to compare mRNA expression levels of the β chemokines MIP-1α, MIP-1 β and RANTES in CD34+ cells with CD4 and CCR5 co-expression, derived from HIV-1 infected patients with expression levels in cells from healthy HIV negative consenting donors using reverse transcriptase polymerase chain reaction (RT-PCR) techniques, to determine whether or not an upregulation of β chemokines genes in these CD34+ cell subsets occurs in response to an in-vivo HIV-1 infection. Transcriptional upregulation if present would strengthen the above hypothesis and suggest an autocrine effect of β chemokines in blocking an HIV-1 infection of CD34+ cell subsets in-vivo.

Aim: To compare mRNA expression levels of the β chemokines MIP-1α, MIP-1 β and RANTES in CD34+ CD4+ cells with CCR5 co-expression, from HIV-1 infected patients with expression levels in CD34+ CD4+ CCR5+ cells from HIV negative donors using reverse transcriptase polymerase chain reaction (RT-PCR) techniques.

Objectives:

- Relative quantification of β chemokine (MIP-1α, MIP-1 β and RANTES) mRNA in CD34+ CD4+ cells with CCR5 co-expression, from HIV-1 infected patients compared to HIV negative healthy controls using qRT-PCR
- HIV-1 DNA quantification in CD34+ CD4+ cells from HIV-1 infected patients using qPCR

Work done: Subject recruitment, baseline screening, peripheral blood mobilization and collection of CD34 + CD4+ CCR5+ cell subsets, DNA extraction and storage completed in 4 study participants.

Support from CSCR: Flowcytometry experiments are done at CSCR

Collaborations:

- Alok Srivastava, CSCR/CMC, Vellore
- George Varghese, CMC, Vellore
- Anand Zachariah, CMC, Vellore
- Rajesh Kannangai, CMC, Vellore
- John Fletcher, CMC, Vellore
- Jaiprasath, CMC, Vellore

MURUGAN RAMALINGAM, PhD, FNS, FRSB, FRSC
Associate Professor, CSCR

LABORATORY HIGHLIGHTS

Area of Research: Biomaterials and 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. The concept of tissue engineering has emerged as a promising approach to overcome these limitations, as it enables the fabrication of functional tissues and organs by combining patient's or donor's own cells with engineered matrices called scaffolds for regenerative medicine. The three-dimensional (3D) microenvironment is one of the key factors to engineer a physiologically functional tissues and organs, which are investigated in our lab using biomimetic-, micro- and nano-technologies as follows.

(i) Development of Gradient Biomaterials for Stem Cell Screening

This project involves the design of combinatorial platform using biomaterials in the form of nanofibers with a linear composition or modulus gradient libraries that facilitates high-throughput screening of stem cell's response to 3D microenvironment in terms of their 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 engineer tissues and organs. Screening the effect of scaffold composition and characteristics towards stem cell behavior is the key selection criteria of 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 high-throughput screening platform to screen human bone marrow-derived mesenchymal stem cells (hBMSCs) (see Figure 1)

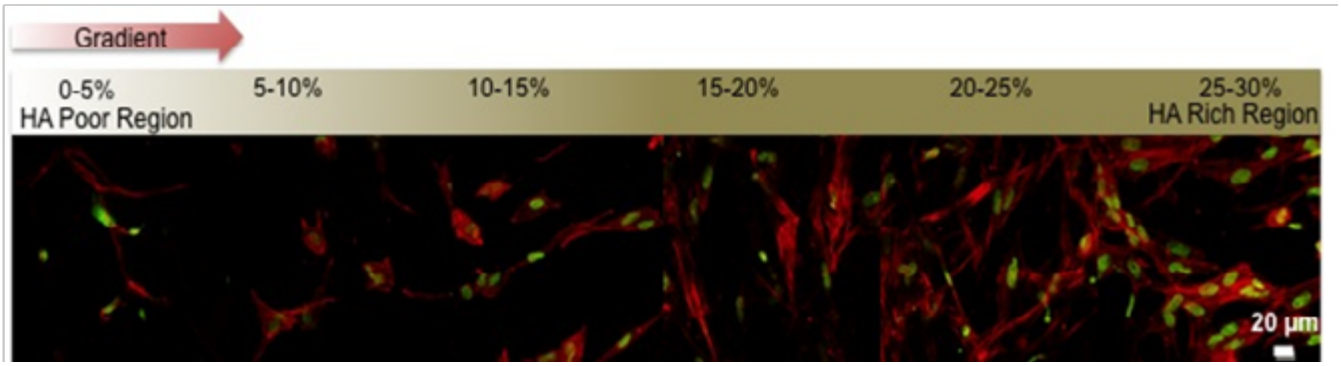


Figure.1. A representative confocal image of hBMSCs responses to gradient microenvironment. HA denotes hydroxyapatite, a major bone mineral substance.

(ii) Development of Shape Memory Biomaterials for Stem Cell Delivery

The aim of this project is the development of injectable and shape-memory biomaterials in the form of hydrogel 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 cellular compatibility with hBMSCs. The results of this study confirmed the injectable nature of the gels as well as its ability to regain the initial/original shape after deformation (shape-memory) (see Figure 2) and cellular compatibility with hBMSCs.

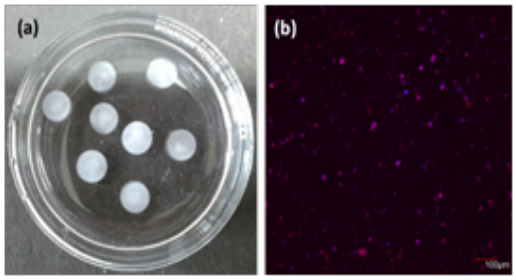


Figure 2. Photographic image of (a) shape memory gels made of GelMA and (b) hBMSCs encapsulated gel suitable for transplantation.

The major finding of this study suggest that GelMA-derived gels may serve as a carrier system for the delivery of stem cells. In addition, the gels have also been subjected to use as substratum for scalable culture of mesenchymal stem cells for tissue engineering applications.

(iii) Development of Thermo-Responsive Biomaterials for Tissue Engineering

The aim of this project is the development of injectable and thermo-responsive gels (also called thermogels) for the use of stem cell delivery, growth factor delivery and tissue engineering. 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 gel 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 3). In addition, the gels are also being tested as a magnetized carrier system for growth factors to enhance the osteogenesis and to track the system upon implantation.

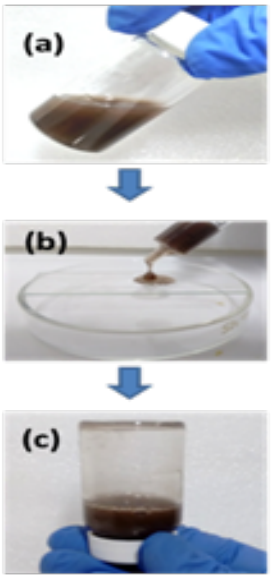


Figure 3. Photographic image of a magnetized thermogel made of chitosan and nHAp: (a) sol state at room temperature, (b) injectability of thermogel and (c) at gel state at physiological temperature.

(iV) Development of Bio-Inks for Organ Printing

The aim of this project is the development of bio-inks, based on alginate/polyacrylamide biomaterials, for the use of 3D printing of human organs (flat, hollow and complex structures). 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 4). 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.

