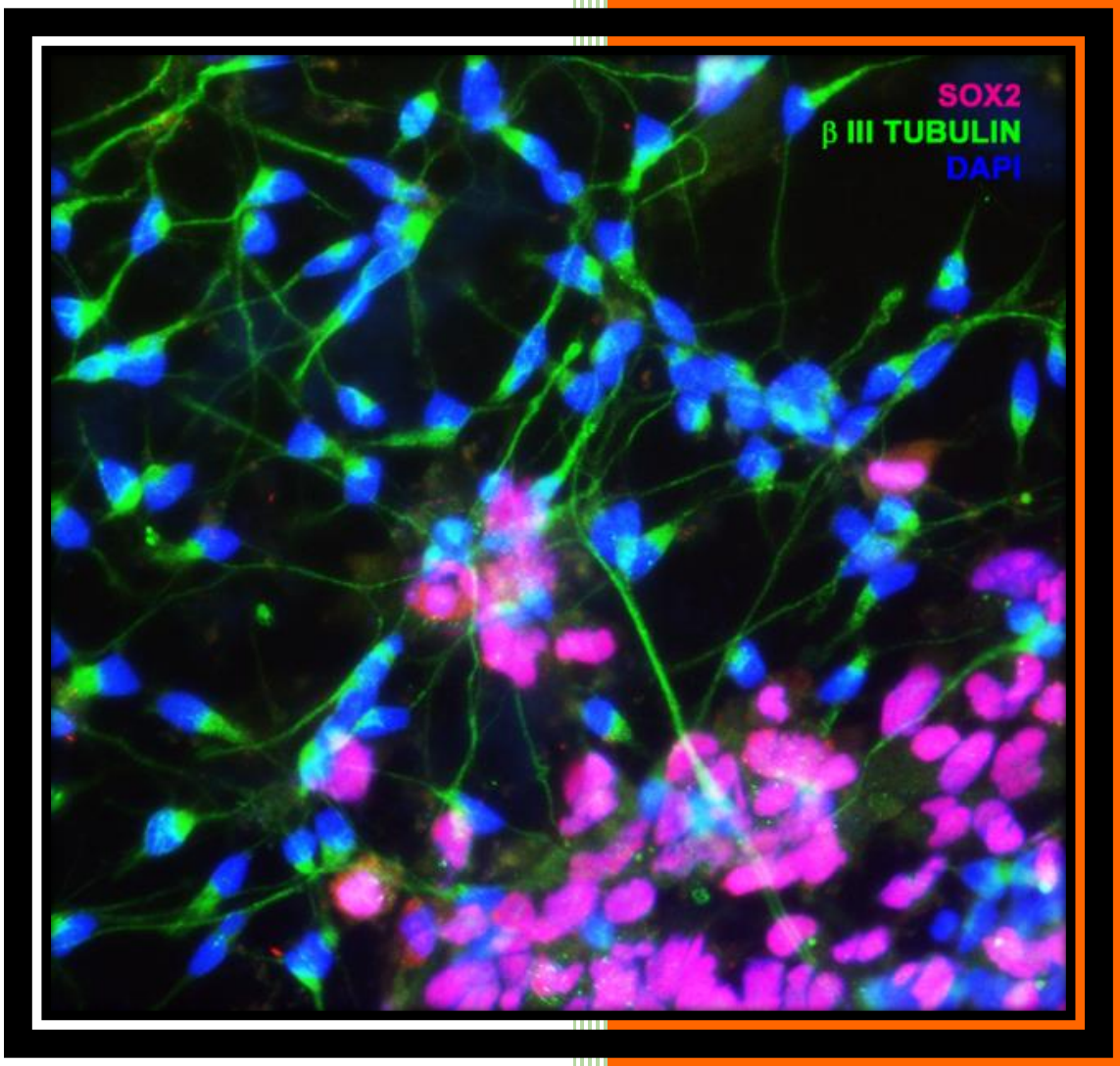
2012-2019

MIRM - REPORT



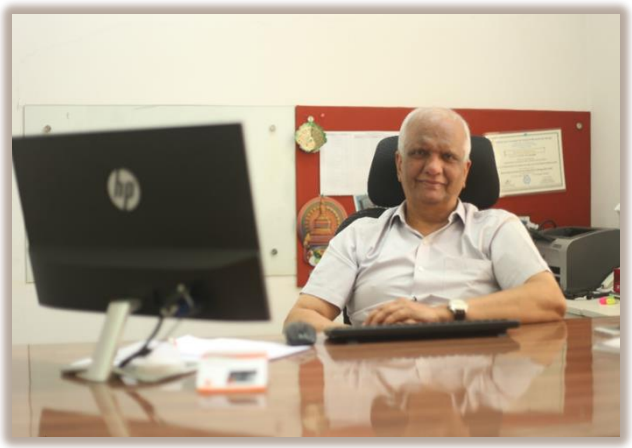
MANIPAL ACADEMY
OF HIGHER EDUCATION

(Deemed-to-be-University under Section 3 of the UGC Act, 1956)

MANIPAL INSTITUTE OF REGENERATIVE MEDICINE

CONTENTS

FROM THE DEAN'S DESK



On behalf of the Manipal Institute of Regenerative Medicine (MIRM) formerly School for Regenerative Medicine, Bangalore (SORM-B), Manipal Academy of Higher Education (MAHE), I am pleased to present to you "MIRM 2012-2019". This booklet encapsulates the research and teaching contributions made by MIRM in the highly specialized fields of stem cell biology and regenerative medicine.

MIRM was conceptualized in the year 2006 as a nascent research arm of the newly established stem cell company Stempeutics. It became an independent teaching cum research department of Manipal Academy of Higher Education (MAHE) under the name of Manipal Institute of Regenerative Medicine (MIRM) in 2008. It moved to its current campus in Yellahanka in 2012. The booklet describes the work done in MIRM/SORM these last seven years (April 2012 to March 2019).

Faculty members of MIRM have worked on stem cells from the embryos, fetal and neonatal tissues and from adult tissues of human and animal origin and have found some very important properties of these cells such as regulation of insulin production and secretion; formation of neurons and heart cells; mechanisms behind their survival and their influence on neighboring cells and tissues. A significant amount of this work is done in primary human cell cultures and has relevance for understanding the basic biology of stem cells. Recent research endeavors in MIRM

are geared towards medical applications and translational research. Over the past years methods have been standardized to deliver insulin producing cells in the pancreas; assays have been established using stem cells to assess the tissue specific toxicity of new and existing drugs; more efficient methods for regeneration of damaged liver and degenerated neurons in the brain are being developed. In addition to the research output about 70 M.Sc., 4 M.Phil. and 8 Ph.D. students have passed their exams from MIRM and awarded their degrees.

This booklet has been compiled by current and previous faculty members of MIRM who need to be thanked for their efforts. An electronic version of the booklet can be seen on the MIRM page on the Manipal University and a print version is available upon request.

I hope the readers would find the information useful and contact my office for further information.

Gopal Pande

MASTERS IN REGENERATIVE MEDICINE

MIRM has offered a post graduate M.Sc. course in Regenerative Medicine since 2007. The syllabus was last modified in 2009. In the intervening years this area has developed rapidly and several new concepts have emerged in the field. Therefore we are proposing to revise the syllabus of this course from the academic year 2017-18 onwards, with the course being renamed as 'Stem Cell Technology and Regenerative Biology'.

The guiding principle of this course revision is to make the syllabus more project oriented and vocational without compromising on the academic and theoretical content of the existing syllabus. The Practical module shall include a 15 day exercise at the end of Semester II to give training in scientific project writing to the students. In addition, the revised course shall offer elective topics and the students to do two in house projects- one of short (3 month) and one long duration (6 months).

SOME SALIENT FEATURES OF THE REVISED SYLLABUS ARE AS FOLLOWS:

The course structure would be modular and each module shall be handled by an assigned faculty member and guest speakers. The theory classes would be of 90 minutes duration comprising 1 hour of formal lecture followed by 30 minute discussion/tutorial session that shall comprise clarifications and questions from the students and evaluation of the students by faculty through short tests, quizzes and group discussions. Student's performance in the discussion/tutorial sessions shall be used for their internal assessments and grading. The afternoon practical sessions, in each module would be in line with the topics covered during the theory classes in the morning sessions. All key fundamental concepts and practicals, related to regenerative medicine, shall be covered during Semester I and II.

At the end of Semester II students would be given exercises to write and assess scientific projects and critique scientific publications in the form of journal club presentations. Lectures in Semester III would be on advanced elective topics. Students shall opt for their elective subject and their allotment would be done based upon their performance in the previous semesters.

During Semester III students would have only 10 lectures on advanced topics in the field and would do practicals related to those topics. They would also do a short project with their assigned PIs.

Projects for Semester IV shall be formulated under the supervision of the PIs selected/ allotted for their elective subjects in Semester III.

SEMESTER I:

There will be four main modules and fifteen sub-modules in the theory lectures

	MODULE
1	**BIOMOLECULES**
	Proteins
	Carbohydrates
	Lipids
	Nucleic Acids
2	**LABORATORY METHODOLOGIES, ETHICS, STATISTICS AND BIOINFORMATICS**
	Physico-Chemical Principles of Lab Techniques
	Statistical Principles in Biology
	Bioinformatics
	Clinical Research, Bioethics and Regulatory Guidance
3	**ARCHITECTURE OF CELLS, TISSUES AND ORGANS**
	Basic Cell Structure and Functions
	Tissue organization and functions
	Organ Structure and Functions
	Integrated Cell Biology
4	**EMBRYONIC DEVELOPMENT AND CELL DIFFERENTIATION**
	Early Embryonic Development
	Mid to Late Embryonic Development
	Tissue specific Stem Cells

For each sub module there would be a corresponding practical after the lectures

SEMESTER II:

There will be four main modules and sixteen sub modules in the theory lectures

	MODULE
1	**INTERCELLULAR COMMUNICATION IN STEM CELL NICHES**
	Introduction to stem cell niches and niche regulation
	Types of stem cell niches
	Epithelial to Mesenchymal transition in stem cells
2	**BIOMATERIALS AND TISSUE ENGINEERING**
	Properties and fabrication of Biomaterials
	Types of intercellular junctions
	Tissue Printing Ex vivo Organogenesis
	Current Challenges in Cell and Tissue Engineering
3	**CLINICAL APPLICATIONS OF STEM CELLS**
	Stem Cells Application in Ocular Diseases
	Stem Cells Application in Endocrine Diseases
	Stem Cells Application in Neurological Diseases
	Stem Cells Application in Immunotherapy
	Stem Cells Application in Hematological Disorders
4	**NON CLINICAL APPLICATIONS OF STEM CELLS**
	Stem Cells in Drug Screening and Toxicology
	Disease Modeling with Stem Cells
	Stem Cells in Tissue Remodeling
	Stem Cells as Discovery Tools

For each sub module there would be a corresponding practical after the lectures

SEMESTER III:

Electives:

- ✓ Immune Regulation of Stem Cell Homeostasis
- ✓ Stem Cells in Cancers and Regeneration – Wnt signaling Pathway Regulation
- ✓ Understanding the Ubiquitin Proteasome System (Ups) In Neurogenesis and Neurodegenerative Disorders
- ✓ Developing Temporal Lobe Epilepsy Model Using Zebra Fish
- ✓ Studying the Functional Role of Inflammatory and Immunomodulators in Human Disease Models
- ✓ Designing Biological/Acellular Matrices and Synthetic Substrates for The Growth And Differentiation Of Embryonic Stem Cells
- ✓ Deciphering the hypoxic influence on angiogenesis
- ✓ Effect of Retinoic Acid on the Cell Cycle of Pluripotent Stem Cells

In Semester III the students shall be assigned a specific Principal Investigator (PI), among the MIRM Faculty, based on their choice and their ranking in the previous semesters. Each PI will offer 10 Theory Classes, each of 90 min duration and General Practicals of about 170 hours. Following which the students will be offered a 'Mini Project' that shall be completed under the guidance of the respective PI. The performance of students shall be evaluated by internal and external examinations as detailed later.

