CSCR

CENTRE FOR STEM CELL RESEARCH

(a unit of inStem, Bengaluru)

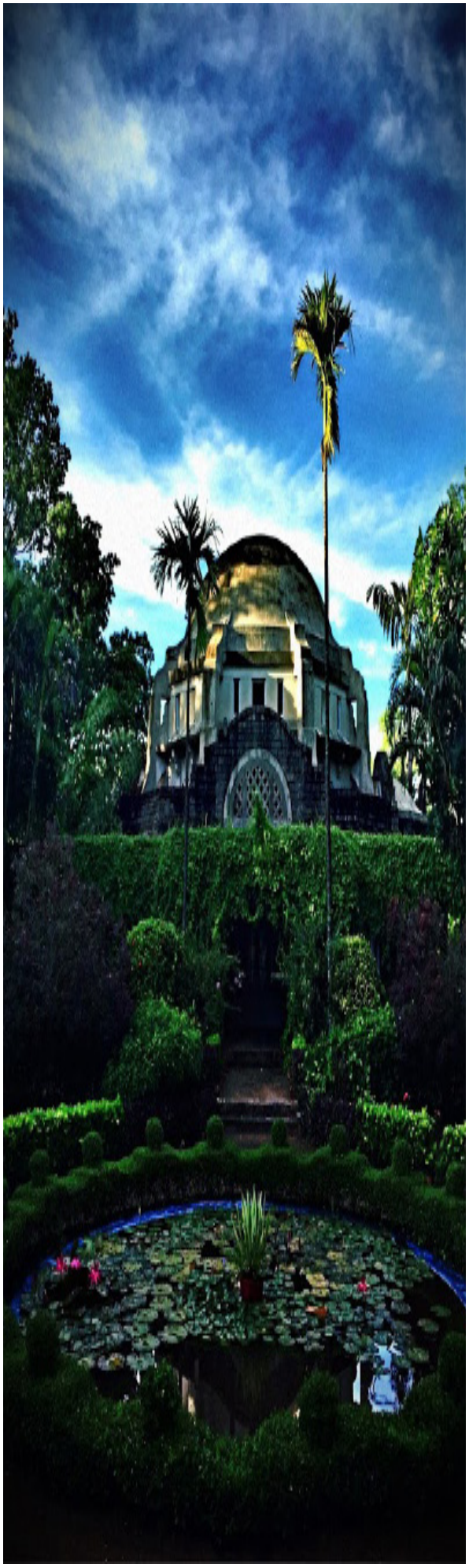
Christian Medical College Campus, Bagayam, Vellore

ANNUAL REPORT

2018-19

inStem
CSCR
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 the Institute for Stem Cell Biology and Regenerative Medicine (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 egenerative 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. 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 small and large animals studies have been completed with differentiated MSCs on indigenous scaffolds with successful outcome. Osteoarthritis prevention is another area that is being explored. There is a new focus on using biomolecules on scaffold for regeneration with in vitro studies completed and ongoing large animal studies. The continued follow up for pilot human physeal regeneration with culture expanded autologous chondrocytes has shown success at 5 years and a phase 1 clinical trial has been initiated. The group has also achieved success in physeal regeneration using hydrogel scaffolds in large animal model. A first of its kind pilot study on human bone defect regeneration study has been completed and further work is ongoing in the area of bone regeneration using biomolecules. A new phase I/II clinical trial is initiated in collaboration with Karolinska Institutet for treatment of osteogenesis imperfecta using fetal liver mesenchymal stem cells. Under international collaboration the work on non-invasive manipulation of physeal cartilage and muscle derived stem stem cell for sphincter repair continues.

2. 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 preclinical 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.

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.

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.

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 immune cell therapy, particularly CAR T cells, Sunil Martin's laboratory is working to develop this technology for applications in human cancers along with Aby Abraham as a clinical partner who is also working towards developing gamma delta T-cell based therapies.

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

Scientific Research Profile

VRISHA MADHURI, MS, MCh
Professor, Department of Pediatric Orthopedics, CMC, Vellore
Adjunct Scientist, CSCR

LABORATORY HIGHLIGHTS

Our lab focuses on regenerative strategies using cell-based therapy for musculoskeletal disorders.