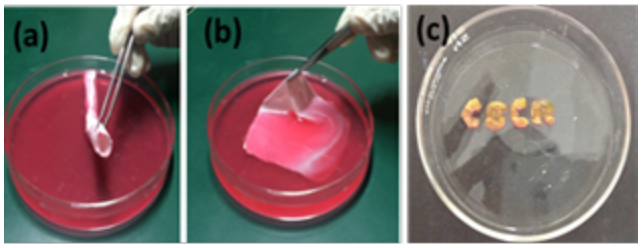


Figure 4. In-house custom made three-dimensional tissue/organ's structures (tubular (a) and flat (b)) and printability of bio-inks (c), made of polymeric biomaterials.

In a pilot study, we have developed hBMSCs encapsulated alginate/acrylamide-based gel-like systems and studied the cellular behavior in vitro. The preliminary results confirmed that the stem cells cultured in 3D gels show morphology and cellular behavior in resemblance to the native tissue-like microenvironment as well as adequate mechanical stability. Furthermore, the optimized biomaterial composition facilitated its printability and structural stability. This kind of bio-inks might be used in engineering various human tissues and organs.

Funding

- DST, CMC, CSCR

Honors and awards

- Adjunct Professor, Tohoku University, Japan
- Fellow, Royal Society of Chemistry, UK
- Fellow, Institute of Nanotechnology, UK
- JBT Best Paper Award from the American Scientific Publisher, USA
- Best Poster Award, 7th India-Japan International conference on Science and Technology: Future Challenges and Solutions, held at University of Mysore, India (2016).
- Best Poster Award, International Conference on Nanoscience and Nanotechnology (ICNN'16), held at VIT University, India (2016),
- Best Poster Award, The National Conference on Emerging Biomaterials (NCEB-2016), held at Bharathiar University, India (2016).

Publications:

Journals

- Sukhwinder Bhullar, Deepti Rana, Huseyin Lekesiz, Ayse Celik Bedeloglu, Junghyuk Ko, Yonghyun Cho, Zeynep Aytac, Tamer Uyar, Martin Jun, Murugan Ramalingam. Design and fabrication of auxetic PCL nanofiber membranes for biomedical applications. Materials Science and Engineering: C 81 (2017) 334-340.
- D. Rana and R. Murugan. Enhanced proliferation of human bone marrow-derived mesenchymal stem cells on tough hydrogel substrates. Mater. Sci. Eng. C 76 (2017) 1057-1065.
- Prakash Parthiban, D. Rana, Esmaiel Jabbari, Nadia Benkirane-Jessel and R. Murugan. Covalently immobilized VEGF-mimicking peptide with gelatin methacrylate enhances microvascularization of endothelial cells. Acta Biomater. 51(2017)330-340.
- 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 tissue formation and function. J. Tissue Eng. Reg. Med. 11 (2017) 582-595.
- Yingying Chen, Jiaju Lu, Bangrui Chen, Shuo Wang, Deepti Rana, R. Murugan, Yueteng Wei, Xiaodan Sun, Lingyun Zhao, and Xiumei Wang. PFS-functionalized Self-assembling Peptide Hydrogel for the Maintenance of Human

Adipose Stem Cell in Vitro. J. Biomater. Tissue Eng (2017).

- Deepti 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. 11 (2017) 942-965.
- Deepti Rana, Sampath Kumar and R. Murugan. Impact of Nanotechnology on 3D bioprinting. J. Bionnanosci. 11 (2017) 1-6.
- R. Jayasree, S. Indrakumar, Deepti Rana, R. Murugan and T. S. S. Kumar. Bone mineral-like nanoscale amorphous calcium phosphate derived from egg shells. J. Bionnanosci. 11 (2017) 297-300.
- Deepti Rana, Xiumei Wang, Thomas Webster and R. Murugan. Biomimetic Nanohydroxyapatite Synthesized with/without Tris-Buffered Simulated Body Fluid: A Comparative Analysis. J. Nanosci. Nanotech 17 (2017).
- R. Jayasree, K. Madhumathi, Deepti Rana, R. Murugan, Rakesh Nankar, Mukesh Doble and T. S. S. Kumar. Development of egg shell derived carbonated apatite nanocarrier system for drug delivery. J. Nanosci. Nanotech 17 (2017).
- Sukhwinder Bhullar, Deepti Rana, Burçak Kaya Ozsek, Mehmet Orhan, Martin BG Jun, Harpal Buttar and R. Murugan. Development of silver-based antibacterial nanofiber composites by airbrushing. J. Nanosci. Nanotech 17 (2017).
- Deepti Rana, Aleya Tabasum and R. Murugan. Cell-laden alginate/polyacrylamide beads as carrier for stem cell delivery: preparation and characterization. RSC Advances 6 (2016) 20475-20484.
- Maria Leena, Deepti Rana, Thomas Webster and R. Murugan Accelerated synthesis of biomimetic nanophase hydroxyapatite using stimulated body fluid. Mater. Chem. Phys 180 (2016) 166-172.
- Sukhwinder Bhullar, Deepti Rana, Burcak Kaya Ozsel, Ramesh Yadav, Ginpreet Kaur, Meena Chintamaneni, Harpal Buttar, Martin B.G. Jun and R. Murugan. A Comparative Study of the Antibacterial Activity of Rosemary Extract Blended with Polymeric Biomaterials. J. Bionanoscience 10 (2016) 326-330.
- 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. Tissue Eng 6 (2016) 549-562.
- R. Murugan, Alicia El Haj, Thomas Webster and Seeram Ramakrishna. The role of nanotechnology in stem cell research. J. Nanosci. Nanotech. 16 (2016) 8859-8861.
- R. Murugan and R. V. Shaji. Advances in induced pluripotent stem cells: Nanomaterial perspectives. J. Bionanoscience 10 (2016) 163-170.
- Deepti Rana, Maria Leena, Manasseh Nithyananth, Renu Pasricha, Geetha Manivasagam and R. Murugan. Control of stem cell fate and function by polymer nanofibers. J. Nanosci. Nanotech. 16 (2016) 9015-9021.
- Deepti Rana, R. Keerthana, Maria Leena, Constanza Jiménez, Javier Campos, Paula Ibarra, Ziyad S. Haidar and R. Murugan. Surface functionalization of nanobiomaterials for application in stem cell culture, tissue engineering and regenerative medicine. Biotechnology Progress 32 (2016) 554-567.
- S. Ahadian, R. Obregón, J. Ramón-Azcón, H. Shiku, R. Murugan, T. Matsue. Carbon-based nanomaterials for stem cell differentiation and tissue regeneration. J. Nanosci. Nanotech 16 (2016) 8859-8861.
- 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. 16 (2016) 8948-8952.

Text Books

- R. Murugan and S. Ramakrishna. Nanofiber Composites for Biomedical Applications. Woodhead Publishing Series, Elsevier Publication, UK (2017) 540 pages.
- X. Wang, R. Murugan, X. Kong and L. Zhao. Nanobiomaterials: Classification, Fabrication and Biomedical Applications. Wiley Publication, USA (2017) 450 pages.
- Z. Haidar and R. Murugan. Bioceramics: Principles and Applications. Wiley-Scrivener Publication, USA, ISBN: 978-1-119-16029-8 (2017) 500 pages.
- A. Vishwakarma, X-P. Wang, P.T. Sharpe, S. Shi and R. Murugan. Stem Cell Biology and Tissue Engineering in Dental Sciences. Elsevier, UK (2015) 932 pages.