The purpose of conducting Semester III in the above manner is to give the students hands-on-training in advanced techniques of Stem Cell Biology and train them to organize their experimental data as a scientific project report.

Elective topics would be added or revised in the following years.

SEMESTER IV:

DISSERTATION PROJECT

In this semester the students will do a detailed Project under the supervision of the respective PIs allocated in semester III

The schedule for the semester shall be as follows:

1. Project work: Jan to May; Submission & Presentation: June

MIRM engages in basics and industrial research related to stem cells. The Research pregame is conducted by six independent PIs whose research activities are described in the subsequent pages.

S. JYOTHI PRASANNA

Dr. S. Jyothi Prasanna did her Ph.D. from the Indian Institute of Science, Bangalore (2005). She has been with MIRM from 2007 till date.

Key words: Immunomodulation of stem cell networks, immune system-stem cell niche interactions during regeneration and degeneration, Hepatobiology

BACKGROUND AND OBJECTIVES:

Inflammation is a pivotal part of injury and regenerative mechanisms should prevail in an inflammation premise. Most degenerative diseases are often associated with chronic inflammation which skews the balance aberrantly towards either progressive injury and tissue damage or aberrant injury resolution leading to functional impairment. Endogenous stem cells as well as transplanted stem cells should mediate reparative benefits and regeneration in an inflammatory milieu for disease correction. The prime focus of the group is to understand the reciprocal association between immune cells and stem/progenitor cells both in *in vitro* and *in vivo* disease contexts with a broader motive to better injury resolution and functional recovery of cellular and organ homeostasis. Towards this broad goal, injury, repair and regeneration group is currently focusing on: Understanding the contribution of cellular and humoral components of the immune system in shaping stem cell responses and regeneration in damaged cells/tissues. Developing immune targeting strategies to promote tissue regeneration in degenerative diseases deciphering the influence of inflammation on connective tissue stem cell renewal and differentiation choices

WORK DONE

Connective tissue remodeling and immune infiltration are two critical components exhibiting paradoxical roles in promoting inflammation as well as injury resolution. Imbalances and abnormal thresholds of these processes disrupts permissive regenerative processes by impacting endogenous stem cell renewal and differentiation choices. Current projects in the group are modelled towards understanding the interface between connective tissue stem cell precursors, the Mesenchymal stem cells and molecular and cellular players of the immune system.

Influence of pro-inflammatory cytokines on Mesenchymal stem cell fate choice and immune-plasticity Work conducted in the lab over the past few years have evaluated the impact of predominant pro-inflammatory cytokine, IFNγ on MSC physiology with particular reference to their immune modulatory potential and differentiation into connective tissue lineages: two key facets driving tissue repair in transplantation and regeneration settings. IFNγ signaling augmented immune modulatory functions of MSCs in a tissue specific fashion but compromised the competence of MSC to undergo adipogenesis. In contrast terminal events associated with osteogeneis and chondrogenesis were enhanced in IFNγ exposed MSCs. A detailed analysis of biochemical mediators and molecular events associated with attenuated adipogenesis unravelled IFNγ induced metabolic fluctuations including insulin resistance, IDO induced tryptophan depletion, alterations in AMPK-Sirtuin1-p38 MAP kinase axis and autophagy inhibition. Pathway specific inhibitor screens aimed at rescuing adipogenesis from IFNγ exposed MSCs identified TGF□□receptor signaling as the prime mediator. Work is in progress to unwind the molecular interlinks and miRNA targets binding IFNγ induced TGF□□receptor signaling and metabolic perturbations in lineage choice decisions of MSCs under inflammatory stress.

Identification of transcriptional networks controlling MSC fate choice during tissue stress and inflammation: Despite huge clinical focus on reparative and regenerative benefits of transplanted MSCs in degenerative disease a huge lacunae exists in our understanding of precise roles of endogenous MSCs in degenerating tissue niches. This ambiguity arises due lack of knowledge in molecularly defining multi-potent self-renewing MSCs endowed with immune modulatory capabilities. Loss of MSC renewal and skewed differentiation choices is marked by reduced immunomodulation. To explore the contribution of common transcription factors driving contrasting MSC immune-plasticity/stem cell plasticity fluctuations, a screen was conducted to explore transcription factors modulated upon early differentiation of MSCs to any of the

connective tissue lineages. Zinc finger transcription factor, Zbtb16 was induced in most tissue specific MSCs upon differentiation and targeted knockdown of Zbtb16 in bone marrow derived human MSCs compromised MSC multi-lineage differentiation. MSCs exposed to inflammatory stimuli and MSCs from ovarictomised mice deficient in estrogen signaling inputs exhibited promiscuous Zbtb16 expression in absence of differentiation induction indicating loss of MSC renewal. Studies are ongoing to identify upstream molecular mediators driving Zbtb16 expression by decoding Zbtb16 promoter occupancy and analyzing Zbtb16 gene targets in MSCs exposed to chronic inflammation and hormonal insufficiencies challenging MSC lineage integrity and immune plasticity.

Elucidating the relevance of MSC-Macrophage crosstalk in repair and regeneration: Macrophages are endowed with unique functional plasticity equipping them to respond to ever changing environments. Each phase of inflammation and injury resolution are marked by unique macrophage polarized states. Preponderance of specific macrophage subsets during pathology emphasizes importance of these polarization shifts. We hypothesized that mechanisms existing at the MSC-macrophage interface could rationalize efficient immunomodulation as well as repair upon MSC transplantation by driving macrophage polarization shifts.

To test the above hypothesis, macrophage polarization programs and functional status was evaluated in MSC-macrophage co-cultures. MSCs seem to sense macrophage polarized states and reciprocally regulate their effector functions in an activation state dependent manner. MSCs promoted macrophage differentiation, enhanced respiratory burst and potentiated microbicidal responses in naïve macrophages. Functional attenuation of inflammatory M1 macrophages was associated with a concomitant shift towards alternatively activated M2 state in MSC-M1 co-cultures. In contrast, alternate macrophage (M2) activation was enhanced in MSC-M2 co-cultures. Elucidation of key macrophage metabolic programs in naïve macrophage/MSC, M1/MSC and M2/MSC co-cultures indicated a paradigm shift in macrophage bioenergetics status upon MSC co-culture. MSC-educated M1 macrophages underwent metabolic shift from glycolysis to OXPHOS alongside activation of M2 polarization programs. MSC secreted Prostaglandin E_2 was identified as a key bioactive factor instructing metabolic reprograming in macrophages and driving macrophage polarization shifts.

Functional significance of MSC-mediated macrophage activation shifts was further validated by protection conferred in cell culture models of endothelial injury. In nutshell, we

propose a novel role for MSC secreted factors induced at the MSC-macrophage interface in re-educating macrophages by manipulating metabolic programs in differentially polarized macrophages. Attempts are underway to test the relevance of these mechanisms in context of Diabetes, a disease primed and sustained by metabolically manipulated inflammatory macrophages.

Nucleotide catabolism in inflammation, injury resolution and MSC mediated reparative functions: Existence of nucleotide signaling during embryonic development as well as in multiple adult tissues including the immune system emphasizes its physiological omnipresence. Basal levels of cellular ATP released into the extracellular environment facilitate autocrine and paracrine signaling. However, persistent presence of ATP stress signal at the injury interface hampers tissue repair and regeneration. Preliminary studies in the lab indicate the existence of an elaborate battery of surface nucleotidases on MSCs which cleaves these stress signals. However the functional relevance of these enzymes in MSC physiology and their

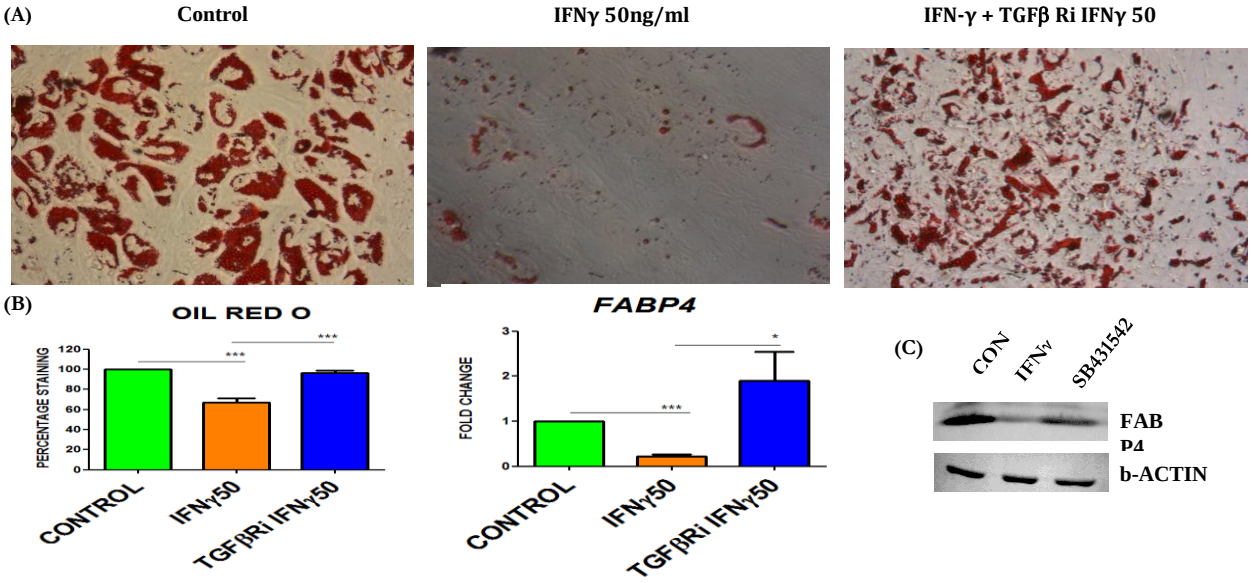


Fig: Inhibition of TGFb receptor signaling rescues adipogenesis in IFNg exposed Mesenchymal stem cells. (A, B) is Oil O Red staining and quantification of MSC derived adipocytes (C) depicts immnoblot analysis of FABP4, a mature marker for terminally differentiated adipocytes . Control are untreated MSCs subjected to adipogenic induction

contribution towards MSC mediated tissue repair is elusive. The theme of this project is to investigate the role of ectonucleotidase mediated nucleotide catabolism on MSC physiology, their differentiation choices, immunomodulation and reparative abilities

Ancillary projects:

Trans-differentiation of Mesenchymal stem cells to functional hepatocytes: With several studies implying the regenerative roles of MSCs, we were curious to test if multipotent human mesenchymal stem cells cross intrinsic lineage restrictions and trans -differentiate to non-mesodermal lineages upon exposure to developmentally significant cues. A systematic chronological exposure of hepatogenic cues to multipotent MSCs resulted in MET but failed to initiate hepatic fate induction. A dedicated approach of overexpressing key early endoderm fate committing transcription factors in MSCs equipped them with competence to respond to extrinsic hepatic cues and trans-differentiate into albumin secreting hepatocytes. Induced hepatocytes exhibited robust expression of CYPs, enzymes involved in detoxification of drugs and xenobiotic responses indicating hepatic maturation. Drug screening studies are underway to evaluate if induced hepatocytes generated from MSC through trans-differentiation approaches could be used as a platform to test hepatotoxicity.

FUTURE PLANS

Research endeavors of our group over the past few years indicate a key role for inflammatory cytokine sensing and macrophage cross-talk with stem cells as pivotal players in orchestrating repair responses. With this background understanding, future directions are aimed towards evaluating these interactions in pathological niches of chronic inflammation/progressive degeneration (Diabetes) and aberrant connective tissue homeostasis (postmenopausal osteoporosis/tumors). We believe that in depth molecular insights into these interactions in animal models of disease will open up novel therapeutic possibilities to remodel pathological niches.