1. Boost to Brittle Bones (BOOST2B): This phase I/II trial aims to evaluate the safety and efficacy of intravenous and intraosseous infusion of allogeneic expanded fetal mesenchymal stem cells for the treatment of severe Osteogenesis Imperfecta. We are in the process of recruiting patients for the trial (n=15).

2. Physeal regeneration: This phase I trial aims to evaluate the safety of autologous iliac crest physeal chondrocytes to treat physeal bars in children. We are in the process of recruiting patients for the trial (n=15).

3. Genetic Heterogeneity in patients with OI: Genetic profiling to screen 150 children with OI by NGS for all known genes (targeted sequencing will be performed using Illumina).

4. Musculoskeletal stem cell targeting (MUSTER):
   1. Muscle-derived stem cells in the treatment of anal sphincter injury in a rat model – We have isolated quiescent satellite cells using a single cell surface marker and established rat anal sphincter injury model.
   2. Treatment of osteochondral and segmental bone defects using functionalised scaffold with or without MSC- We have standardized the animal model by creating critical size defects in a goat model (n=6 each).

5. Effect of shock wave treatment on growth plate cartilage: A novel model of growth retardation in rat metatarsal bone was established using a Gli1 inhibitor. Shockwave treatment may counteract the effect of inhibitor-induced growth retardation.

6. Bone regeneration: We have completed 2-4 years follow up in all 10 children treated with hydroxyapatite loaded with MSC. The response is satisfactory in an infected nonunion while the results cannot be extrapolated to bone diseases with underlying genetic conditions.

7. Differentiation of mesenchymal stem cells into chondrocytes by sustained delivery of miRNAs using chitosan hydrogel: A combination of miRNAs was effective in chondrogenesis of MSCs without any growth factors. A new (liposome-based) method of transfecting plasmid, mRNA and siRNA/miRNA in a 3D condition has been identified.

Ongoing studies & Funding

Project 1: Transplantation of autologous iliac crest physeal chondrocytes cultured in monolayer to treat Physeal bars in children
Funding agency: DHR
Budget: Rs. 74.93 lakhs

Project 2: BOOST2B (Indo-Swedish)
Funding agency: DBT
Budget: Rs. 480.024 lakhs

Project 3: MUSTER (Indo-Danish)
Funding agency: DBT
Budget: 99.77 lakhs

Project 4: Molecular genetic analysis of Osteogenesis imperfecta in Indian Children
Funding agency: ICMR
Budget: Rs. 69 lakhs

Project 5: In vivo effect of shockwave on rabbit growth plate
Funding agency: IRB/CSCR
Budget: Rs. 10.00 lakhs

Completed project:

Title: Differentiation of MSCs into chondrocytes by sustained delivery of miRNAs using chitosan hydrogel.
Funding agency: DST
Budget: Rs. 74 lakhs

Honors and awards:

1. Sowmya Ramesh - Travel Grant - 57th Annual European Society for Paediatric Endocrinology Meeting, Greece.
2. Sowmya Ramesh received the Svenska frimurare (Frimurare Barnhus Foundation) scholarship from the Queen Silvia of Sweden, October 10, 2018.
3. Sowmya Ramesh- Elected as Treasurer for the Tissue Engineering and Regenerative Medicine Society-Asia Pacific region (TERMIS-AP) society - SYIS.
4. Karthikeyan Rajagopal – 3rd place oral - A novel protocol to produce articular cartilage in a sustained delivery system using RNAi technology - Annual Research Day (Ph.D. Specialty).
5. Sowmya Ramesh - 1st place oral - Efficacy of a novel cell-laden dressing in the topical treatment of partial-thickness burns in a rat model- Annual Research Day (PG Surgical Specialty).
6. Ashis Kumar - Selected on merit basis for the Whole Genome Sequencing workshop organized by the Accelerator program for Discovery in Brain disorders using Stem cells-Institute of Bioinformatics and Applied Biotechnology (IBAB), Bengaluru, December 2018.
7. Dr. Vrisha Madhuri- Appointed Member 4th Programme Advisory Committee IMPRINT2 (Impacting Research Innovation and Technology Version 2), SERB, DST
8. Dr. Vrisha Madhuri - Elected National Delegate for APPOS from April 2019.
9. Dr. Vrisha Madhuri - Appointed as a member of the Reconstituted Task force of Bioengineering DBT.
10. Dr. Vrisha Madhuri - Appointed as a member of DBT Biomedical engineering and Biodesign Technical Expert Committee October 2018.