Book Chapters

- 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 (2017) 109-141.
- 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, Murugan Ramalingam and Seeram Ramakrishna (Eds.), Elsevier Publication, UK (2017) 225-252.
- Lakshmipriya Manickam, Deepti Rana, Akshay Bhatt and R. Murugan. Nanofiber Composites in Gene Delivery. In Nanofiber Composite Materials for Biomedical Applications, Murugan Ramalingam and Seeram Ramakrishna (Eds.), Elsevier Publication, UK (2017) 253-274.
- G. Manivasagam, R. Asokamani, A. Jaiswal, and R. Murugan. Mechanical characterization of nanofiber composites. In Nanofiber Composite Materials for Biomedical Applications, Murugan Ramalingam and Seeram Ramakrishna (Eds.), Elsevier Publication, UK (2017) 117-156.
- Chetna Dhand, Neeraj Dwivedi, Harini Sriram, Samiran Bairagi, Deepti Rana, Lakshminarayanan Rajamani, R. Murugan, and S. Ramakrishna. Nanofiber Composites in Drug Delivery. In Nanofiber Composite Materials for Biomedical Applications, Murugan Ramalingam and Seeram Ramakrishna (Eds.), Elsevier Publication, UK (2017) 197-224.
- Deepti Rana, Greeshma Ratheesh, S. Ramakrishna and R. Murugan, Nanofiber Composites in Cartilage Tissue Engineering. In Nanofiber Composite Materials for Biomedical Applications, Murugan Ramalingam and Seeram Ramakrishna (Eds.), Elsevier Publication, UK (2017) 325-344.
- Lakshimipriya Manickam, Deepti Rana, and R. Murugan. Ceramic Nanofiber Composites. In Nanofiber Composite Materials for Biomedical Applications, Murugan Ramalingam and Seeram Ramakrishna (Eds.), Elsevier, UK (2017) 31-54.
- 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, Murugan Ramalingam and Seeram Ramakrishna (Eds.), Elsevier Publication, UK (2017) 507-528.
- R. Murugan and S. Ramakrishna. Introduction to Nanofiber Composite Materials. In Nanofiber Composite Materials for Biomedical Applications, Murugan Ramalingam and Seeram Ramakrishna (Eds.), Elsevier Publication, UK (2017) 1-30.
- Deepti Rana, R. Keerthana, Maria Leena, Renu Pasricha, Geetha Manivasagam and R. Murugan. Surface Functionalization of Biomaterials. In Stem Cell Niche Biology and Engineering, A. Vishwakarma and Jeff Karp (Eds.), Elsevier, USA (2017) 1-13.
- Deepti Rana, Keerthana Ramasamy, Samad Ahadian, Geetha Manivasagam, Xiumei Wang and R. Murugan. Polymeric Nanobiomaterials. In Nanobiomaterials: Classification, Fabrication and Biomedical Applications, Xiumei Wang et al. (Ed.), John-Wiley Publication, USA (2017) 65-84.
- Lakshimipriya Manickam, Akshay Bhatt, Deepti Rana, Serge Ostrovidov, Renu Pasricha, Xiumei Wang and R. Murugan. Nanopatterning Techniques. In Nanobiomaterials: Classification, Fabrication and Biomedical Applications, Xiumei Wang et al. (Ed.), John-Wiley Publication, USA (2017) 189-210.
- G. Magesh, G. Vasanth, A. Revathi, Geetha Manivasagam and R. Murugan. Metallic Nanobiomaterials. In Nanobiomaterials: Classification, Fabrication and Biomedical Applications, Xiumei Wang et al. (Ed.), John-Wiley Publication, USA (2017) 39-63.
- Samad Ahadian, Farhad Batmanghelich, Raquel Obregón, Deepti Rana, Javier Ramón-Azcón, Ramin Banan Sadeghian and R. Murugan. Carbon-based Nanobiomaterials. In Nanobiomaterials: Classification, Fabrication and Biomedical Applications, Xiumei Wang et al. (Ed.), John-Wiley Publication, USA (2017) 85-104.

Invited talks

- Injectable Biomaterials for Tissue Engineering. Advanced Institute for Materials Research, Tohoku University, Japan.
- Biomaterials and stem cells for tissue engineering, VIT University, India

Courses 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.

Academic activities

- 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, 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 of Journal of Stem Cell Research and Therapy, USA
- Editorial Board Member of Journal of Biomimetics, Biomaterials, Tissue Eng.
- 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
- Scientific Committee, Ministry of Business, Innovation and Employment, New Zealand.
- Member of Grant Reviewing Committee, Foundation for Polish Science (FNP).
- Scientific Committee Member, The FFSCI Europe, Croatia.
- Doctoral Committee Member, VIT University, India.
- Doctoral Committee Member, Periyar Maniammai University, India.

Collaborators

Internal:

- Alok Srivastava, CSCR/CMC, Vellore
- Sanjay Kumar, CSCR, Vellore
- R. V. Shaji, CSCR/CMC, Vellore
- Manasseh Nithyananth, CMC, Vellore
- Manish Baldia, CMC, Vellore
- Mathew Joseph, CMC, Vellore
- Jeyanth Rose, CMC, Vellore

External:

- Geetha Manivasakam, 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
- Esmaiel Jabbari, University of South Carolina, USA
- S Bhullar, University of Victoria, Canada
- Seeram Ramakrishna, National University of Singapore
- Serge Ostrovido, Tohoku University
- Tomokazu Matsue, Tohoku University, Japan
- Ziyad Haidar, Universidad de los Andes, Chile
- Nadia Jessel, University of Strasbourg, France
- Tomasz Trzeciak, Poznan University of Medical Sciences, Poland
- Hala Zreiqat, University of Sydney, Australia
- Xiumei Wang, Tsinghua University, China
- Neil Davies, University of Cape Town
- Thomas Franz, University of Cape Town, South Africa

JEYANTH ROSE, MS
Associate Professor, Department of Ophthalmology, CMC, Vellore
Adjunct Scientist, CSCR

PROJECT-1

Project title: Efficacy of placenta derived Mesenchymal Stem Cells in reducing corneal scarring in an Ex-vivo organ culture model of post mortem human corneas

Funding source: Fluid major research grant / CSCR.

Duration: Nov 2016 to March 2018

Brief description: The aim of this project is to use intrastromal injection of Placenta derived Mesenchymal stem cell as a treatment for corneal scar. Briefly the Placenta derived MSC's will be extracted from the placenta of consented LSCS donor by enzymatic digestion. Five pairs of post mortem human corneas harvested for corneal transplant excluded for clinical use, of a grade equal to fair or better will be chosen for the study. Both eyes of each pair will have a superficial keratectomy with a standardized protocol. The eyes will be randomly assigned to receive a test or sham injection. The test eye will have an intrastromal injection of 3x100000 placenta derived mesenchymal stem cells, pre-labelled with Dil in 2 microlitrers of PBS. The control eye will have an intrastomal injection of carrier without cells. The eyes will be maintained in an organ culture model system at the air liquid interface over a 28 days period. All assessment will be done on the 28th day onwards.

Objectives:

- To evaluate the viability and distribution of placenta derived Mesenchymal stem cells in the corneal stroma in an ex-vivo organ culture model of human corneal scarring over a 30 days period.
- To compare the influence of intrastromal MSC's on corneal transparency in an ex-vivo organ culture model of evolving corneal scarring.
- To compare the influence of MSC's on the basic histopathology of the cornea.
- To compare the influence of MSC's on markers of Fibrotic corneal scarring.
- To investigate the mechanism of action of MSC's in corneal scarring.