LIST OF STUDENTS

1) **Anoop babu Vasandan** is an MPhil in Microbiology from Bharatidasan University. He drives the stem cell-macrophage interaction studies

2) **Sowmya Jahnavi** is a DST Inspire Fellow with a MSC in Industrial Microbiology from University of Madras. She is exploring the mechanisms driving IFN signaling and connective tissue perturbations.
3) **Chandanala Shashank** is a Masters in Life Sciences and a DBT JRF who has recently initiated studies on extracellular nucleotidases and is exploring the roles of nucleotide catabolism in modelling MSC immune-plasticity.
4) **Vaishali Garg** holds a dual master's degree. She is a MSC in Biotechnology from MS University, Baroda and has MS in general biology from Georgia State University, Atlanta. She investigates the transcriptional networks controlling MSC differentiation decisions
5) **David Luther is a MSc. in Regenerative Medicine from MIRM.** He is developing different animal models of liver injury to study cell-cell interactions critical for liver regeneration

HONORS AND AWARDS

1. **International Patents**
2. **Methods of Preparing Mesenchymal Stem Cells, Compositions and Kit Thereof**
3. Publication number US20110229965 A1 **Publication date:** Sep22, 2011 PCT number PCT/IB2010/055424
4. Poster presentation award at International conference of Stem cell Research organized by SCRFI (Stem cell Research Forum of India), 2007
5. Travel grant award ISSCR (International Society for Stem Cell Research) Cairns, Australia June 17^{th}-20^{th} 2007
6. Travel Grant award ISSCR (International Society for Stem Cell Research) Barcelona, Spain July 8-11 2009
7. BD Biosciences Research grant award 2011

LIST OF 5 SIGNIFICANT PUBLICATIONS

1. Vasandan AB**,** Jahnavi S, Shashank C, Prasad P, Kumar A, **Prasanna SJ**. Human Mesenchymal stem cells program macrophage plasticity by altering their metabolic status via a PGE(2)-dependent mechanism**. Nature Sci Rep. 2016** Dec 2;6:3 8308.

2. Sheshadri P, Ashwini A, Jahnavi S, Bhonde R, **Prasanna J** & Kumar A. Novel role of mitochondrial manganese superoxide dismutase in STAT3 dependent pluripotency of mouse embryonic stem cells. **Nature Sci Rep. 2015** Mar 30;5:9516. doi:10.1038/srep09516.
3. Vasandan AB, Shankar SR, Prasad P, Sowmya Jahnavi V, Bhonde RR, **Jyothi Prasanna S.** Functional differences in mesenchymal stromal cells from human dental pulp and periodontal ligament. **J Cell Mol Med. 2014** Feb;18(2):344-54
4. **Prasanna S.J.,** V. Soumya Jahnavi. Wharton's Jelly Mesenchymal stem cells as off-the-shelf cellular therapeutics: A closer look into their regenerative and immunomodulatory properties **The Open Tissue Engineering and Regenerative Medicine Journal , 2011, *4*, 28-38** (Bentham publications)
5. **Prasanna SJ,** Gopalakrishnan D, Shankar SR, Vasandan AB. Pro-inflammatory cytokines, IFNgamma and TNFalpha, influence immune properties of human bone marrow and Wharton jelly mesenchymal stem cells differentially. **PLoS One. 2010** Feb 2;5(2)

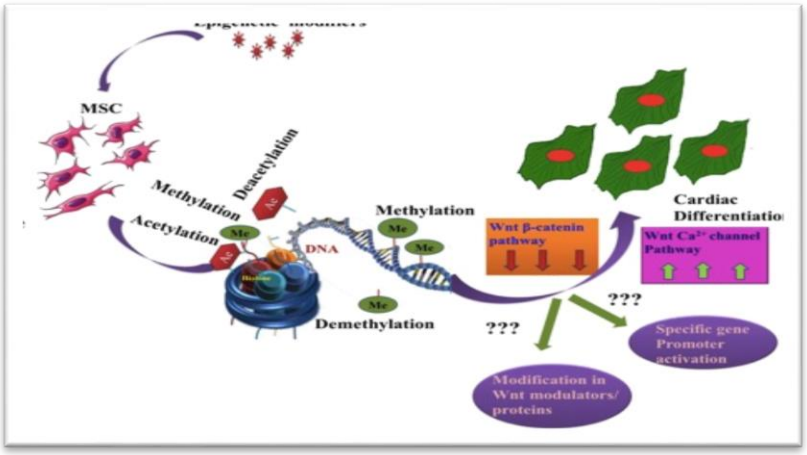
SUDHA WARRIER

Dr. Sudha Warrier obtained her Ph.D. in Reproductive Biology/Biochemistry/ Reproductive from the Life Sciences Dept., Univ of Madras, India. And her Postdoc from Duke-University, Dept of Biochemistry, North Carolina, USA(2002-2003) and Medical College of Ohio, Dept. of Molecular Medicine, Ohio, US (2003-2004). She has been with MIRM from 2009 till date.

Key words: Wnt signaling in stem cells, sFRP, cancer stem cells, cardiac regeneration, neurodegeneration, Alzheimer's disease, epigenetic modifications

BACKGROUND AND OBJECTIVES

The hallmark features of stem cells are their ability to self-renew and generate differentiated cell types. Wnt signaling has been implicated in a wide range of processes in stem cells and cancers. One of the key defining features of stem cells viz., self-renewal and pluripotency is regulated by the Wnt signaling pathway, which forms the basis of the use of Wnt proteins and their antagonists in the modulation of the fate of stem cells in culture. My lab focuses on the role of Wnts in stem cells in cardiac differentiation and neurogenic differentiation. In addition, with a stress on cardiac differentiation we are also delineating the epigenetic changes both at the histone and DNA level during cardiomyocyte differentiation. Another closely related but diverse area of stem cells in Wnt signaling has a prominent role is in cancer stem cells (CSCs) in which we are developing Wnt based therapeutics to target the CSCs of glioblastoma, breast and head and neck cancers.



WORK DONE

Amalgamated collaborations and joint research grants with Australia, Singapore and Malaysia in the areas of cardiac regeneration, cancer stem cells and neurodegeneration.

MY KEY RESEARCH FINDINGS INCLUDE

1. Establishment an inherent angiogenic property of amniotic membrane stem cell- Placenta 2012
2. Designing a therapeutic combination of Wnt and chemotherapeutic for brain tumor stem cells- PloS One 2015
3. Functional cardiomyocytes were obtained from discarded perinatal stem cells

4. A novel biomarker model for Alzheimer's disease by sFRP-mediated inhibition of the Wnt signaling pathways – Int J Biochem Cell Biol 2016- FIG 4

FUTURE PLANS:

Area 1: Cancer stem cells (CSCs) from solid tumors (glioma, head and neck and breast cancers) and drug targeting approaches, characterization of radio- and chemorefractory properties of cancer stem cells, regulation of CSC profile by Wnt signalling. I focus on studying the signalling mechanism that regulates chemoresistance of cancer stem cells. In active collaboration with Prof Arun Dharmarajan of Curtin Univ, Australia, Prof Gautam Sethi and Prof Alan Prem Kumar of National Univ of Singapore, we have been developing antagonists targeting Wnt - ß catenin pathway as a drug against cancer stem cells.

Area 2: Mesenchymal stem cells (MSCs) as a cell source for cardiac regeneration, role of epigenetic modification in cardiac differentiation from MSCs and ESCs, role of Wnt antagonism in multilineage differentiation, immunomodulatory properties of MSCs.

Area 3: Alzheimer's disease stem cell based model for the drug testing platform for neurodegenerative disorders, pluripotent stem cells from amniotic membrane for testing multilineage differentiation potential- In collaboration with National Univ of Singapore.

Area 4: Traditional medicine and natural compounds for improving the quality of stem cells, and as potent inhibitors of CSCs, and as combinatorial treatment with synthetic inhibitors- In collaboration with National Univ of Singapore

Other areas- Use of nanoscaffolds for MSC encapsulation and differentiation, Analysis of MSC for their anti-tumorigenic properties, Development of natural scaffolds for tissue engineering the culture of MSC for therapeutics

LIST OF STUDENTS

1. Ms G Bhuvanalakshmi
2. Ms Manasi Patil
3. Mr Saurabh Mandal
4. Mr Naisarg Gamit

HONORS AND AWARDS

1. **Travel award for attending the European Molecular Biology Organization (EMBO) meeting in Mannheim, Germany, Sept 2016 to present a paper titled-**
2. Epigenetic modifiers facilitate robust cardiomyocyte differentiation of chick embryonic mesenchymal stem cells accompanied by changes in the Wnt ß-catenin pathway
3. **Travel award from Wnt-EMBO organization to attend the Wnt meeting in Brno, Czech Republic in Sept 2016, to present a paper titled-**
4. Attenuation of Wnt signaling by diosgenin, a steroidal saponin, causes increased chemosensitization and apoptosis of breast cancer stem cells
5. Dr TMA Pai Gold Medal for outstanding research for the academic year 2015 in Manipal University awarded in the March 2016.
6. Curtin University, School of Biomedical Sciences Strategic Travel Fellowship, to visit Prof Arun Dharmarajan's lab, Curtin Univ, Univ of Western Australia, and CSIRO, Sydney, Jul-Aug 2013.
7. **Chairperson- IC-SCRT- Institutional Committee for Stem Cell Research (IC-SCRT)-** Chairperson at SRM University, School of Bioengineering, Chennai, India

LIST OF 5 SIGNIFICANT PUBLICATIONS:

1. sFRP-mediated Wnt sequestration as a potential therapeutic target for Alzheimer's disease. **Warrier S**, Marimuthu R, Sekhar S, Bhuvanalakshmi G, Arfuso F, Das AK, Bhonde R, Martins R, Dharmarajan A. **Int J Biochem Cell Biol.** 2016 Jun;75:104-11. doi: 10.1016/j.biocel.2016.04.002. Epub 2016 Apr 7 – **Citation- 2;** Impact factor- 4.1
2. Secreted frizzled-related protein 4 inhibits glioma stem-like cells by reversing epithelialto mesenchymal transition, reducing drug resistance, and increasing apoptosis (2015)- **PloS One.** June 2015; 10(6): e0127517. Bhuvanalakshmi G, Frank Arfuso , Michael Millward, Arun Dharmarajan and **Sudha Warrier* - Citation- 6;** Impact factor- 3.7
3. Cancer stem-like cells from head and neck cancers are chemosensitized by the Wnt antagonist, sFRP4, by inducing apoptosis, decreasing stemness, drug resistance, and epithelial to mesenchymal transition. **Cancer Gene Therapy.** 2014 Sep;21(9):381-8. doi: 10.1038/cgt.2014.42. Epub 2014 Aug 8. **Sudha Warrier***, Bhuvanalakshmi G, Frank

Arfuso, Gunesh Rajan, Michael Millward, and Arun Dharmarajan – **Citation – 20;** Impact factor- 2.5

4. Multifunctional Properties of Chicken Embryonic Prenatal Mesenchymal Stem Cells-Pluripotency, Plasticity, and Tumor Suppression. **Stem Cell Rev.** 2014 Dec;10(6):856-70. Bhuvanalakshmi G, Arfuso F, Dharmarajan A, **Sudha Warrier***- **Citation- 3;** Impact factor- 3
5. Secreted Frizzled Related Proteins: Implications in Cancers, Rohit Surana, Sakshi Sikka, Wanpei Cai, **Sudha R Warrier**, Eun M Shin, Hong-Jie G Tan, Frank Arfuso, Simon A Fox, Arun M Dharmarajan, Alan Prem Kumar. **Biochimica Biophysica Acta** 1845 (2014) 53–65). **Citation – 33;** Impact factor- 9

ANUJITH KUMAR

Dr. Anujith Kumar did his PhD from the Indian Institute of Science, Bangalore (2001-2007) and his Postdoc from the Katholieke University, Leuven (2007-2011). He has been with MIRM from 2010 till date.