Publication:

Vrisha Madhuri, Sowmya Ramesh, Harikrishna Varma, Suresh Babu Sivadasan, Bibhudatta Sahoo, Annie John, Francis Fernandez, Karthikeyan Rajagopal, Vikram Mathews, Balakumar B, Vivek Dutt Dinesh, Sanjay Kashinath Chilbule, Sridhar Gibikote, Alok Srivastava. First report of a tissue-engineered graft for proximal humerus gap non-union following chronic pyogenic osteomyelitis in a child. Journal of Bone and Joint Surgery Case Connector – Accepted for publication

Collaborations

International Collaborations:

1. Henrik Daa Schrøder, University of Southern Denmark, Denmark
2. Jorgen Kjems, Department of Molecular Biology, University of Aarhus, Denmark
3. Moustafa Kaseem, Endocrinology, University of Southern Denmark, Denmark
4. Lars Savendahl, Pediatric Endocrinology, Karolinska University Hospital, Sweden
5. Cecilia Gotherstrom, Division of Obstetrics and Gynecology, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden.

National collaborations (Both Inter and intra institutional collaborations):

1. Jyotsna Dhawan, Centre for Cellular & Molecular Biology, Hyderabad
2. Prabha D. Nair, Tissue Engineering and Regeneration Technologies Division, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Trivandrum
3. Harikrishna Varma, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Trivandrum.
4. Nihal Thomas, Department of Endocrinology, CMC, Vellore
5. Sukriya Nayak, Department of General Surgery, Unit -4, CMC, Vellore
6. Vikram Mathews, Department of Haematology, CMC, Vellore
7. Madhavi. K., Department of Radiology, CMC, Vellore
8. Shyam, Department of Radiology, CMC, Vellore
9. Thomas Paul, Department of Endocrinology, CMC, Vellore
10. Alok Srivastava, CSCR / Department of Haematology, CMC, Vellore
11. Srujan Marepally, CSCR, Vellore
12. Dolly Daniel, CSCR / Department of Transfusion medicine & Immunohematology, CMC, Vellore
13. Antonisamy, Department of Biostatistics, CMC, Vellore

ELIZABETH VINOD, MD
Assistant Professor, Department of Physiology, CMC, Vellore
Adjunct Scientist, CSCR

PROJECT-1

Project title: Chondroprogenitor cells in Platelet Rich Plasma for treatment of osteoarthritis and osteochondral defects in rabbit knee model.

Funding source: CSCR Core Grant

Duration: March 2018 - June 2020

Brief description of the project:

Articular cartilage is an avascular tissue with low potential for self-repair. Cell based therapeutics aim to produce tissue that closely mimic the mechanical and biochemical properties of native cartilage. Recently, the use of articular cartilage derived CP (classified MSCs) have gained popularity in its potential role for cartilage repair. Key features of these multipotent progenitors include differential adhesion to fibronectin, high replicative potential with maintained potency, ability to form large colonies from a small seeding density and importantly primed chondrogenic potential by nature. Their positive immunomodulatory properties make them amenable to allograft strategy. The use of PRP has achieved recognition in its applications towards the treatment of local cartilage defects and osteoarthritis improving the quality of cartilage repair. The rationale for its application is largely dependent on its principal components of growth factors such as TGFβ known to stimulate cellular anabolism, possess anti-inflammatory properties and interact with fibrinogen contributing to its scaffolding effect.

In this study, we aim to isolate chondroprogenitor cells from rabbit knee articular cartilage, culture and characterize them, to create an allogeneic bank. Monosodium Iodoacetate induced early grade osteoarthritis will be created in bilateral hind limbs, following which labelled-chondroprogenitors with platelet rich plasma will be injected intra-articularly. Similarly, osteochondral defects will be created in the trochlear groove of both knees and labelled CPs resuspended in appropriate volume of PRP will be delivered into the defect. At the end of 6 and 12 weeks, healing in both knees will be assessed by synovial fluid analysis, immunohistochemistry studies and histologically using OARSI/ Wakitani scoring

We hypothesize that the regenerative potential of chondroprogenitor cells with its restrictive differential potential, combined with the ability of PRP to modulate proliferation and provide essential growth factors crucial for its survival will work in synergy towards achieving functional cartilage.