Work Done:

- Isolation, characterization and cryopreservation of MSC's from placenta is complete and they are ready for injection.
- Surgeon has established a standardized protocol of superficial keratectomy with aseptic precautions
- Organ culture system has been optimized in the lab with a pilot project, minimizing the risk of contamination and infection.
- Safe transport chain of specimen from the hospital to the lab has been secured.
- Corneal transparency quantification by laser scatter method has been innovated and standardized in the lab. A gradation scale has been established and is ready for publication.
- IHC markers and dilution protocols have been standardized in the pathology lab.
- The concentration and frequency of tgf beta to induce corneal scarring in the organ culture model is being optimized.

Support from CSCR: Partial funding support, lab space and infrastructure

Publications:

- Rose, J., Wankhar, S., Joshua, A., Korah, S., Kuriakose, T. An innovative model to quantify corneal transparency in donor corneal buttons. South Asian Journal of Experimental Biology, UAE, 6, Jul. 2017.

Collaborations:

- Sanita Korah, Department of Ophthalmology, CMC, Vellore
- Thomas Kuriakose, Department of Ophthalmology, CMC, Vellore
- Rutika Dodeja, Department of Ophthalmology, CMC, Vellore
- Deepthi Kurien, Department of Ophthalmology, CMC, Vellore
- Charles Immanuel, Department of Ophthalmology, CMC, Vellore
- Srinivasan, Department of Ophthalmology, CMC, Vellore
- Augustine Thambaiah, CSCR
- Alok Srivastava, CSCR/ CMC, Vellore
- Suresh Devasahayam, Department of Bioengineering, CMC, Vellore
- Syrpailyne Wankhar, Department of Bioengineering, CMC, Vellore
- Geeta Chacko, Department of Pathology, CMC, Vellore
- Tripti Jacob, Department of Anatomy , CMC, Vellore
- Joe Varghese, Department of Biochemistry, CMC, Vellore
- Vinay Oomen, Department of Physiology, CMC, Vellore
- Mindy Fox, University of Cincinnati, USA
- Winston Kao, University of Cincinnati, USA

PROJECT-2

Project title: A Bioengineered corneal substitute using decellularized human donor cornea rejected for corneal transplant

Funding source: Fluid major research grant / CSCR

Duration: Oct 2016 to Oct 2018

Brief description:

Our study aims at finding an alternative substitute for lamellar corneal transplants, using human donor corneas rejected for corneal transplant because of poor grade. The huge gap between demand and supply and the poor utilization rates of donated corneas (because of poor endothelial function and other undesirable characteristics) has prompted the need for a substitute that can be banked and replaced as and when required. We chose to derive a scaffold from unused human corneal donor buttons, to keep the system as physiological as possible.

Human donor cornea rejected for corneal transplant grade fair or poor will be decellularized using a described protocol. The success of decellularization will be tested using H & E staining, DNA quantification, DAPI staining and immunohistochemistry. The decellularized corneas which will be the substitute we are aiming to develop will be tested for corneal transparency using Font recognizer, Laser quantification, digital photography. Biomechanical strength will be measured with a strip extensiometer and young's modulus will be found. The parameters will be compared with the control arm of the study, which will be post mortem donor corneas with similar inclusion criteria but prior to the decellularization process.

Objectives: The objectives of this project include the following:

- To design a biomimetic stromal scaffold using decellularized human corneal stroma
- To measure the transparency, biomechanical strength and morphology of the decellularized
- scaffold.

Work Done:

- Acquired the material and reagents required for the decellularization process. And have pilot run corneas through the process.
- Optimizing the storage process of corneas, once decellularized.
- Standardized scale for quantifying corneal transparency using laser scatter image analysis.
- Specific highlights of the project: A novel idea to increase utilization of donor corneas.

Support from CSCR: Infrastructure/ Lab space / mentorship from faculty

Publications:

- Rose, J., Wankhar, S., Joshua, A., Korah, S., Kuriakose, T. An innovative model to quantify corneal transparency in donor corneal buttons. South Asian Journal of Experimental Biology, UAE, 6, Jul. 2017.

Collaborations:

- Sanita Korah, Department of Ophthalmology, CMC, Vellore
- Thomas Kuriakose, Department of Ophthalmology, CMC, Vellore
- Deepthi Kurien, Department of Ophthalmology, CMC, Vellore
- Shalmili Lalgudi, Department of Ophthalmology, CMC, Vellore
- Murugan Ramilingam, CSCR
- Srypailyne Wankar, Department of Bioengineering, CMC, Vellore

Other investigators:

- Deepti Rana, JRF
- Aarwin Joshua, Optometry Technician
- Thomas Cherian, MBBS student
- Sharon Poorima, MBBS student
- Manna Winford, MBBS student
- Mahima Keziah, Optometry student
- Arthi, Optometry student
- Shalin Arambhan, Optometry student

RESEARCH DEVELOPMENT OFFICE (RDO)

Research Development Office (RDO) at the Centre for Stem Cell Research (CSCR) was created to provide executive and administrative support to research and development activities of the Centre. RDO assists the CSCR faculty and students in various activities related to their research. These include funding source information dissemination, assistance with proposal development and management of research projects. RDO coordinates funding information and provides services related to research administration. It also helps faculty and students identify sources of funding for research and scholarly activities.

RDO also assists in formulating research policies, implementing research administration strategies and developing initiatives to enhance research and funding performance. The Office undertakes research information management and provides administrative support for the development of research. Besides, the Office coordinates management of research projects and initiatives supported by CSCR and also the internal and external research assessment exercises, research capacity building programs as well as national / international research collaborations.

The Office coordinates meetings of research groups and communicates with funding agencies / collaborators to keep track of the progress of research work at the Centre. RDO helps in organizing various scientific meetings that take place at CSCR. Preparation and submission of project reports to the funding agencies and collation of annual report of the Centre is also coordinated by this office.

RDO liaises with the researchers and administration of the Centre to develop new initiatives and to set policies aimed at planning for the improved quality of research and to ensure their proper and timely implementation. It also monitors the implementation of research plans to ensure timeliness and results in relation to planned objectives and expected outcomes. To a certain extent, it also helps the researchers in CSCR in timely utilization of funds.

In the past year, this office has focused its work towards these goals. Several collaborations were established and MoUs signed for the research work that is ongoing at CSCR. For the gene therapy program, research agreements were signed between CSCR and the University of Florida and Emory University, USA. Collaborations are being established with the Government of Odisha and Indian Institute of Public Health Bhubaneswar for development of a population-based control program for thalassemia and sickle cell disease in Odisha (under the NAHD program). Plans to collaborate with an industry partner in India for development of a gene therapy product for hemophilia B is in its final stages. Several research grant applications were submitted in the past year for both intramural / extramural funding. The process of submission and management of grants at the Centre was also streamlined. These efforts were coordinated by this office.

Md. Manzoor Akheel
Research Development Office

Core Facilities and Instrumentation

CSCR Core Facilities:

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

Molecular Biology Core Facility:

- Technical Officer: Mr. A. Rajesh
- Technical Staff: Ms. J. Saranya
- Faculty Support: Dr. R. V. Shaji

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, and an Applied Biosystems QuantStudio 12K Flex Real-time PCR for high throughput analysis.

I. Genetic Analyzer:

Genetic Analyzer 3130 is a 4 capillary series system with Electro-osmotic flow suppression polymers (EOF). This system gives you all the advance automation with hands free operation and superior performance. This system provides compatibility with the existing application software systems, long-term reliability, automated polymer delivery system, enhanced thermal control, and optimized for multiple application.