Key words on Area of Research: Proteasomes, induced pluripotent stem cells, disease modeling, neuronal lineage, Diabetes, Pancreatic beta cells

BACKGROUND AND OBJECTIVES:

My scientific zeal was initiated during my Masters in Biochemistry from JJM Medical college, Davangere. During the course I was fascinated by the biochemical events that dictates the shelf-life pattern of proteins in a cell. These inquisitiveness and loads of desires to be among the scientific fraternity lead me to obtain my Ph. D. in Biochemistry from one of the premier institute of India, Indian Institute of Science (IISc), Bangalore in 2008. Doctoral thesis involved the study on role of intracellular protein degradation, which lead to identification, and biochemical characterization of aminopeptidase N (PepN) in prokaryotes, a functional analogue of eukaryotic

proteasome. During final days of PhD tenure, the haunting question, which prompted in mind, was how to understand the role of proteasome in cellular physiology with developmental perspective. I realized that "***we can't fix a problem unless we understand what the problem is***" and to understand the problem which fascinated me required a "developmental lens". This made me to take a break from protein degradation and to do a postdoc in stem cell biology at Katholieke University, Leuven, Belgium. During post doc tenure the major focus was on understanding the plasticity of adult cells and their ability to transdifferentiate to multiple lineages. This enabled me to understand different events that occur during the development of different derm layers. Obtained hands on experience on different features of ESCs and iPSCs and their differentiation ability to different lineages. After understanding the developmental cascades, in particular, neural and pancreatic cells, I joined MIRM in august 2011 as an independent faculty to deploy my post doc experience and nurture my initial passion of understanding the role of proteasome during lineage specification.

WORK DONE

Regulating stem cell fate and differentiation:

i) Proteasome in MSC cell fate specification: We cannot cure illness and disease unless we understand the basic biology that governs cellular behavior. Cellular homeostasis is majorly maintained by intracellular protein degradation pathway led by the proteasomes. Ubiquitin Proteasomal System (UPS) orchestrate a complex set of functions that culminate in clearance of damaged, unwanted and mis-folded proteins from the cells. UPS is composed of ligases, which attach targeted protein to Ubiquitin, and ubiquitin relays the protein to proteasome for further degradation. The 26S proteasome is a multisubunit complex protein comprised of a 20S catalytic core that coordinate peptide bond cleavage and a 19S regulatory complex that recognizes ubiquitin-tagged substrates, cleaves ubiquitin chains, unfolds substrates and translocates into the catalytic chamber of the 20S core. Targeting the 26S proteasome with the proteasome inhibitor bortezomib (Velcade, PS-341) is in clinical practice as a therapy for patients with myeloma or mantle cell lymphoma. Hitherto, there is substantial knowledge available on the transcriptional and translational regulation of factors regulating the stem cell fate. However, the post-translational modification by UPS that governs the steady state of proteins remains largely an untapped area. Hence, understanding the role of proteasomes in governing the cell fate choices is one of the core

interests of our laboratory. Currently we are in the process of utilizing mesenchymal stem cells (MSCs) as the model system to study the effect of modulating proteasomal sub units on the proliferation and differentiation potentials of these cells. After a detailed screening procedure, we have identified a key regulatory subunit that preferentially governs the adipogenic potential of MSCs but not osteogenesis. Loss and gain of function and proteasome chemical inhibitor approach conclusively elucidated the key role of the regulatory subunit in MSC fate choice. Further indepth role of the subunit will be studied in perspective of multiple myeloma and their resistance to bortezomib. In all, this study gears towards opening a new avenue in the field of intracellular protein degradation and allowing deeper understanding of their importance in stem cell biology, disease modeling and regenerative medicine.

ii) Cross-talk between Proteasome and antioxidant enzyme in ESC Physiology: To maximally utilize ESCs either as a developmental model or as a therapeutic cell, it is paramount to understand the complex network that defines their self-renewal and pluripotency. We, in our laboratory, decoded the untapped role of Manganese Superoxide Dismutase (MnSOD), in pluripotency and self-renewal of mESCs. In-depth mechanistic insight further demonstrated MnSOD to stabilize the turnover of pluripotent proteins at the post-translational level by modulating proteasomal activity (*Sheshadri et al., Sci Rep., 2015*). MnSOD being a mitochondrial resident enzyme, further we are attempting to understand how the cross-talk between proteasome and MnSOD is regulating mitochondrial integrity and its biogenesis.

The unique quality of MnSOD lies in the fact that $MnSOD^{-/-}$ leads to neonatal lethality in mice due to neurodegeneration and cardiomyopathy. This prompted us to better understand the functional aspects of MnSOD and its role in development and reprogramming of mouse cells. Recent findings in our laboratory indicate that abrogation of MnSOD affects ectodermal differentiation, specifically neural progenitor formation of mESCs, designating neurons as the choice of lineage specification of MnSOD during development. Upon reprogramming, MnSOD facilitates transdifferentiation of Mouse Embryonic Fibroblasts (MEFs) to neural progenitors, reiterating the prominent role of MnSOD in neural lineage specification.

iii) Decoding the role of *Zic3* in early neural development using stem cell model system: An extremely complex transcription factor circuitry regulates the development of eukaryotes in a stage specific manner. Certain transcription factors, which are master regulators of pluripotency also play key role in lineage specification. Owing to its paradoxical role in

maintenance of pluripotency and neural lineage commitment, Zinc finger transcription factor in Cerebellum3 (ZIC3) gains our research interest. ZIC3 has been shown to be associated with left-right axis symmetry. In order to conclusively elucidate the role of Zic3 in derm layer specification, our lab is utilizing ESC model system and its directed tri-lineage differentiated counterpart. Using various reporter lines and DNA-protein interaction studies, we are attempting to understand the downstream targets of Zic3 during neural development and also in future, we intend to perform protein interaction studies to effectively unravel the ZIC3 interactome. By and large, this study aims at contributing to the existing knowledge in the field of transcriptional regulation of neurogenesis.

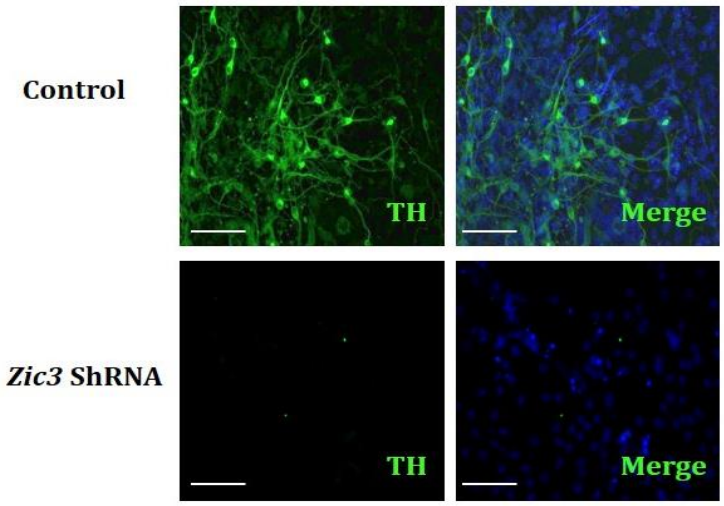


Fig: Embryonic Stem Cells Differentiated to OB like neurons were transduced with Zic3 ShRNA and analyzed for the expression of tyrosine hydroxylase (TH). Immunofluorescence analysis showed down-regulation of TH expression upon Zic3 knockdown compared to control, inferring the expression of TH in OB neurons to be regulated by Zic3.

Deconstructing Pancreatic developmental events:

i) Modulating proteasomal activity to augment the *in vitro* derivation of functional β-cell like cells from hESCs: Type 1 diabetes (T1D) is manifested mainly by autoimmune destruction of the insulin-secreting pancreatic β-cells leading to absolute lack of β-cells. Derivation of functional β-cells from stem cells has provided an potential alternative cell source for diabetic therapy. Much of the protocols directing hESCs to insulin-producing cells have courted controversy and the reliability of these protocols is still questionable. Of note, the β-cells derived from the existing protocols were fraught with limitation of being immature multihormonal cell type and also resulted in formation of teratoma post transplantation. Our laboratory considered these lacunae in the field of pancreatic differentiation and focused on modulating the steady state

level of Key transcription factors that regulate pancreatic cell fate. We are targeting the ubiquitin-proteasomal degradation machinery to achieve better stability of pancreatic lineage committing TFs, which would in turn translate to a higher percentage and homogenous population of β-cells.

ii) Stage specific evaluation of proteasomes role during pancreatic development using genetically modified induced pluripotent stem cells (iPSCs): Due to high similarities between iPSCs and ESCs, iPSC is garnering larger attention in the field of developmental and translational biology. To understand the role of proteasome during pancreatic development, we are utilizing the iPSCs derived from mice fibroblast horboring stage specific promoter tagged to a reporter system. In this process we are able to generate Pdx1-GFP, Ngn3-YFP and insulin promoter-YFP iPSCs. iPSCs will be subjected for pancreatic differentiation and based on the expression of the reporter, wherein Pdx1 marks the early pancretic progenitors, Ngn3 markes endocrine progenitor and insulin marks the mature islet cells, stage specific cell type will be fractionated. The purified reporter positive and negative population will analysed for proteasomal subunit and activity. By this approach we are attempting to precisely unravel the role of protein degradtaion during pancreatic development in a stage specific manner.

FUTURE PLANS:

In future we have planned to decrypt the interactome of proteasomal component as well ubiquitinases and deubiquitinases in myeloma and proteasomal inhibitor bortezomib resistant patients. We are also in the process of screening for proteasomal subunits and its activity along with other peptidase activities as marker for myeloma and their drug resistant counterpart. One of the other interesting projects, which we are planning to introduce, is to understand the relevance of proteasome mediated neural inputs in Duchene muscular dystrophy (DMD) disease using induced pluripotent stem cell model system. Among the genetic disorders known to date, DMD is the second most commonly occurring genetically inherited disease in humans. At present, there is no effective therapy to restrain the lethal progression of the disease. DMD is well characterized in the purview of the skeletal musculature. Though, the major clinical issue in DMD is the skeletal muscle pathology, there has been very little clinical investigation regarding the role played by the absence or disruption of dystrophin on central nervous system (CNS) function and how proteasome is involved in the manifestation of the symptoms. Significant stride has been made in utilizing iPSCs as a model in understanding the underlying mechanism of several disorders. We are

planning to derive iPSCs from diseased blood cells and differentiate them to neurons to resolve the role of proteasome in the lack of sufficient neural input to muscle functioning in DMD.

LIST OF STUDENTS

1. ***Preethi Sheshadri****:* Preethi completed Masters from Vellore Institute of Technology in Microbiology and she has been focusing on unraveling the importance of antioxidant enzymes in self-renewal and fate specifications of stem cells.
2. ***Ashwini:*** Ashwini is a M.Sc. graduate in regenerative medicine from MIRM, Bangalore. Her interest lies in understanding the role of intracellular protein degradation in stemness, differentiation and subsequently in disease phenotypes.
3. ***Meenal Francis:*** Meenal completed her Masters from Bangalore University. She is currently working on pancreatic beta cells derived from pluripotent stem cells, specifically trying to elucidate the role of non-beta cells on beta cell physiology in diabetic mouse model systems.
4. ***Smitha****:* Smitha has done her Masters in Biotechnology from Bangalore University and currently works on an early transcription factor ZIC3 and tries to decipher its role in stem cell fate choices. Also her study involves understanding ZIC3 interactome.