Work done: Cell culture and animal interventions have been completed. Knee joints have been harvested for processing. Blinded histological grading and data analysis are in progress.

PROJECT-2

Project title: Comparison of chondrogenic potential between cell sorted chondroprogenitors, fibronectin assay derived chondroprogenitors and chondrocytes derived from human articular cartilage.

Funding source: CMC Fluid Research Grant

Duration: December 2019 - 2021

Brief description of the project:

Cell based therapy optimization is constantly underway since regeneration of genuine hyaline cartilage is under par. Extensive work on chondrocytes has afforded valuable information to their use in cartilage repair, although questions pertaining to their behavior in culture remain unanswered. Although single source derivation of chondrocytes and chondroprogenitors is advantageous, lack of a characteristic differentiating marker between chondrocytes and chondroprogenitors obscures clear identification of either cell type which is essential to create a biological profile

and is also required to assess cell type superiority for cartilage repair. Our previous study was the first attempt, where characterization was performed on the two cell populations derived from the same human articular cartilage samples using flow cytometry, gene expression studies were done using RT-PCR, growth kinetics and tri-lineage differentiation were also studied. Our results suggest that sorting chondroprogenitors based on a combination of surface markers instead of isolation using fibronectin adhesion assay would yield a population of cells primarily composed of chondroprogenitors.

In the present study, based on the obtained knowledge we aim to isolate and culture chondrocytes and chondroprogenitors (fibronectin adhesion assay) cells from normal articular cartilage of human knee joints and characterize them by FACS. P1 chondrocytes will be sorted using a combination of CD markers to obtain chondroprogenitors. The chondrogenic potential of cell sorted chondroprogenitors, fibronectin assay derived chondroprogenitors and chondrocytes will be compared using RT PCR studies for Chondrogenic and hypertrophic markers, FACS for markers of enhanced chondrogenesis, positive and negative MSC markers. Trilineage differentiation and staining will also be done.

Results obtained will provide us comparative information about the cell population with higher chondrogenic potential, thus translatable results in terms of enhanced chondrogenesis and reduced hypertrophy; both indispensable for the field of cartilage regeneration

Publications:

1. Vinod E, Vinod Francis D, Manickam Amirtham S, Sathishkumar S, Boopalan PRJVC. Allogeneic platelet rich plasma serves as a scaffold for articular cartilage derived chondroprogenitors. Tissue Cell. 2019 Feb;56:107–13.

2. Elizabeth Vinod, Deepak Vinod Francis, Tripti Jacob, Soosai Manickam Amirtham, Solomon Sathishkumar, Praghalathan Kanthakumar, and Vinay Timothy Oommen. Autologous Platelet Rich Fibrin as a Scaffold for Chondrocyte Culture and Transplantation: An in Vitro Bovine Study. Journal of Clinical Orthopaedics and Trauma. 2019.

3. Elizabeth Vinod, Upasana Kachroo, Ozlem Ozbey, Solomon Sathishkumar, and P. R. J. V. C. Boopalan. Comparison of Human Articular Chondrocyte and Chondroprogenitor Cocultures and Monocultures: To Assess Chondrogenic Potential and Markers of Hypertrophy. Tissue Cell. 2019; 57: 42–48.

Support from CSCR: Funding support, lab space and core lab facilities

External Collaborations:

Ozlem Ozbey, Akdeniz University, Antalya, Turkey
Sabareeswaran Arumugam, , Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram
Anjali Goyal, Smt NHL Municipal Medical College, Ahmedabad

Internal Collaborations:

Soosai Amirtham Manickam, Department of Physiology, CMC, Vellore
Upasana Kachroo, Department of Physiology, CMC, Vellore
P.R.J.V.C Boopalan, Department of Orthopaedics, CMC, Vellore
Solomon Sathishkumar, Department of Physiology, CMC, Vellore