II. Real-Time PCR:

QuantStudio 12 K system is designed for maximum throughput, outstanding flexibility with 5 inter-changeable blocks, scalability and user friendly. This system is widely used in gene expression analysis, SNP genotyping, copynumber analysis, digital PCR technology, Micro RNA and other noncoding RNA analysis.

Radioactivity Core Facility

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

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

Flow Cytometry Core Facility:

- Technical Officer : Mr. A. Rajesh
- Technical Staff: Ms. J. Saranya
- Faculty Support: Dr. Sanjay Kumar

Flow cytometry is a pivotal tool in stem cell isolation and their in vitro expansion procedure. Many intra and extra-cellular parameters can be analyzed and systematically evaluated with high speed and precision. The Flow Cytometry Core Facility currently houses following instruments:

I. FACS Aria III:

The BD FACSAria III sorter is a superior multicolor performance, and legendary ease-of-use that opened the complex world of cell sorting to a broader audience of researchers and wider range of applications. A patented flow cell with gel-coupled cuvette and patented octagon and trigon detection system allow the system to achieve unrivaled sensitivity and resolution. BD FACSAria III cell sorter with a 5 laser 11 colour setup. The BD FACSAria III system has a throughput of 70,000 events per second and can do 4-way sorting and single cell sorting. Wavelength choices include 561-nm (Y/G), as well as the 488-nm (Blue), 633-nm (Red), 405-nm (Violet), and 375-nm (UV) lasers.

II. FACS Celesta:

BD FACS celesta is a multi-laser flow cytometer with 3 laser and 12 colour setup for delivering high sensitivity and performance. All configurations have blue (488-nm) and violet (405-nm) lasers, which has been paired with a yellow-green (561-nm) laser, for your application needs.

III. FACS Calibur:

The BD FACS Calibur platform allows users to perform both cell analysis in a single benchtop system. This has 2 lasers, 488nm (Blue) and 633nm (Red), 4 colour system and is routinely used for intracellular and surface markers analyses.

The Flow Cytometry core aims to conduct regular workshops related to multicolor flow sorting and cell analysis for human resource development in state-of-the-art flow cytometry techniques and provides support to various departments in selecting appropriate antibodies panels and experimental designs. An offline workstation with a FlowJo license is also available and networked for data sharing and post-acquisition data analysis.

Histopathology Core Facility:

- Technical staff: Mrs. Esther Rani and Mr. Ashok Kumar
- Faculty Support: Dr. Noel Walter

Special stains standardized:

Histology Special Stains:

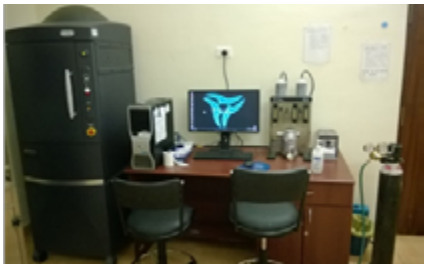
Alcian Blue, Perl's Prussian Blue Iron Stain, Periodic Acid Schiff, Masson Trichrome, Gordon Sweet Reticulin, Acid Fast Bacillus stain for Mycobacterium Tuberculosis, Toluidine Blue, Masson Fontana, Verhoeff's elastic stain, Sirus Red stain, Hematoxylin and Eosin (H&E), Safaranin-O, Elastic van Gieson, Paraffin blocks and tissue mounting, Unstained slide sections for specific immunostaining

Cytology:

Cytospin preparation, Cell block preparation, Cryostate sectioning.

In vivo Small Animal Imaging System

- Technical Officer : Mr. A. Rajesh
- Faculty Support: Dr. Sanjay Kumar

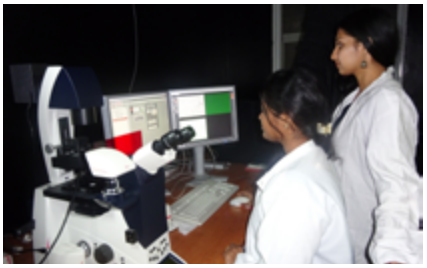


The IVIS Spectrum CT supports low dose microCT for longitudinal imaging. It features 3D optical tomography for fluorescence and bioluminescence and has sensitive detection for real time distribution studies for both fluorochromes and PET tracers.

Imaging Core Facility:

- Technical Officer: Mr. A. Rajesh
- Faculty Support: Dr. Saravanabhavan Thangavel

I. Inverted Fluorescence Microscope

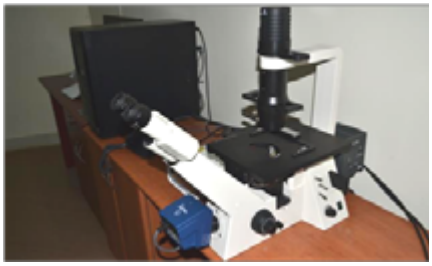


The Leica DMI6000B is an inverted fluorescence microscope comprising of 6 interchangeable filters for detecting various fluorochromes. It has two independent cameras – DFC295 for high resolution bright-field imaging and DFC360 FX for high frame rate fluorescence imaging. It is also equipped with a fluorescence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

II. Light Microscopes

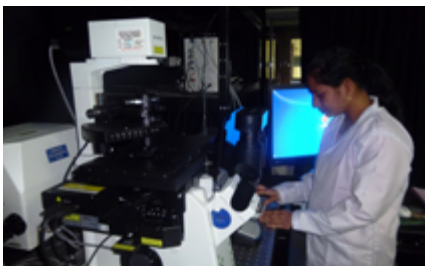
Leica DMIL (upright) and Leica DMI1000 (inverted) are available for users to perform routine light microscopy imaging. Both microscopes are provided with an interchangeable Leica DFC290 camera for high resolution bright-field imaging. The Leica DMI1000 is also installed in the tissue culture facilities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

III. Inverted Fluorescence Microscope



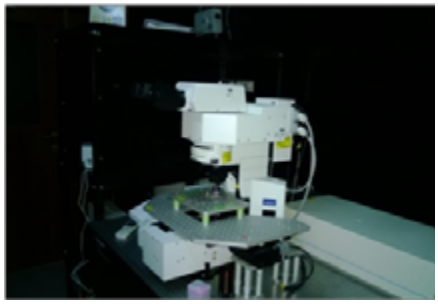
A Carl Ziess Axiovert 40 CFL equipped with 3 filters (DAPI, FITC and TRITC) for routine fluorescence imaging is available, along with a ProgRes C3 camera module for image acquisition.

IV. Laser Scanning Confocal Microscope System



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



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.

VI. 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.

CSCR Laboratory Animal Facility:

- Veterinary Officer: Dr. Vigneshwar R. / Dr. Arunprabhakaran V.
- Technical Staff: PavithraR., Esther Rani J., and Ashok Kumar S.
- Faculty Support: Dr. Sanjay 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 well-being, 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 an experiment on small laboratory animals vide registration no. Reg. 88/PO/RcBi-S/Rc-L/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 six animal rooms. The facility has got double corridor system to facilitate unidirectional movement of personnel. The clean corridor is for the movement of the animal facility staff and animal users only. The dirty corridor is for the movement of unsterile bedding, cages, and trolleys. Animals are maintained within individually ventilated microisolator caging (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 the airborne micro-organisms. The cages are constructed and designed in a specific way to ensure a clean 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 the Small animal to live imaging system, Multiphoton microscope and Small animal irradiator with Co-60 as a 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 eleven different strains of mice - including knockout and SCID strains and a single strain of rat. The majority of rodent strains are bred under strictly inbred conditions. For the very first time in India, we procured NSG mice, the most immunodeficient strain for our institution from Jackson Laboratory, United States. Animals were quarantined in a prescribed manner; on an observation all the animals were found to be healthy enough. Animals are put under breeding now.