HONORS AND AWARDS

1. GATE Aptitude test: 99.2 percentile, All India 26th Rank
2. Travel grant award ISSCR (International Society for Stem Cell Research) Toronto, Canada 17th-20th 2011
3. BD Biosciences best Research grant on stem cells award 2012

LIST OF 5 SIGNIFICANT PUBLICATIONS:

1. **Kumar A**, Declercq J, Eggermont K, Agirre X, Prosper F, Verfaillie CM. Zic3 induces conversion of human fibroblasts to stable neural progenitor-like cells. ***J Mol Cell Biol.*** 2012; 4: 252-5. **(IF: 8.4)**
2. **Kumar A**, Lo Nigro A, Gysemans C, Cai Q, Esguerra C, Nelson-Holte M, Heremans Y, Jiménez-González M, Porciuncula A, Mathieu C, Binas B, Heimberg H, Prosper F, Hering B, Verfaillie CM, Barajas M. Reversal of hyperglycemia by insulin-secreting rat bone

marrow- and blastocyst-derived hypoblast stem cell-like cells. ***PLoS One***. 2013 May 9;8(5):e63491. **(IF: 3.2)**

3. Declercq J, Sheshadri P, Verfaillie CM, **Kumar A**. Zic3 enhances the generation of mouse induced pluripotent stem cells. ***Stem Cells Dev***. 2013; 22(14): 2017-25. **(IF: 3.7)**
4. Sheshadri P, Ashwini A, Jahnavi S, Bhonde R, Prasanna J, **Kumar A.** Novel role of mitochondrial manganese superoxide dismutase in STAT3 dependent pluripotency of mouse embryonic stem cells. ***Sci Rep***. 2015; 5: 9516. **(IF: 5.3)**
5. Porciuncula A*, **Kumar A***, Rodriguez S, Atari M, Araña M, Martin F, Soria B, Prosper F, Verfaillie C, Barajas M. Pancreatic differentiation of Pdx1-GFP reporter mouse induced pluripotent stem cells. ***Differentiation.*** **2016 May 12**. **(IF-3.8) (* Contributed equally)**

D. ANANDH

Dr. Anandh obtained his M.Phil., Ph.D. in Neurophysiology from the National Institute of Mental Health and Neurosciences, Bangalore (2004). He did his Postdoc from the Dept. of Neurosurgery, Duke University, North Carolina, USA and the Dept. of Physiology, Otto-von-Guerick University, Germany. He has also been a Research Associate at the Dept. of Neurology, University of Tennessee, Tennessee, USA. He has been with MIRM Since July 2012.

Key words: Neurodegeneration, Neuroregeneration, Stem Cell Therapy, Hippocampus, Secretome.

BACKGROUND AND OBJECTIVES:

Neurodegenerative diseases are devastating diseases wherein there is no appreciable treatment available so far. The primary objective of our research group is to understand the molecular, biochemical and cytoarchitectural changes that lead to progressive neurodegeneration and behavioural impairments in temporal lobe epilepsy, Parkinson's disease and Alzheimer's disease. From the neuroprotection perspectives, we are actively exploring the possibilities of stimulating endogenous stem cells for functional recovery by means of novel drug treatments and

stem cells and stem cells "based" therapy. We have reported that transplanting stem cells in brain injured rodents can prevent neurodegeneration, neuroinflammation and improves cognitive functions. Currently, we are involved in understanding the molecular mechanisms of neuroprotection conferred by transplanted cells in protecting hippocampal neurons and ameliorating behavioral co-morbidities that are commonly observed in temporal lobe epilepsy, Parkinson's disease and Alzheimer's disease.

WORK DONE:

In the past five years, we have been exploring the neuroprotective potential of adult mesenchymal stem cells and its secretome in functional recovery following hippocampal neurodegeneration in *in vitro* as well as *in vivo* conditions. In particular, we are investigating the neuroprotective properties of dental pulp stem cells (DPSC) as compared to commonly used bone marrow mesenchymal stem cells (BM-MSC). We demonstrated that neural crust originated dental pulp stem cells are far superior in inducing hippocampal neurogenesis that was well correlated with early hippocampal functional recovery as compared to BM-MSC treated rodents. We also demonstrated that the neuroprotective potential of MSC transplantation could be recapitulated with MSC secretome treatment as well. Our research is funded by SERB, DBT, DST and intramural funding.

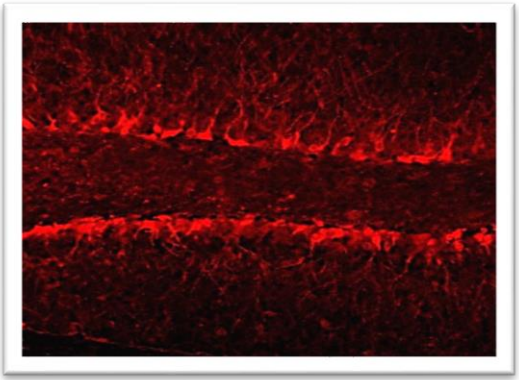


Fig: Hippocampal Neurogenesis Detected by Doublecortin.

FUTURE PLANS:

In line with translational approach, we will be exploring the migration potential of DPSC as compared to BM-MSC into the CNS following systemic injections in hippocampus damaged rodents. Such study would shed light on whether DPSC express CNS specific chemoattractants which may not be expressed in commonly used BM-MSC. This would help clinicians to select appropriate MSC for treating neurodegenerative/neurological diseases. Furthermore, we are

planning to identify the crucial factors present in MSC secretome essential for neuroregeneration that would lead to stem cells “based” therapies for neurodegenerative/neurological diseases.

LIST OF STUDENTS

1. Ms. Chaitra Venugopal,
2. Mr. Pradeep Kumar
3. Mr. Harish
4. Mr. Christopher Samir
5. Ms. Siva Priya

LIST OF 5 SIGNIFICANT PUBLICATIONS:

1. Venugopal C, Shamir C, Senthilkumar S, Babu JV, Sonu PK, Nishtha KJ, Rai KS, Shobha K, Anandh Dhanushkodi. Dosage and Passage Dependent Neuroprotective Effects of Exosomes Derived from Rat Bone Marrow Mesenchymal Stem Cells: An In Vitro Analysis. Curr Gene Ther. 17(5):379-390; 2018 Impact Factor: 2.8
2. Venugopal C, Prasad YSHC, Shobha K, Pinnelli VB, Anandh Dhanushkodi. HEK-293 secretome attenuates kainic acid neurotoxicity through insulin like growth factor-phosphatidylinositol-3-kinases pathway and by temporal regulation of antioxidant defense machineries. Neurotoxicology. pii:S0161-813X(17)30233-4, 2018. Impact Factor: 3.8
3. Sanap A, Chandravanshi B, Shah T, Tillu G, Anandh Dhanushkodi, Bhonde R, Joshi K. Herbal pre-conditioning induces proliferation and delays senescence in Wharton's Jelly Mesenchymal Stem Cells. Biomed Pharmacother. 93:772-778; 2017; Impact Factor: 2.8
4. Shamir C, Venugopal C, Anandh Dhanushkodi. Dental pulp stem cells for treating neurodegenerative diseases. Neural Regen Res. 10(12):1910-1,2017 Impact Factor: 2.0
5. Venugopal C, Chandanala S, Prasad HC, Nayeem D, Bhonde RR, Dhanushkodi A. Regenerative therapy for hippocampal degenerative diseases: lessons from preclinical studies. J Tissue Eng Regen Med. 2017 Feb;11(2):321-333. doi: 10.1002/term.2052. Epub 2015 Jun 29.

MANASA NUNE

Dr. Manasa Nune completed her Ph.D. in 2015 from the Centre for Nanotechnology & Advanced Biomaterials (CeNTAB), SASTRA University, Thanjavur, Tamil Nadu. She worked as a Post-doctoral Research Associate in 2016 at the Chemistry of Physics & Materials Unit of Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Bangalore. She is working as an Assistant Professor in MIRM since July 2017 till date.

Keywords: Tissue engineering, Synthetic and natural biomaterials, Embryonic stem cells, Nerve regeneration, Uterine tissue repair

BACKGROUND AND OBJECTIVES:

Biomaterials that are used as scaffolds must provide a suitable microenvironment for the cells to enhance the growth of tissue. In addition to providing the 3-D geometry, the scaffolds must support cell attachment, proliferation, infiltration, differentiation, and aid new tissue formation. Thus, the chemical composition, physical structure, and presence of bio-functional moieties in the scaffold are all important attributes for a biomaterial to be used as a scaffold in tissue engineering. Traditionally, different biomaterials both of natural and synthetic origin have been used in tissue engineering.

Embryonic stem cells (ESCs) hold a great potential for the cell replacement therapy and regenerative medicine as they are capable of unlimited self-renewal, cell growth and retain the pluripotency to differentiate into all cell types in the body. Current challenge is to grow ESC on feeder free and serum free conditions. The approach is to grow the acellular matrices on the polymer based substrates where they could be detachable and portable for further in vitro and in vivo applications. The novelty in our research is to focus to mimic and create the extra cellular matrix milieu to support the ESC growth and to maintain pluripotent state and to generate ‘portable’ acellular matrices on the synthetic polymeric substrates.

Clinical use of autologous Schwann cells (SCs) for the treatment of nerve injuries is challenged and of limited use due to difficulty in obtaining clinically useful numbers. So therefore, several groups have attempted alternative approaches to obtain good SC yield for transplantation

by differentiating several types of stem cells to SCs. Our approach is to use a cell free method where the acellular matrices are derived from the SCs and functional capacity of the cells is evaluated. Current scaffold fabrication techniques permit for only few molecular cues to be integrated into the scaffold, this method uses cells as “natural factories” for deposition of various cell-secreted peptides. We would like to assess whether such matrices could promote the growth and differentiation of the ESCs to motor neurons. We hypothesise to see if the myelination phenomenon is retained in the SC generated matrices.

WORK DONE:

1. Acellular matrices were prepared and characterised from Mouse embryonic fibroblasts (MEFs) in different conditions.
2. Synthetic polymer films made up of Polycaprolactone were prepared by manual coating and spin coating methods. MEFs were cultured on both the types of PCL films and acellular matrices were prepared
3. Acellular matrices were also spin coated on the PCL films which could be used as potential substrates for MESC culture as well as other tissue engineering purposes.
4. Mouse Embryonic Stem Cells have been cultured on the acellular matrices which showed better proliferation than the MEF feeder layers

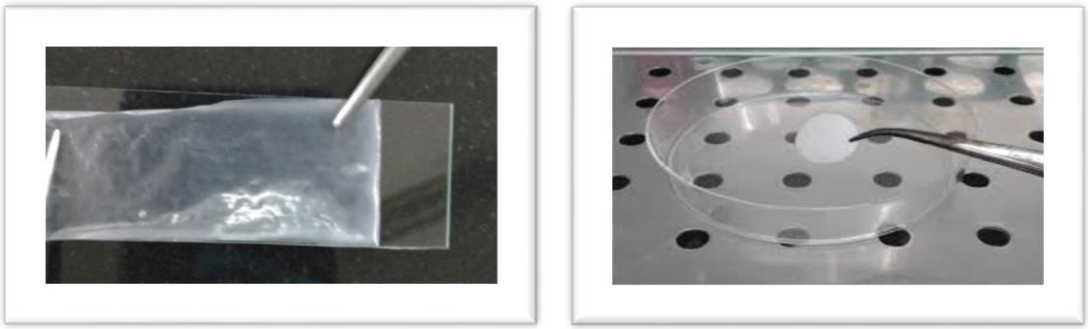


Fig: PCL films by solvent casting and spin coating methods

FUTURE PLANS

1. Design of novel tissue engineered materials for treatment of human uterine tissue defects and applications in uterine tissue engineering
2. Myelin secreting Schwann cell based matrices for the treatment of demyelinating nerve disorders
3. Innovative applications of acellular matrices as biomaterials for Embryonic stem cell maintenance, lineage specific cell expansion and differentiation

LIST OF STUDENTS

1. S. Hemalatha (M.Sc. Elective student - 2018)
2. Vishaka Vijaykumar Pillai (M.Sc. Elective student - 2018)
3. Maheshwari BC. (M.Sc. Elective student - 2019)
4. Steffi Zimran (B.Tech. MIT, Manipal - present)
5. Srividya H. (Ph.D. student - present)