ALOK SRIVASTAVA, MD, FRACP, FRCPA, FRCP

Professor, Department of Haematology, CMC, Vellore

Adjunct Scientist / Head, CSCR

Scientific Areas of Research

There major focus during 2018-19 has been the gene therapy program for haemophilia and the major haemoglobin disorders. The field outreach program for the control of thalassemia and sickle cell disease in Odisha has also been launched. I also continue to support the on-going workrelated to the development of assays for AAV antibodies as well as the program on banking of iPSCs from HLA haplotype identical individuals. The details are outlined below:

A. The gene therapy program

1. CLINICAL TRIAL FOR GENE THERAPY OF HEMOPHILIA B

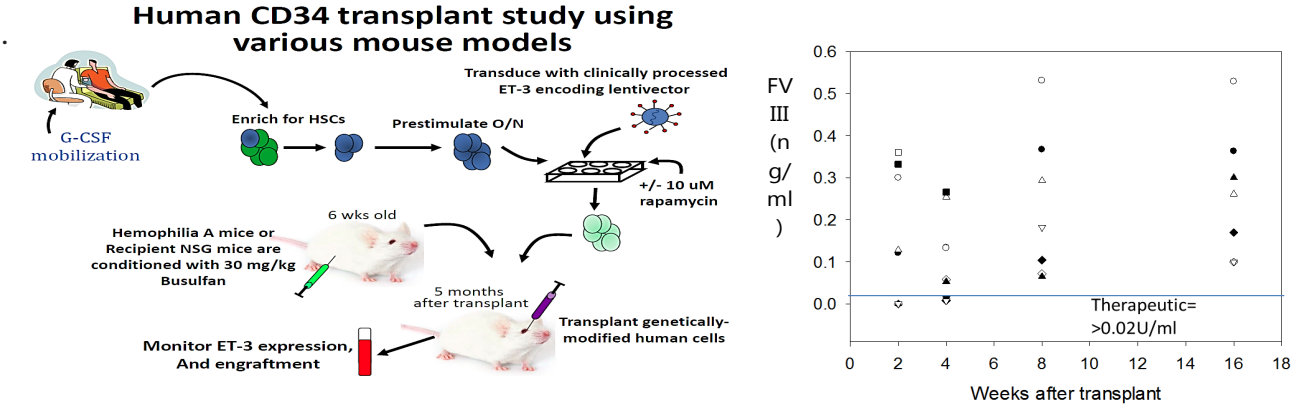
After generation of a suitably efficient transgene and packaging it in the AAV3 vector which was tested to two mouse models for in vivo efficacy, as also reported last year, there was a setback in the plans for this clinical trial as the Powel Gene Therapy Center (PGTC) at the University of Florida at Gainesville, Florida, USA was unable to produce the vector at the right titers to allow for a cost effective production of enough quantity of the vector for the IND enabling proposed non-human primate studies and the subsequent clinical trial.

Since then we have explored the option of several other sites (academic and contract manufacturing organizations) for getting this AAV production done. Two challenges have not made this possible - at the academic sites, which have the capability for producing this vector, there is waiting list of products lined up for gene therapy clinical trials (in fact, this is the biggest single challenge in the development of gene therapy products at present in the world - lack of capacity within existing GMP facilities for production of the required products) while the cost of production at the contract manufacturing organizations is way beyond the budget available to us in this clinical trials, in the range of US$ 1 million for the requirements of this trial.

We have a in principle agreement from the GMP facility at the Cincinnati Medical Center which also has considerable experience in producing vectors for gene therapy trials, of making this vector for us by the end of 2019. If that happens, we will be able to proceed with the clinical trial plans after testing it in the NHP models at the Emory University. This is certainly a disappointment but we are hopeful that we will be able to proceed with the clinical trial later this year / early next year in 2020 with this very promising transgene and vector.

2. LENTI VIRAL VECTOR GENE THERAPY FOR HEMOPHILIA A

Apart from this, a novel lentiviral vector-based gene therapy for haemophilia A has also been developed over the last 2 years in collaboration with the scientists at the Emory University, Atlanta, USA (AS). (Hum Gene Ther. 2018, 29:1183-1201 - see figure below) A proposal for a phase 1 clinical trial has been reviewed by the CDSCO and has received technical clearance. Certain regulatory issues are to be resolved. These are being addressed and the final requiremnst will be submitted within the enxt 4-6 weeks. In the meantime, the US FDA has already approved this product for a phase 1 IND in USA in June,2019. (Annexure 2) An agreement has also been reached with our collaborators in USA for full freedom to operate in India with this product, if found successful in this trial. This product, therefore, which has been developed with joint collaborative research and is accessible to us for further licensing, manufacture and use in India, should be considered to be like an 'Indian' product for all regulatory purposes.