	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	Outbred strain	Sentinel animals, Pseu-dopregnancy	Charles River, UK
5	B6.129S4-F8tm1Kaz/J	Mutant Stock; Targeted Mutation	Hemophilia A	Jax Lab, US
6	B6.129P2-F9tm1Dws/J	Congenic; Mutant Strain	Hemophilia B	Jax Lab, US
7	B6;129S4 Pou5f1tm1Jae/J.	Mutant Stock; Targeted Mutation	OCT-GFP model	Jax Lab, US
8	B6.129-Adamts13tm1Dgi/J	Congenic; Mutant Strain	Thrombotic Thrombo¬cytic Purpura	Jax Lab, US
9	B6.CB17-Prkdcscid/SzJ	SCID	Transplantation studies	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
12	NSG	SCID	Immunodeficient	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 a 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 often conducted by PCR. All report of QC is maintained in CSCR-Laboratory Animal Facility.

Protocols established

SOP's for Subcapsular renal cell transplantation and Retro-orbital injection and timed pregnancy were created.

Current Good Manufacturing Practice Facility (cGMP Facility)

- Technical Officer: Mr. Augustine Thambaiah
- Technical Staff: Ms. Aleya Tabasum
- Faculty Support: Dr. Aniket Kumar, Dr. Vrisha Madhuri, Dr. Alok Srivastava

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.

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 forclinical 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 theregulatory 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 was involved in the culture and expansion of autologous MSC for a clinical trial headed by Dr.VrishaMadhuri (Department of Paediatric Orthopaedics, CMC, Vellore), titled "Treatment of large segmental bone defects with custom made tri-phasic hydroxyapatite 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 remaining 5 patients with no report of any adverse reaction.

We are currently involved in the standardization of protocols under laboratory conditions and will be translated to GMP conditions in the future, for the following projects:

1. Gamma delta T cell-based immunotherapy for blood cancers. Centre for Stem Cell Research, CMC Campus and Department of Haematology, CMC.
2. Establishing a protocol for expansion of Natural Killer cells. Centre for Stem Cell Research, CMC Campus and Department of Haematology, CMC.

cGMP facility is also involved with the following research projects:

1. 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

- A successful protocol was established for the isolation and culture of fibroblast from human skin

2. 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.

- An ex vivo organ culture model was established in which the human eye was maintained in sterile condition for a period of 28 days. This model will be used for further analysis by scientist from the department of ophthalmology.

Access

Access to the facility is limited only to 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

FIRST ANNUAL SYMPOSIUM ON CELL AND GENE THERAPY
5 & 6 AUGUST, 2016

The Centre for Stem Cell Research (CSCR, a unit of inStem, Bengaluru) organized the 1st Annual Symposium on Cell and Gene Therapy on 5th & 6th August, 2016. This symposium brought together scientists, physicians and all others interested in and responsible for developing this field in the country. The symposium was supported by the Department of Biotechnology (DBT) and Indian Council of Medical Research (ICMR). The program this year focussed on cell and gene therapy in haematological disorders, ocular disorders, skin & musculoskeletal conditions, and cancer therapy. Over 100 delegates from across the country and 14 speakers from around the world took part in the symposium.

Dr. VijayRaghavan, Secretary, DBT and Dr. Soumya Swaminathan, Secretary, Department of Health Research (DHR) and Director General, ICMR addressed the participants through video link. The first day of the symposium focussed on haematological disorders, musculoskeletal regeneration, and cancer cell and gene therapy. The key note address of the symposium was delivered by Prof. Michele De Luca. He discussed the evolution of epithelial stem cell therapy for skin diseases. The second day of the symposium had various discussions on cell therapy for ocular disorders and genome editing. The symposium was streamed live to the NCBS / inStem campus in Bengaluru so that a wider range of participants could view the presentations in real time and participate actively.

CSCR plans to hold this meeting on an annual basis to help promote this area of research in India through cross-discipline dialogue and collaborations on a diverse range of inter-connected issues relevant to the field.

Number of participants:

- International speakers: 06
- National Speakers: 08
- Delegates: 29
- CMC / CSCR participants: 83
- Total number: 126

Participating institutes:

International:

1. Chiba Cancer Centre, Japan
2. Emory University School of Medicine, Atlanta, USA
3. Karolinska Institute, Sweden
4. San Raffaele Telethon Institute for Gene Therapy, Italy
5. University of Modena and Reggio Emilia, Italy

National:

1. ACTREC, Tata Memorial Cancer Centre, Mumbai
2. All India Instiute of Medical Sciences, New Delhi
3. Central University of Kerala, Nileshwar
4. Indian Council of Medical Research, New Delhi
5. Indian Institute of Technology, Delhi
6. Indian Institute of Technology, Kanpur
7. Institute for Stem Cell Biology and Regenerative Medicine, Bangalore
8. Institute of Life Sciences, Bhubaneswar
9. L. V. Prasad Eye Institute, Hyderabad
10. Narayana Netralaya, Bangalore
11. National Centre for Biological Sciences, Bangalore
12. National Centre for Cell Sciences, Pune
13. Rajendra Prasad Eye Institute, New Delhi
14. Sankara Nethralaya, Chennai
15. Stempeutics Research Pvt. Ltd., India
16. University of Hyderabad, Hyderabad
17. Vellore Institute of Technology, Vellore

The 2nd Annual Symposium on Cell and Gene Therapy is scheduled on 7 & 8 September, 2017.

Education and Training

EDUCATION AND TRAINING

I. PhD Program

CSCR has an active PhD programme and the students can register for PhD under Sree Chitra Thirunal Medical Science and Technology (SCTIMST), Thiruvananthapuram, CSCR or Thiruvalluvar University. One student has registered for PhD in 2016-2017.

Thesis Submitted

- Mr. Salar Abbas
- Ms. Sumitha E
- Mr. Janakiraman R
- Ms. Kannan VM
- Ms. Savitha V
- Ms. Divya M
- Ms. Ezhil Pavai M
- Ms. Sreeja K

PhD Completed

- Mr. Thiyagaraj M
- Mr. Syed Mohammad Musheer Aalam
- Ms. Sumitha PB

II. Other training programs:

Short term student projects (Bi-annual)

S. No	Name	Duration	Qualification	University	Project title	PI /Lab
1.	Ms. Shreya Mishra	Jan 16 - Jun 16	B.Tech Biotech	SRM University	Decoding the epigenetic machinery in stem cell maintenance and differentiation	Dr. Shaji / Lab-2
2.	Mr. Nandha Kumar	Jan 16 - Jun 16	M.Sc - Biotech	Thiruvalluvar University	Immunofluorescence analysis of pluripotency markers in induced pluripotent stem cells	Dr. Shaji / Lab-2
3.	Ms. Ramya G	Jan 16 - Jun 16	M.Sc - Biotech	Thiruvalluvar University	Characterization of induced pluripotent stem cells by real time PCR analysis of pluripotency markers	Dr. Shaji / Lab-2
4.	Ms. Diana D	Jan 16 - Jun 16	M.Sc - Biotech	Thiruvalluvar University	Phenotypic characterization of human wharton's jelly-derivedmesenchymal stem cells and cloning of htert transgene in second generation lentiviral expression plasmid	Dr. Sanjay/ Lab- 3