LIST OF 5 SIGNIFICANT PUBLICATIONS

1. Manasa Nune, Anuradha Subramanian, Uma Maheswari Krishnan, Swaminathan Sethuraman. Peptide Nanostructures on Nanofibers for Peripheral Nerve Regeneration. Journal of Tissue Engineering & Regenerative Medicine 2019; 13:1059–1070. (SCI, IF-4).
2. Manasa Nune, Shivaprasad Manchineella, T. Govindaraju, K.S. Narayan. Melanin incorporated electroactive and antioxidant silk fibroin nanofibrous scaffolds for nerve tissue engineering. Material Science & Engineering C 2019; 94: 17-25. (SCI, IF-5)
3. Manasa Nune, Anuradha Subramanian, Uma Maheswari Krishnan, Suraj Sasidhara Kaimal, Swaminathan Sethuraman. Self-assembling peptide nanostructures on aligned poly (lactide-co-glycolide) nanofibers for the functional regeneration of sciatic nerve. Nanomedicine 2017; 12 (3): 219-235. (SCI, IF-5)
4. Manasa Nune, Uma Maheswari Krishnan and Swaminathan Sethuraman. PLGA Nanofibers Co-electrospun with Designer Self Assembling Peptides for Peripheral Neural Regeneration. Material Science & Engineering C 2016; 62: 329–337. (SCI, IF-5)

5. Manasa Nune, Uma Maheswari Krishnan and Swaminathan Sethuraman. Decoration of PLGA Electrospun Nanofibers with Designer Self-Assembling Peptides: A "Nano-on-Nano" Concept. RSC Advances 2015; 5: 88748–88757. (SCI, IF-2.936)

HONORS, AWARDS & PRESENTATIONS:

1. Received Senior Research Fellowship (CSIR-SRF) award from Council of Scientific and Industrial Research (CSIR), Human Resource and Development Group, Government of India in 2014.
2. Received best poster award at the 5th Indian Peptide Symposium organized by Indian Peptide Society on September 24-25, 2015, at JNCASR, Bangalore, India
3. Oral presentation at the International Conference on Nano Science and Technology (ICONSAT-2018) held at Indian Institute of Science, Bengaluru, India during March 21-23, 2018.

SHAGUFTA PARVEEN

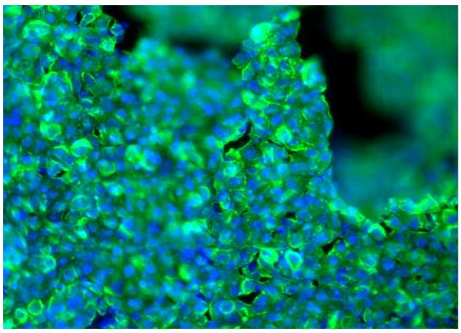
Dr. Shagufta Parveen is an Assistant Professor at MIRM. She did her PhD under Dr. Pawan Kumar Gupta and has a Master's degree in Microbiology from Bangalore University. She has been with MIRM since 2008. She is working on Generation of Induced Pluripotent stem cells.

Key Words on Research interests: Reprogramming; Induced pluripotent stem cells; Disease models; Cardiomyocytes.

BACKGROUND AND OBJECTIVES:

Generation and Application of stem cells: I am interested in using the Reprogramming technology to generate stable, transgene free induced Pluripotent stem cell lines of both diseased and healthy individuals. The focus is to use these pluripotent stem cell lines to understand disease biology and to generate therapeutically useful cells. I have been working on the following two aspects in the recent past.

Generation and characterization of Turner Syndrome Induced pluripotent stem cell line: Individuals with Turner syndrome suffer from a range of abnormalities the most important of which is infertility. My pluripotent stem cell model of Turner's syndrome should serve as a tool to study the developmental abnormalities of foetus as well as the placenta that lead to early embryo lethality and profound symptoms like infertility in 45 XO survivors. I am currently exploring the difference in differentiation of these pluripotent stem cells into three germ layers at a cellular level.



TURNER SYNDROME INDUCED PLURIPOTENT STEM CELL LINE EXPRESSING THE PLURIPOTENCY MARKER TRA 1-60

KARYOTYPE OF THE IPSC LINE WITH ONLY ONE X CHROMOSOME

Figure: Turner Syndrome Pluripotent stem cell line

Differentiation of induced pluripotent stem cells into Cardiomyocytes: Induced pluripotent stem cell (iPSC) technology has emerged as an important tool to generate large scale cardiomyocytes to treat cardiomyopathies as well as in drug discovery and cardiac disease modelling. The phenomenon of retention of epigenetic memory is related to the cell of origin and influences iPSC differentiation propensity. Thus it was logical to generate iPSC derived cardiomyocytes from a source of mesodermal origin. Hence I isolated mesenchymal stomal cells (PMSCs) from chorionic villi of normal term human placenta and converted them to integration free Placental induced pluripotent stem cells (P-iPSCs). Analysis of these P-iPSCs revealed a fully reprogrammed pluripotent state as evidenced by their stemness marker profile and their ability to differentiate both in vitro and in vivo. These P-iPSCs differentiated into beating cardiomyocytes (CM) expressing major cardiac specific transcription factors (NKX2-5, GATA4) and structural proteins (vMHC and cTNT). They also expressed cardiac gap-junction protein, Connexin-43.

PUBLICATIONS:

1. **Shagufta Parveen,** M. M. Panicker, Pawan Kumar Gupta. Generation of an induced pluripotent stem cell line from chorionic villi of a Turner syndrome spontaneous abortion. Stem cell research. **Stem Cell Research** 19 (2017) 12–16
2. **Parveen, Shagufta**. 2018. "Establishment and Characterization of Induced Pluripotent Stem Cells from Placental Mesenchymal Stromal Cells." **Stem Cell Research** 27:15–20.

SUJA ANN MATHEW

Dr. Suja Ann Mathew, is an Assistant Professor and has been with MIRM since 2007 till date. She did her Ph.D. under Dr. Ramesh Bhonde and has a Master's Degree in Biotechnology. Her interest lies in deciphering the phenomenon of angiogenesis during development and under diseased conditions.

Key words: Angiogenesis, Placental Mesenchymal Stromal Cells, Hypoxia, Wound healing

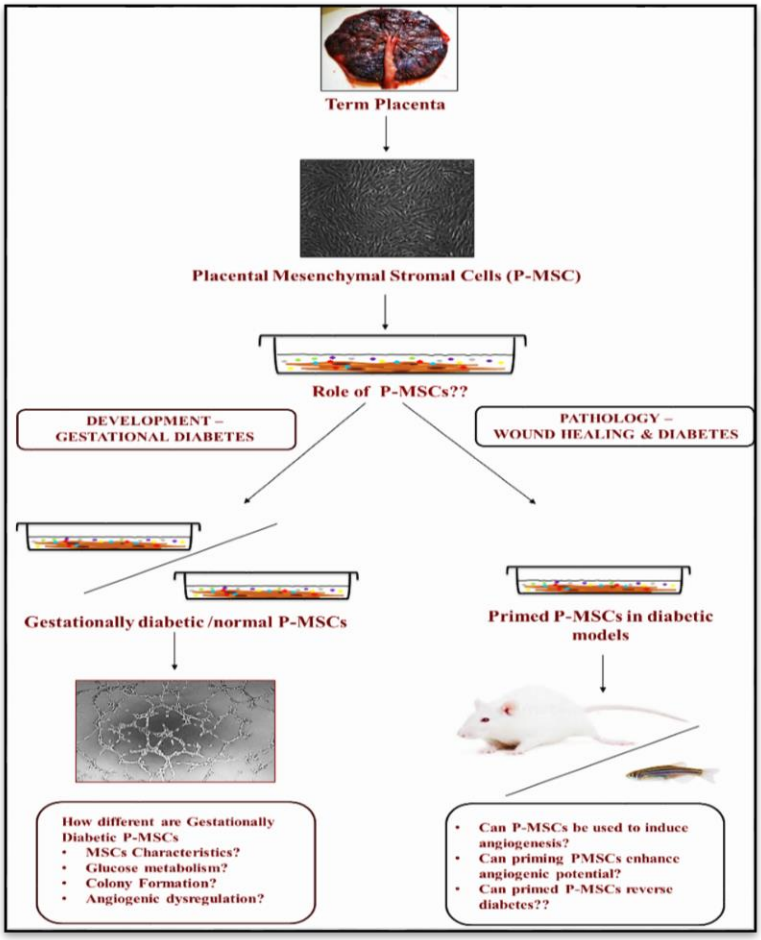
BACKGROUND AND OBJECTIVES

The placenta being a rich source of MSC can be looked at as an alternative source for therapy. We therefore studied placenta derived Mesenchymal Stromal Cells (P-MSC) to understand their potential for angiogenesis. We aim to look at the property under two aspects – Development; the role of PMSCs towards placentation and its dysregulation during gestational diabetes, and Pathology; the role of PMSCs contributing towards enhanced angiogenesis under pathological conditions like chronic and diabetic wounds.

WORK DONE:

P-MSC and Angiogenesis: One of the themes that Dr. Bhonde envisioned, is the idea of 'trash to treasure'. So we looked at source that would do just that!! The term-placenta, is usually incinerated post-delivery. But it is blessed with a rich pool of Mesenchymal Stromal cells. Thus

we opted to study the placenta as an alternative source for obtaining Mesenchymal stromal cell (MSC). Placenta is also preferred over several other sources as it is an easily available source, does not involve any invasive procedures and is ethically more favorable. The isolated and characterized cells subjected to angiogenic cues were observed to form *in vitro* angiogenic tubes, and angiogenic markers like VEGF, bFGF, PDGF. We found that the P-MSC possess angiogenic potential that can be further analyzed.



P-MSC and Development: Pregnancy is often referred to be in a ‘diabetogenic state. An increase in insulin resistance is seen with the progression of pregnancy. Diabetes has been seen to lead to both cellular and molecular level dysfunction. Earlier studies done on GDM derived placenta and cord blood samples showed enhanced epigenetically modified genes predominant in the metabolic disease pathways, even under modest hyperglycemia! It is importance for us to see if the results that are evident at term is maintained post culture of the cells under normal culture conditions. More important will be to determine if such cells can be used for therapy. One of our aims to study MSCs isolated from placenta of both normal and diabetic mothers and compare them, with a hope of developing an *in vitro* model for diabetes.

P-MSC and Pathology: Wound healing is a natural phenomenon essential to compact the normal wear and tear in the human body. But impaired wound healing has been of concern in several conditions like diabetes, burns, ageing, etc. We also looked at factors that could be used to prime

P-MSC to modulate their angiogenic potential and wound healing process, like hypoxia and polyunsaturated fatty acids. These experiments have been validated in mouse models.

FUTURE PLANS

With the understanding gained over the period of my PhD, I hope to look deeper into the phenomenon of angiogenesis/ wound healing both under developmental and pathological conditions. We have also initiated work in zebra fish models, which we further hope to develop into a possible diabetic model.

LIST OF SIGNIFICANT PUBLICATIONS

- ✓ **Mathew SA**, Bhonde RR. Omega-3 polyunsaturated fatty acids promote angiogenesis in placenta derived mesenchymal stromal cells. Pharmacol Res. 2018 Apr 14;132:90-98. doi: 10.1016/j.phrs.2018.04.002. [Epub ahead of print]
- ✓ **Mathew SA**, Bhonde R., Mesenchymal stromal cells isolated from gestationally diabetic human placenta exhibit insulin resistance, decreased clonogenicity and angiogenesis. Placenta. 2017 Nov; 59:1-8.
- ✓ **Mathew SA**, Chandravanshi B, Bhonde R., Hypoxia primed placental mesenchymal stem cells for wound healing. Life Sci. 2017 Aug 1; 182:85-92.
- ✓ Mathews S, **Mathew SA**, Gupta PK, Bhonde R, Totey. Glycosaminoglycans enhance osteoblast differentiation of bone marrow derived human mesenchymal stem cells. J Tissue Eng Regen Med. 2014 Feb; 8(2):143-52.
- ✓ **Mathew SA**, Rajendran S, Gupta PK, Bhonde R., Modulation of physical environment makes placental mesenchymal stromal cells suitable for therapy. Cell Biol Int. 2013 Nov; 37(11):1197-204.