This approach involving transplanted of gene corrected autologous haematopoietic stem cells has become even more critical to explore for haemophilia in India as our preliminary data on the AAV serology distribution in the country is showing that >50% of the patients wimay be ineligible for current AAV-based gene therapy due to pre-existing anti-AAV antibodies. Though this approach is well established for several diseases including the major haemoglobin disorders, this is the first such proposal for haemophilia in the world. It is also the first proposal for a clinical trial of gene therapy in India.

Haplobanking

This novel and unique project aimed at creating a bank of induced pluripotent stem cells (iPSCs) from normal individuals with homozygous HLA haplotypes. It is part of an international consortium working in this area. http://www.gait.global/ These haplo identical iPSCs could then serve as a source for cell therapy for many individuals with different organ dysfunctions. Please see report of Dolly Daniel and R.V. Shaji for details of this program in collaboration with DATRI stem cell donor registry.

Other areas of work related to stem cell transplantation / gene therapy

Apart from the work described above, I also continue to be involved with clinical hematopoietic stem cell transplantation (HSCT) and research related with it particularly in the area of HSCT for thalassemia major. An Indian Society for Blood and Marrow Transplantation has been established. The Indian Stem Cell Transplant Registry for hematopoietic stem cell transplantation done in India will now be part of this scientific society's activities. I also continue the vice chair of the Asia-Pacific Blood and Marrow Transplant Group.

The Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis has established a task force for gene therapy for haemophilia. I chair this taskforce which includes members from the European Medicinal Authority and also engages with the USA FDA. The aim of this group is to provide guidance on development of products for gene therapy.

Community outreach – Creating a model for control of thalassemia and sickle cell disease

This program is led along with Profs. Kuryan George and Shantidani Minz along with several other senior colleagues from the departments of Community Health, Haematology, Transfusion Medicine and Immunohaematology, and Obstetrics and Gynecology at CMC, Vellore. I am helping getting it started by coordinating the planning of this program – a unique event in terms of scale and complexity in this field in the world.

In the last year, several aspects of this program have progressed. Six districts have been identified to implement the first phase of this program. Towards increasing capacity and capability for treatment of major haemoglobin disorders in Odisha, workshops are being arranged at different levels (State / Regional / District levels) for doctors / other healthcare workers of Odisha to train them on different aspects of management and prevention of sickle cell disease and thalassemia in the State. The field program is expected to be launched within the next 2-3 months. Training in genetic diagnosis is also being initiated as a part of this project. Prenatal diagnosis for thalassemia will be initiated in the State by September, 2019. The Odisha government and NHM have also been advised to identify centres where stem cell transplantation (SCT) can be established. Once this is done, training can also be provided at CMC, Vellore to build the expertise for SCT in Odisha.

Two new technologies are being developed within this field program - a proteomic (MALDITOF) based method for high throughput low cost analysis of variant haemoglobins and a low cost novel technology genetic diagnosis of globin gene defects. Both these are with overseas industry collaboration.

Selected publications

1. Vrisha Madhuri, Sowmya Ramesh, Harikrishna Varma, Suresh Babu Sivadasan, Bibhudatta Sahoo, Annie John, Francis Fernandez, Karthikeyan Rajagopal, Vikram Mathews, Balakumar B, Vivek Dutt Dinesh, Sanjay Kashinath Chilbule, Sridhar Gibikote, Alok Srivastava. First report of a tissue-engineered graft for proximal humerus gap non-union following chronic pyogenic osteomyelitis in a child. Journal of Bone and Joint Surgery Case Connector – Accepted for publication, July 1, 2019

2. Doering CB, Denning G, Shields JE, Fine EJ, Parker ET, Srivastava A, Lollar P, Spencer HT. Preclinical Development of a Hematopoietic Stem and Progenitor Cell Bioengineered Factor VIII Lentiviral Vector Gene Therapy for Hemophilia A. Hum Gene Ther. 2018 Oct;29(10):1183-1201