5.	Ms. Kavitha V	Jan 16 - Jun 16	M.Sc - Biotech	Thiruvalluvar University	Phenotypic characterization of human bone marrow-derivedmesenchymal stem cells and cloning of SV40 large t antigen transgene in second generation lentiviral expression plasmid	Dr. Sanjay/ Lab- 3
6.	Mr. Ashish Kumar	Jan 16 - Jun 16	M.Sc - Biotech	Loyola College	Mutation analysis of EXT1 and EXT2 genes in patients with Hereditary Multiple Exostoses in Indian population	Dr. Vrisha / Lab-4
7.	Ms. Sanjana R	Jan 16 - Jun 16	B.Tech Biotech	VIT University	Differentiation of mesenchymal stem cells to chondrocytes on hydrogels	Dr. Vrisha / Lab-4
8.	Ms. Priya Dharmalingam	Jan 16 - Jun 16	M.Tech - Medical Biotech	Dr.M.G.R Educational and Research Institute University	Lipoic acid derivatives for improving safety and efficacy of cationic liposome mediated transfections	Dr. Srujan / Lab-5
9.	Ms. Anagha Venugopal	Jan 16 - Jun 16	M.Tech -Biomedical Engineering	VIT University	Osteogenic potential of MSCs using 3D nanomaterial	Dr. Murugan/ Lab- 8
10.	Ms. Priyatharshini M	Jan 16 - Jun 16	M.Tech (Integrated) Biotech	Bharathidasan University	Chondrogenic potential of MSCs using injectable gels	Dr. Murugan/ Lab- 8
11.	Mr. Brijesh Lohchania	Jul 16 -Dec 16	M.Tech Biotech	Sharda University	Development of novel liposomal formulations for generating iPSCs	Dr. Srujan / Lab-5
12.	Mr. Vemuru Dinesh	Jul 16 -Dec 16	M.Sc - Biotech	VIT University	Development of multifunctional nano carrier system carrying STAT3 & RNA for treating psoriasis	Dr. Srujan / Lab-5
13.	Ms. Anantha Mary Jeba T	Jul 16 -Dec 16	MSc -Integrated Biotech	VIT University	Development of Integration- Deficient Lenti Virus (IDLVs) for targeted genome editing	Dr. Saravana / Lab-5
14.	Mr. Akshay S. Bhatt	Jul 16 -Dec 16	M.Tech Biotech	VIT University	Expansion & culture of stem cells in three dimension	Dr. Murugan/ Lab- 8

15.	Ms. Aditya Karunanithi KN	Jul 16 -Dec 16	B.Tech Biotech	Periyar Maniammai University	Decellularized scaffolds for stem cells-barl tissue engineering	Dr. Murugan/ Lab- 8
16.	Mr. Manigandan V	Jul 16 -Dec 16	M.Sc - Biotech	VIT University	Synthesis and characterization of gelatin methacrylate for bioprinting	Dr. Murugan/ Lab- 8
17.	Ms. Krithicaa Narayanaa	Jan 17 - Jun 17	B.Tech - Biotech	SRM University	RNAi screening in stem cell differentiation	Dr. Shaji / Lab-2
18.	Ms. Sangeetha S	Jan 17 - Jun 17	M.Sc - Biotech	Thiruvalluvar University	Hypoxia cultured human wharton's jelly derived mscs exibit modulation in oxidative stress signalling	Dr. Sanjay / Lab-3
19.	Ms. Lakshmi S	Jan 17 - Jun17	M.Sc - Biotech	Thiruvalluvar University	Hypoxia cultured human wharton's jelly derived mscs exibit differential expression of immunomodulatory genes	Dr. Sanjay / Lab-3
20.	Mr. Mohammed Abrar Basha	Jan 17 - Jun 17	B.Tech - Genetic Eng.	Bharath university	Mutation analysis of WISP3 gene in patients with clinical symptoms of progressive pseudorheumatoid dysplasia	Dr. Vrisha / Lab-4
21.	Ms. Anushna Sen	Jan 17 - Jun 17	B.Tech - Biotech	VIT University	Stem cell based tissue engineering	Dr. Vrisha / Lab-4
22.	Mr. Brijesh Lohchania	Jan 17 - Jun 17	M.Tech - Biotech	Sharda University	Diosgenin, Tomatidine & Solasodine for improving safety and efficacy of cationic liposomes mediated transfections	Dr. Srujan / Lab-5
23.	Mr. Akshay S. Bhatt	Jan 17 - Jun17	M.Tech Biotech	VIT University	Development Chitosan Based Injectable Magnetic Thermogel for Biomedical Applications	Dr. Murugan/ Lab- 8
24.	Mr. Manigandan V	Jan 17 - Jun 17	M.Sc - Biotech	VIT University	Cell-laden hydrogels for tissue engineering	Dr. Murugan/ Lab- 8
25.	Mr. Bhargav D Sanketi	Jan 17 - Jun 17	BE - Biotech	R V College of Engineering, Bangalore	Therapeutic genome editing for inherited hematological disorders	Dr. Mohan / Lab-9

26.	Ms. Sharada Gopal	Jan 17 - Jun 17	BE - Biotech	R V College of Engineering, Bangalore	Therapeutic genome editing for the treatment of Sickle cell disease	Dr. Mohan / Lab-9
27.	Ms. Shalini Ramasamy	Jan 17 - Jul 17	M.Sc - Biotech	VIT University	Genome editing in 293.T cells, using CRISPR/cas 9 nucleases and truncated guide RNAs	Dr. Saravana / Lab-5
28.	Ms. Swathy Radhakrishnan	Jan 17 - Jun 17	M.Sc - Biotech	Cochin University	Increasing the efficiency of genome editing	Dr. Saravana / Lab-5
29.	Ms. Sumitha B	Jul 17 - Dec 17	M.Sc Biotech (Integ.)	VIT University	RNAi Screening	Dr. Shaji / Lab-2
30.	Ms. Antra Pant	Jul 17 - Dec 17	M. Pharm.	National Institute of Pharmaceutical Education and Research, Guwahati	Cationic lipids derived from vegetable oils as delivery vehicle for CRISPR/Cas9 system.	Dr. Srujan / Lab-7
31.	Ms. Swapna L	Jul 17 - Dec 17	M. Pharm.	National Institute of Pharmaceutical Education and Research, Guwahati	Maleimide lipids for delivering CRISPR/ Cas9 system.	Dr. Srujan / Lab-7
32.	Ms. Dhakshanya Predheepan	Jul 17 - Dec 17	B.Tech - Biotech	Anna University	Development of injectable biocompatible polymer gel for stem cell transplantation	Dr. Murugan/ Lab- 8
33.	Mr. Subhajit Hazra	Jul 17 - Dec 17	B.Pharm.	Maulana Abul Kalam Azad University	Development of bioink for printing of stem cells and analyze their different potential	Dr. Murugan/ Lab- 8