HONORS, AWARDS AND PRESENTATIONS

- ✓ **Travel award funded by the National Institutes of Health (NIH)** for attending the Endothelial Cell Phenotypes in Health and Disease, Gordon Research Conference, Italy, July 15 - 20, 2018
- ✓ **First Prize for Poster Presentation**. Suja Ann Mathew, Sowmya Rajendran, Pawan Kumar Gupta, R. Ramesh Bhonde. "Modulation of Physical Environment makes Placental

Mesenchymal Stem Cells Suitable for Therapy" at the International Stem Cell Summit - 2013 held at Life Line Clinics and Multi-Specialty Hospital, Chennai. January 04-06, 2013.

- ✓ **Introduction to the Biology of Cancer** by Johns Hopkins University on Coursera. Certificate earned at Thursday, January 10, 2019 9:12 AM GMT (https://www.coursera.org/account/accomplishments/certificate/HSQPV36RZ7PU)
- ✓ **Suja Ann Mathew**, R. Ramesh Bhonde. "Priming Placental Mesenchymal Stromal Cells with Hypoxic Stress and Poly Unsaturated Fatty Acids To Enhance Their Angiogenic Potential". at the Gordon Research Conference - Endothelial Cell Phenotypes in Health and Disease, held at the Renaissance Tuscany II Ciocco, Italy, July 15 - 20, 2018
- ✓ **Suja Ann Mathew**, R. Ramesh Bhonde. Gestational Diabetic Placenta, a blessing in disguise??? At the Manipal Poster Presentation, Manipal University, Manipal-576104
- ✓ **Suja Ann Mathew**, R. Ramesh Bhonde. "Can Placental Mesenchymal Stem Cells act as surrogate β Cells???" at the All India Cell Biology Conference- 2013 help at Indian Institute of Science. December 22nd -24th, 2013
- ✓ Faculty at the **Stem Cell Workshop, 2011** at Manipal Institute of Regenerative Medicine.
- ✓ Demonstrator at the **Stem Cell Workshop, 2009** at Manipal Institute of Regenerative Medicine.

UTTARA CHAKRABORTY

Dr. Uttara Chakraborty joined as an Assistant Professor in the Manipal Institute of Regenerative Medicine from January 2019. She conducts classes (theory and practical) for Masters Students and is involved in making question paper sets for the course. She has been working on developing a State-of-the-Art Flow cytometry, cell sorting and Cell imaging facility in this Institute.

Key words: Flow cytometry, Cell sorting, Stem cell applications, Scientific Imaging, cutaneous wound healing and regeneration

BACKGROUND AND OBJECTIVES:

Uttara Chakraborty obtained her PhD in Fungal Biotechnology from Bose Institute, Jadavpur University, Kolkata (2008) and did her post doctoral research as a DBT-RA in Molecular Mycology (2008-2012) from the MBGU department of Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR) Bengaluru, Karnataka. As a post doc she started working on inter-specific parasexual hybridization in Candida sp., and studied the inter-relationship of ploidy and biological fitness of these micro-organisms in terms of pathogenicity in mice models. She later continued working in JNCASR as the Facility In-charge of the Flow Cell and started her training in the field extensively through various Cytometry Courses in India and also by attending courses from Excyte Cytometry.

Flow cytometry Facility

Microscopy Facility

Her recent interests include:

- ✓ Establishing a shared resource laboratory (SRL) in flow cytometry (FCM) and light microscopy
- ✓ Initiating research on Role of host-mycobiome interaction in cutaneous wound healing and regeneration.
- ✓ Stewarding the "MYO Cytometer" (Make your own) in collaboration with Dr. William Telford from National Cancer Institute, NIH, Bethesda, USA

WORK DONE:

- ✓ Initiated revamping the existing microscopy and the flow cytometry facilities of MIRM.
- ✓ Took regular Semester II theory and practical classes for the MSc students
- ✓ Submitted a project under the DBT, RRSFP scheme titled "Establishing a Shared Resource Laboratory for Development and Analysis of Immune Cell/Stem Cell Subset Phenotyping Strategies for Detection, Diagnosis and Treatment of Communicable and Non-Communicable Human Diseases"
- ✓ Conducted In-house training workshops for basic flow cytometry and microscopy

AREAS OF INTEREST, EXPERTISE AND RESEARCH

Areas of interest:

- ✓ Flow cytometry (FCM) and cell sorting for rare population detection and cellular proliferation studies, single cell sorting, circulating tumor cell detection, multicolor immunophenotyping panel designing, ploidy analyses of human commensal yeasts, confocal and superresolution microscopy technologies
- ✓ Member of the International Society for Advancement of Cytometry (ISAC) and selected by the ISAC Shared Resource Laboratory (SRL) Committee as an ISAC SRL Emerging Leader from 2018 until Dec 31, 2022 (https://isac-net.org/page/SRLEmeringLeaders)

Special interest:

- ✓ Facility management and setting up shared resource laboratory in FCM and Microscopy

Research:

- ✓ Using the model concept of the "MYO" (Make your Own) cytometer, designed and conceptualized by Prof. William Telford, National Cancer Institute, NIH, USA (https://ccr.cancer.gov/Experimental-Transplantation-and-Immunology-Branch/william-g-telford) as an educational tool to teach basics of flow cytometry to research students
- ✓ Role of Host- Mycobiome interaction in cutaneous wound healing and regeneration

HONORS, AWARDS AND POSTERS

1. Presenting Multi-media poster at the CYTO 2019 conference conducted by the ISAC (International Society for Advancement of Cytometry) in Vancouver 22-27 June 2019 on "The Make Your Own Flow Cytometer: An expanding system for flow cytometry education"
2. Co-Chairing a parallel session on New Sorting News I in the CYTO 2019 meeting
3. Selected as an SRL Emerging Leader (Shared Resource Laboratory) from ISAC in 2018 for the duration 2018-2022 by the ISAC Task Force Committee on SRL education
4. Conducted the 17th Indo-US Cytometry workshop in IISc, Bangalore (2016) as Organizing Secretary of the Committee
5. Participated in 3 different Indo-US Cytometry workshops in different states in India and worked as an instructor in modules on apoptosis and cell cycle analyses by flow cytometry in IITR, Lucknow in 2017 for the 18th Cytometry workshop.
6. Presented a poster at the EMBL Conference: CTLS 2016 - Core Technologies for Life Science, Heidelberg, under the session "Teaching and training by and for Life Science core facilities".
7. Conducted several workshops and courses on Basics of flow cytometry and Cell sorting at Indian Institute of Science at the Flow cytometry facility, and conducted regular monthly courses on confocal microscopy in the facility for in-house users of the Bio-imaging facility, Division of Biological Sciences, Indian Institute of Science.
8. Awarded the Prof. Awtar Krishan certificate as the runner up in the flow cytometry quiz organized at C-CAMP, 2013.
9. Awarded for best poster at the Scientific Sessions of the 79th SBCI Annual General Body Meeting held at IISc, Bangalore from 13th- 15th Dec, 2010.
10. Received Eukaryotic Cell Outstanding Young Investigator Award sponsored by the American Society for Microbiology at the 3rd FEBS Advanced Lecture Course on Human Fungal Pathogens, Molecular Mechanisms of Host Pathogen Interactions and virulence.
11. Awarded Young Investigator Award for a poster presentation at the 3rd FEBS Advanced Lecture Course, "Human Fungal Pathogens", May, 2009 in La Colle sur Loup, France.

SANGEETHA NATH

My research interest is broadly on understanding of cellular mechanism of gradual pathology progression in neurodegenerative diseases. Main focus of my research is, how intracellular endo-lysosomal accumulation of amyloidogenic aggregates influence direct cell-to-cell transfer of pathology via tunneling-nanotubes in Alzheimer's and Parkinson's diseases. My passion is inspiring students towards research.

Key words: Neurodegenerative diseases, Alzheimer's disease, Parkinson's disease, Protein aggregation, Prion propagation, cell-to-cell propagation, intercellular transport, tunneling nanotubes and lysosomal exocytosis.

BACKGROUND AND OBJECTIVES

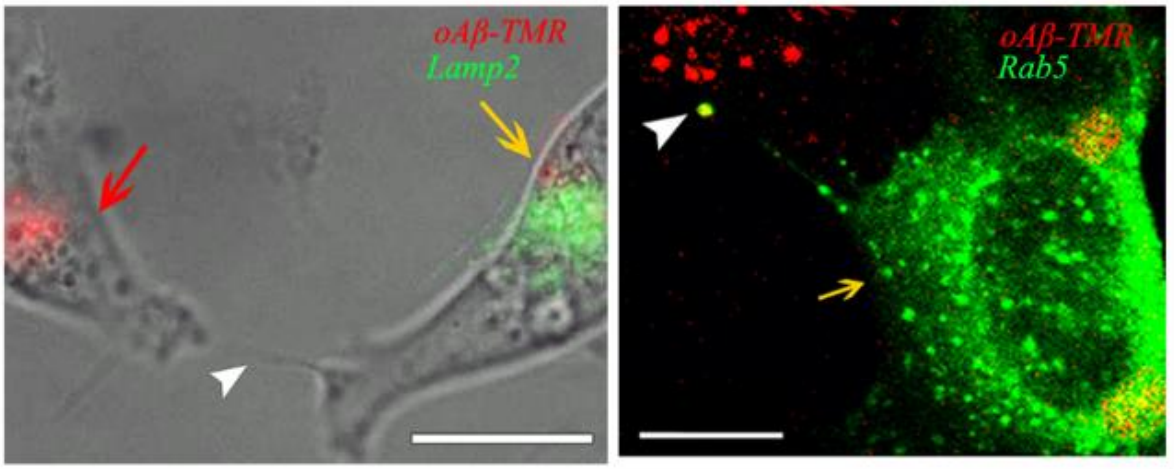
My research interest is broadly on understanding of cellular mechanism of gradual pathology progression in neurodegenerative diseases. During my Ph.D at Indian Institute of Chemical Biology, Kolkata and postdoc at KU Leuven, Belgium; I worked on characterization of transient protein aggregates using biophysical and spectroscopic methods. Later during my research tenure in Sweden at Linkoping University, I worked directly in-vivo to understand protein aggregates deposition related brain diseases, mainly Alzheimer's and Parkinson's diseases. I corresponded two research papers on how spreading of pathology is due to direct cell-to-cell transfer of amyloidogenic oligomers. Microscopic observations showed direct-cell-to-cell transfer via tunnelling nanotubes. Later, I moved to inStem, Bangalore, to learn membrane dynamics and super-resolution microscopes from Prof. Satyajit Mayor of NCBS, India and Prof. Akihiro Kusumi of Kyoto University, Japan in a joint collaboration position. I earnestly believe this expertise will have enormous impacts in revealing mechanism of TNTs formation.

WORK DONE:

My research interest is broadly on understanding of cellular mechanism of gradual pathology progression in neurodegenerative diseases. My PhD work was on characterization of in-vitro transient protein aggregates using biochemical-biophysical and spectroscopic methods. Characterizations of transient aggregates in Alzheimer's and Parkinson's diseases and their

relevance in oxidative cellular stress have also been studied thoroughly (Nath et al. Biophysical J. 2010; Nath et al. Mol. Cell. Neurosc. 2010 and Goodwin et al. Neurochem Int. 2013). During my journey from PhD to postdoc years, I implemented my knowledge of in-vitro protein aggregation directly in-vivo to understand protein aggregates deposition related brain diseases. During my research tenure in Sweden, I corresponded two research papers on how intracellular endo-lysosomal accumulation of amyloidogenic aggregates influence direct cell-to-cell transfer of pathology in Alzheimer's disease (Domert et al. Neurobiol Dis. 2014; Nath et al. J. Neurosci. 2012; Hallbeck et al. Neuroscientist. 2013). I have also worked on the dual role of high cholesterol against lysosomal membrane permeabilization in PD model (Nath et.al. Eur J Cell Biol, 2017).