3. Sullivan S, Stacey GN, Akazawa C, Aoyama N, Baptista R, Bedford P, Bennaceur Griscelli A, Chandra A, Elwood N, Girard M, Kawamata S, Hanatani T, Latsis T, Lin S, Ludwig TE, Malygina T, Mack A, Mountford JC, Noggle S, Pereira LV, Price J, Sheldon M, Srivastava A, Stachelscheid H, Velayudhan SR, Ward NJ, Turner ML, Barry J, Song J. Quality control guidelines for clinical-grade human induced pluripotent stem cell lines. Regen Med. 2018 Oct;13(7):859-866. doi: 10.2217/rme-2018-0095. Epub 2018 Sep 12. PubMed PMID: 30205750

Internal Collaborations::

1. Aby Abraham, CSCR / Department of Haematology, CMC, Vellore
2. Fouzia N. A., Department of Haematology, CMC, Vellore
3. Eunice Sindhuvi, Department of Haematology, CMC, Vellore
4. Anu Korula, Department of Haematology, CMC, Vellore
5. Biju George, Department of Haematology, CMC, Vellore
6. Vikram Mathews, Department of Haematology, CMC, Vellore
7. Sukesh Nair, Department of Immunohematology, CMC, Vellore
8. Asha Mary Abraham, CSCR / Department of Clinical Virology, CMC, Vellore
9. Hubert Daniel, CSCR / Department of Clinical Virology, CMC, Vellore
10. Rajesh Kannangai, Department of Clinical Virology, CMC,Vellore
11. Saravanabhavan Thangavel, CSCR
12. Mohankumar Murugesan, CSCR
13. R. V. Shaji, CSCR / Department of Haematology, CMC, Vellore
14. Dolly Daniel, CSCR / Department of Immunohematology, CMC, Vellore
15. Kuryan George, Department of Community Health, CMC, Vellore
16. Shantidani Minz, Department of Community Health, CMC, Vellore
17. J. P. Muliyil, Retd. Professor, CMC, Vellore
18. Vrisha Madhuri, CSCR / Department of Pediatric Orthopedics, CMC, Vellore
19. Mahendra Rao, inStem, Bengaluru

External Collaborations::

1. Arun Srivastava, Department of Genetics, University of Florida
2. Barry Byrne, Powell Gene Therapy Center, University of Florida
3. Mavis Agbandje-McKenna, Director, Center for Structural Biology, University of Florida
4. Trent Spencer, Aflac Children's Cancer Center, Emory University, Atlanta, USA
5. Chris Doering, Emory University, USA
6. John Lollar, Emory University, USA
7. INTAS Pharmaceuticals, Ahmedabad, India
8. Nezih Cereb, Histogenetics, USA / DATRI, Chennai
9. Raghu Rajagopal, DATRI, Chennai

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

LABORATORY HIGHLIGHTS

Our lab carries out research in two themes at CSCR, gene therapy and iPSC technology.