PERSONNEL AT CSCR

Scientific / Technical Staff

Dr. Alok Srivastava	Head / Adjunct Scientist
Dr. Mohankumar Murugesan	Assistant Investigator
Dr. Saravanabhavan Thangavel	Assistant Investigator
Dr. Murugan Ramalingam	Associate Professor
Dr. Srujan Kumar Marepally	Scientist
Dr. Sanjay Kumar	Scientist
Dr. Vrisha Madhuri	Adjunct Scientist
Dr. Rekha Samuel	Adjunct Scientist *(up to July 2017)*
Dr. R. V. Shaji	Adjunct Scientist
Dr. Aniket Kumar	Adjunct Scientist
Dr. Aby Abraham	Adjunct Scientist
Dr. Asha Mary Abraham	Adjunct Scientist
Dr. Boopalan Ramasamy	Adjunct Scientist
Dr. Christunesa Christudass	Adjunct Scientist
Dr. Dolly Daniel	Adjunct Scientist
Dr. Inian Samarasam	Adjunct Scientist
Dr. Jeyanth Rose	Adjunct Scientist
Dr. Poonkuzhali Balasubramanian	Adjunct Scientist
Dr. Ravikar Ralph	Adjunct Scientist
Dr. Md. Manzoor Akheel	Scientist, Research Development Office
Dr. Arunprabhakaran V.	Veterinary Officer
Dr. Vigneshwar R.	Veterinary Officer
Mr. Augustine Thambaiah	Technical Officer
Mr. Rajesh A.	Technical Officer
Dr. Deepak Jayakumar	Scientific Program Manager (NAHD Program)
Dr. Nishanth Gabriel	Post Doctoral Fellow *(up to February 2017)*
Mr. Syed Mohammed Musheer Aalam	Senior Research Fellow *(up to April 2017)*
Mr. Kannan Thoppil	Senior Research Fellow
Ms. Sumitha P. B.	Senior Research Fellow *(up to May 2017)*

» Mr. Karthikeyan R.	Senior Research Fellow
» Ms. Sowmya R.	Senior Research Fellow
» Mr. Thiyagaraj M.	Senior Research Fellow *(up to July 2017)*
» Mr. Balasubramanian S.	Senior Research Fellow
» Ms. Kasthuri	Senior Research Fellow
» Mr. Franklin Jebaraj Herbert	Senior Research Fellow
» Ms. Smitha I.	Junior Research Fellow
» Mr. Abhirup Bagchi	Junior Research Fellow
» Ms. Krittika Nandi	Junior Research Fellow
» Ms. Sonam Rani	Junior Research Fellow
» Mr. Thulaj Meharwade	Junior Research Fellow
» Mr. Rishav Seal	Junior Research Fellow
» Ms. Aneesha Nath	Junior Research Fellow
» Mr. David Livingstone I.	Junior Research Fellow *(up to February 2017)*
» Ms. Deepti Rana	Junior Research Fellow
» Ms. Abisha Crystal	Junior Research Fellow
» Ms. Rachel Anand Nethala	Junior Research Fellow
» Ms. Nimmi Vincy	Junior Research Fellow
» Ms. Lakshmi Priya	Junior Research Fellow
» Mr. Bandlamudi Bhanu Prasad	Junior Research Fellow
» Mr. Harish Kumar	Junior Research Fellow
» Mr. Delvin K. Pauly	Junior Research Fellow *(up to July 2017)*
» Ms. Divyashree	Junior Research Fellow *(up to June 2017)*
» Ms. Aleya Tabasum	Graduate Technician
» Ms. Dhavapriya B.	Graduate Technician
» Ms. Kalaivani G.	Graduate Technician
» Ms. Saranya R.	Graduate Technician *(up to January 2017)*
» Ms. Saranya J.	Graduate Technician
» Ms. Pavithra R.	Graduate Technician
» Ms. Chitra P.	Graduate Technician
» Ms. Esther Rani J.	Technician
» Mr. Ashok Kumar	Technician
» Mr. Aarwin Joshua	Optometry Technician
» Ms. Saranya J.	Graduate Technician Trainee
» Ms. Jayashree	Graduate Technician Trainee
» Mr. Abdul Muthalli	Graduate Technician Trainee
» Ms. Praveena	Graduate Technician Trainee

Office Staff

» Mrs. Anupama Nambiar	Assistant Manager
» Mrs. Shirley Anandanathan	Secretary
» Mrs. Selvi P.	Clerk Typist
» Mrs. Geetha R.	Accountant
» Mr. Muthukrishnan J.	Multi-tasking personnel
» Mr. Tamil Vanan J.	Asst. Librarian

Attendant / Housekeeping Staff

» Mr. Ramraj	Attendant
» Mr. Nithyanand	Attendant
» Mr. Arun Kumar	Attendant
» Mr. Shankar	Attendant
» Mr. Augustin Vasanthakumar	Attendant
» Mr. Vikas	Attendant
» Mr. Vijay	Attendant
» Mr. Sakthivel	Housekeeping Attendant
» Mrs. Renuka Devi	Housekeeping Attendant

GOVERNANCE OF CSCR

inStem Governing Council

Prof. K VijayRaghavan, Secretary to the Government of India, DBT	Chairman
Prof. Satyajit Mayor, Director, NCBS & inStem (Ex-officio)	Member
Prof. Jyotsna Dhawan, Senior Professor CCMB	Member
Prof. S Ramaswamy, Dean, inStem (Ex-officio)	Member
Prof. Upinder S Bhalla, Dean, NCBS (Ex-officio)	Member
Prof. Apurva Sarin, Dean, inStem (Ex-officio)	Member
Mr. Chandra Prakash Goyal, Joint Secretary (Admin), DBT (Ex-officio)	Member
Smt. Sumita Mukherjee, JS & FA, DBT(Ex-officio)	Member
Prof. Sunil T Chandy, Director, CMC Vellore (Ex-officio)	Member
Prof. Satyajith Rath, Scientist NII	Member
Prof. K Muniyappa, Professor & Chairman, Department of Biochemistry, IISc	Member
Prof. Chandrima Shaha, Director, NII	Member
Prof. Chittaranjan Yajnik, Director, Diabetes Unit, KEM Hospital and Research Centre	Member
Dr. Alka Sharma, Adviser/Scientist 'G', Medical Biotechnology DBT (Ex-officio)	Member
Prof. Alok Srivastava, Head, CSCR & Professor of Medicine, CMC, Vellore (Ex-officio)	Member
Mr. Krishnama Raju, Head-Administration, inStem	Non Member Secretary

CSCR Committee

Director, CMC (Ex-officio)	Chairperson
Director, inStem (Ex-officio)	Member
Dean, inStem (Ex-officio)	Member
Principal, CMC (Ex-officio)	Member
Head, CSCR	Member Secretary

CSCR Core Committee

Dr. Nihal Thomas, Department of Endocrinology	Chairperson
Dr. C E Eapen, Medical Superintendent (Ex-Officio)	Member
Dr. Molly Jacob, Department of Biochemistry	Member
Dr. Asha Mary Abraham, Department of Virology	Member
Dr. R V Shaji, Adjunct Scientist, CSCR	Member
Dr. Alok Srivastava, Head, CSCR	Member Secretary

CSCR Scientist Review Committee

Dr. C E Eapen, Medical Superintendent	Chairperson
Dr. B S Ramakrishna, SRM University	Member (External)
Dr. Molly Jacob, Department of Biochemistry	Member
Dr. Nihal Thomas, Department of Endocrinology	Member
Dr. Asha Mary Abraham, Department of Virology	Member
Dr. Alok Srivastava, Head, CSCR	Member Secretary

SAC 2016

1st Annual Cell and Gene Therapy Symposium - 2016

Centre for Stem Cell Research
(a unit of inStem, Bengaluru)
Christian Medical College Campus
Bagayam, Vellore - 632002
Tamil Nadu, India
Phone: 91-416-2285101, 2285107, 2285120
Fax: +91- 416-2285103
Email: cscr@cmcvellore.ac.in | Website: www.cscr.in