Gene therapy: Novel lentiviral vectors to induce therapeutic levels of HbF

In the gene therapy projects, we are currently working on developing novel lentiviral vectors for gene therapy applications. Induction of fetal hemoglobin sickle cell disease (SCD) and β–thalassemia is a promising approach to ameliorate the disease phenotype. B-cell lymphoma/leukemia 11A (BCL11A) is a transcription factor that represses gamma globin gene (HBG) expression in adults. Down regulation of BCL11A induces HbF levels. Therefore, BCL11A is a prime candidate for targeted therapy aimed at induction of HbF in the patients with haemoglobin diseases. As depletion of BCL11A in hematopoietic stem cells can result in impaired B-cell growth and render aging like changes in HSCs, it is important that the knock out or knock down of BCL11A be erythroid specific. We have generated two novel lentiviral shRNA vectors (CSCREUv1 and CSCREUv5) for the knock down of BCL11A in human erythroid cells. The CSCREUv1 has three DNA hypersensitivity sites, HS2, HS3 and HS4 and a 180 bp HBB promoter. The CSCREUv5 lacks HS4, and has a 266 bp β – globin (HBB) promoter. Both carry a polycistronic GFP-IRES-puromycin-shBCL11a cassette cloned downstream of the HBB promoter. We observed that >90% knock down of BCL11A in transduced HUDEP2 erythroid cells resulting in an increase in HbF expressing cells by nearly 40% for both CSCREU v1 and v5. This experiment was then repeated in the ex-vivo erythropoiesis model. The GFP+ HSPCs were flow sorted and differentiated to erythroid cells using an erythroid culture system. GFP+CD71+CD235a+ erythroid cells showed >80% down regulation of BCL11A, resulting in 40% of the cultured erythroid cells expressing HbF. CSCREUv5 vector lacking HS4 appears to be produced at higher titers. For developing a gene therapy vector, we removed GFP-IRES-puromycin cassette from CSCREU-v5 vector. CD34+HSPCs were transduced with the new vector (CSCREU-v6), differentiated to erythroid cells and analysed for the expression of HbF and BCL11A and HBG. The analysis showed that, compared to the control cells transduced with scrambled shRNA, there was a >70% down regulation of BCL11A resulting in 5-fold upregulation of HBG transcripts and 20% increase in the HbF expressing cells. This was achieved without selecting the transduced erythroid cells. This data shows that this lentiviral vector provides significant erythroid specific knockdown of BCL11A which can result in clinically relevant enhancement of HbF expression in the therapy of SCD and thalassemia. Furthermore, this vector can be used to clone other shRNAs targeted at any of the genes in the erythroid cells to alter their function for the study human erythropoiesis or for therapeutic purposes.

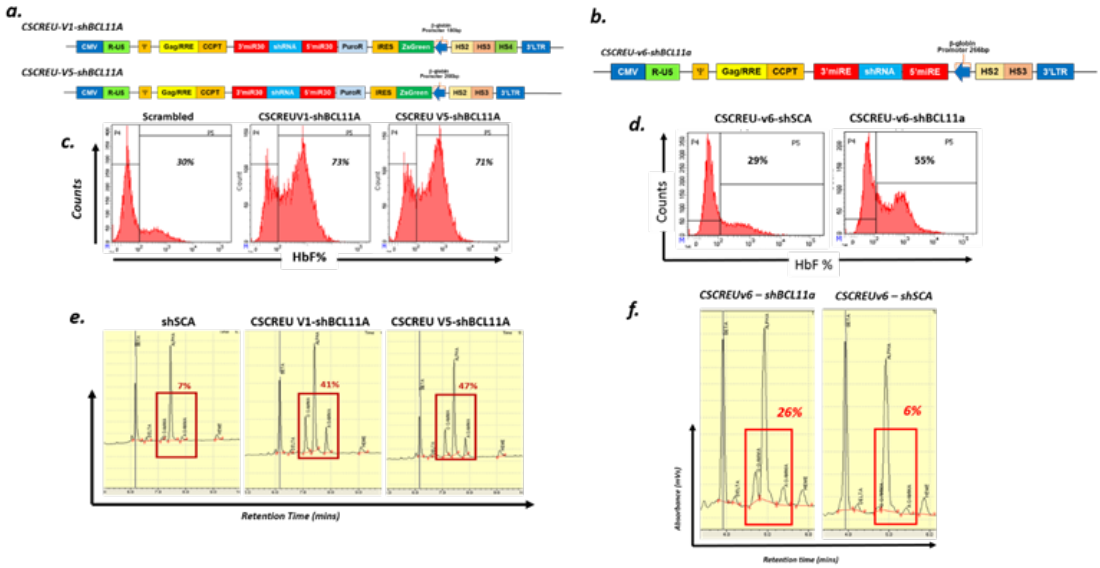


Figure: Induction of HbF expression in cultured erythroid cells after knocking down the expression of BCL11a using CSCREUv1, CSCREUv5 and CSCREU-v6 vectors A) Schematic of the CSCREU-v1 and CSCREU-v5 with shRNA inside a modified mir30 scaffold with ZsGreen-IRES-puromycin cassette, B) Modified CSCREU-v5 (CSCREU-v6) without ZsGreen-IRES-puromycin cassette. (C) and (D) show HbF expression measured by flow cytometry in the erythroid cells derived from transduced HSPCs (E) and (F) show the levels of different globins measured by HPLC. The results were compared with the cells transduced with scrambled (SCA) shRNA.