Fig.1: Formation of TNTs between donor and recipient cells (white arrow head).



Microscopic observations of direct-cell-to-cell transfer of oligomers also indicated formation of tunnelling nanotube (TNT) structures (Ollinger et al. 2019). Over this journey, I realized the tremendous potential that the super-resolution imaging is having to understand the mechanism for relatively unexplored area of membrane nanotubes. Therefore, I have also worked on membrane dynamics and super-resolution microscopes. I earnestly believe this expertise will have enormous impacts in revealing relatively unexplored area of intercellular communication pathway of membrane nanotubes (TNTs).

FUTURE OBJECTIVES

Molecular basis of TNTs formation in response to lysosomal accumulation of amyloid aggregates is mostly unexplored. Our research group wanted to unfold the mechanism behind the formation of TNTs.

Precisely the Future objectives are:

- ✓ Proper structural characterizations of TNTs using quantitative super-resolution imaging.
- ✓ Systematic investigations will be conducted to understand what could be the possible stress signals and their relation with AD pathology
- ✓ Combined experience of AD pathology and membrane dynamics will help to understand how exactly recycling of vesicles and endo-lysosomal pathway involve in TNT formation.
- ✓ Why significant membrane surface expansion precedes TNTs formation? Is this a mechanism for surface area compensation of cell membrane during membrane repair process?

LIST OF STUDENTS

- ✓ Aysha Dilna, MPhil

LIST OF 5 SIGNIFICANT PUBLICATIONS

- ✓ **Nath S**, Eriksson I, Per Bornefall,Ana-Maria VG, Öllinger K. Impact of high cholesterol in a Parkinson's disease model: Prevention of lysosomal leakage versus stimulation of α-synuclein aggregation. Euro J of Cell Biol 96: 99-109; (2017).
- ✓ Domert J, Rao S.B, Agholme L, Brorsson A.C, Marcusson J, Hallbeck M, ***Nath S**. Neuritic cell-to-cell transfer of amyloid-β peptides and induced pathology is dependent on insufficient cellular clearance. Neurobiology of Disease 65:82-92, (2014). * Corresponding author.
- ✓ ***Nath S**, Agholme A, Kurudenkandy F.R, Granseth B, Marcusson J, *Hallbeck M. Spreading of neurodegenerative pathology by neuron to neuron transmission of β-amyloid. J Neurosci. 32:8767-8777 (2012). * Corresponding author. (Published as Issue Highlights in this issue and highlighted in Science News in July 14 (digital), 'Title: Alzheimer's may

be handiwork of 'prion' proteins: A-beta moves from cell-to-cell, spreading destruction.' 2012-page 4-5).

- ✓ **Nath S**, Meuvis J, Hendrix J, Carl S and Engelborghs Y. Early aggregation steps in α-synuclein as measured by Fluorescence Correlation Spectroscopy and Forster Resonance Energy Transfer. Evidence for a contagious conformational state. Biophysical J. 98:1-10 (2010).
- ✓ **Nath S**, Goodwin J, Engelborghs Y, Pountney D.L. Raised calcium promotes α-synuclein aggregate formation. Mol Cell Neurosci. 46:516-26 (2010).

HONORS AND AWARDS

Research Grants:

- ✓ Magnus Bergvalls research grant (Sweden) for the year 2015-2016
- ✓ Gun och Bertil Stohnes research grant (Sweden) for the year 2014-2015
- ✓ Alzheimer fonden (Sweden) awarded research grant for the year 2012-2013
- ✓ Alzheimer fonden (Sweden) awarded research funding for the year 2011-2012

Travel Grants:

- ✓ Fonden för teknisk personal, Linköping University (Sweden) grant for the year 2013-2014
- ✓ Kunt och Alice Wallenbergs Stiftelse, (Sweden) grant for the year 2013-2014
- ✓ **Invited Speaker:** ***Alzheimer's Association International Conference on Alzheimer's Disease,*** Paris, France, 2011
- ✓ **Invited Speaker:** ***International Conference on Electron Microscope,*** Delhi, India, July, 2014
- ✓ **Speaker:** ***Conférences Jacques-monod, Protein folds in diseases,*** Aussois, France, 2009.
- ✓ **Organizer** in *Bangalore Microscopy Course*, 2017-2019
- ✓ Biography published in **Marquis Who's Who in the World**, 2014 and 2015 Editions.
- ✓ **2010-2012 - Post-Doctoral Fellowship**, Awarded by, Linköping University Hospital, Sweden
- ✓ **2007-2009 - Post-Doctoral Fellowship,** Awarded by FWO (Research Foundation, Belgium)
- ✓ **2005-2007 - Senior Research Fellowship,** Awarded by CSIR, India

- ✓ **2001- GATE (Graduate Aptitude Test in Engineering, India)** in Pharmaceutical Technology

MEENAL FRANCIS

Meenal Francis is a senior lecturer with nearly ten years of teaching experience in the field of stem cells. A post graduate in biotechnology from Bangalore University and a PhD scholar working in the field of diabetes under the guidance of Dr. Anujith Kumar, Associate professor, MIRM. Teaching at MIRM since September 2007.

Key words: Diabetes, induced pluripotent stem cells (IPSCs), islets, transplantation, tumor, cancer stem cells, epithelial to mesenchymal transition (EMT)

BACKGROUND AND OBJECTIVES

Current research interest: For long term sustenance apart from traditional medication, cadaveric islet transplantation was thought to be an ideal treatment for diabetes; however, shortage of donors has diverted focus of treatment towards transplantation of *in vitro* differentiated pancreatic β cells for which iPSCs seem a promising candidate. IPSCs are gathering great attention as an alternate to treat degenerative diseases such as diabetes owing to their ability of being autologous and having high proliferative and differentiation potential. Following developmental cues and directing pluripotent stem cells towards pancreatic β cells although have been partially successful, alongside have drawbacks of differentiated cells being heterogeneous, immature and poly hormonal. Moreover, the role of the non-β cells during differentiation and transplantation is yet unknown.

Given the scenario, the project aims at understanding the role of non-β cells derived from genetically engineered iPSCs in reversing *in vivo* hyperglycemia in a Streptozotocin induced diabetic mouse model. This novel line of iPSCs are derived from fibroblasts of double transgenic mice harboring RIP (Rat Insulin promoter)-Cre along with loxed YFP (Yellow fluorescent protein), which will be further on mentioned as RIP-YFP, to address the above issues. During the

course of differentiation towards pancreatic β-cells; cells expressing insulin will also express YFP, which serves as a convenient tool to fractionate the homogenous population of insulin secreting cells. Using this cell line we are trying to delineate the importance of various cellular components while transplantation of pancreatic beta cells derived from IPSCs in diabetic mouse model systems.

<u>An overview of derivation, characterization and differentiation of mouse RIP-YFP-IPSCs towards pancreatic beta cell precursor</u>

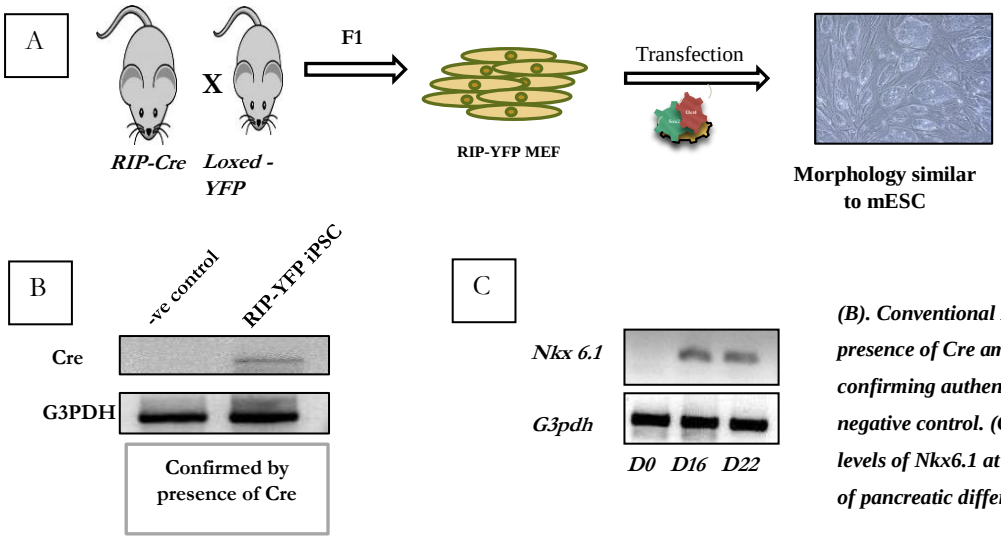


Fig: (A).Schematic representation of the derivation of RIP-YFP-IPSCs and phase contrast microscopic photograph of the derived iPSCs.

(B). Conventional PCR of genomic DNA indicating the presence of Cre amplified by Cre specific primers confirming authentic iPSC cultures. mESCs was used as a negative control. (C) Gel picture indicating expression levels of Nkx6.1 at day 16 and Day 22 compared to Day 0 of pancreatic differentiation.

PAST RESEARCH WORK

A. To study the role of HEF1 (Human Enhancer of Filamentation 1), a focal adhesion protein; in the epithelial to mesenchymal transition in breast carcinomas and other solid epithelial tumors:
The study involved; culture of established breast normal and transformed cell lines, over-expressing and depleting levels of HEF1 and studying its effect on the epithelial and mesenchymal markers by western- blotting, RT-PCR and immunoflourscence.
It also involved immunohistochemical analysis of these markers in relation to HEF1 in non-tumor and tumor breast tissue sections who had been followed up for 5 years. This was done to establish a correlation of expression of HEF1 in breast cancers with the grade of the cancer, to put HEF1 as a possible prognostic marker for meatstasis.

B. To study HEF1 and its correlation to cancer stem cells:
The study involved processing and culturing of cells isolated from human breast tissue (both normal and cancer) as spheres (non-adherent cultures) and primary epithelial cells (adherent

cultures). These cells were then characterized by RT-PCR, immunoflourescence, western blotting and flow cytometry for expression of mesenchymal, epithelial and self-renewal markers.

FUTURE PLANS:

Stem cells, regenerative biology and cellular therapy are the upcoming fields in the current era of medical treatments. There have been various research works world over that show promising therapeutic potentials of stem cells. Yet there is a lot of research that is required in the field.

Alongside teaching, my future plan is to establish a research area in the stem cells arena to fortify the concept of this new medical therapy.

ADJUNCT FACULTY

RAMESH BHONDE

Dr. Ramesh Bhonde obtained his Ph.D. from NIV, Pune and Postdoc from the University of Calgary Canada. He was the Dean of MIRM from April, 2011 till June, 2016 and is currently serving as an Adjunct Faculty at MIRM India.

Key words: Islet engineering, stem cells, diabetes, obesity, Insulin resistance, immune-isolation, islet biology

BACKGROUND AND OBJECTIVES

Generation of islet like cell aggregates (ICAs) from pancreatic and non- pancreatic stem cells, their characterization, cryopreservation, cytoprotection and make them suitable for transplantation. Unavailability of human pancreatic islets and restriction of animal usage for islet isolation for diabetes research and therapeutics prompted us to look for alternative sources of human tissue derived stem cells to get islet equivalents. Earlier work from this lab established the methodology for obtaining ICAs from human dental pulp stem cells (DPSCs) and umbilical cord derived MSCs (UC MSCs).

Objectives:

1. To engineer large number of islets from different exotic sources of mesenchymal stem cells
2. To test defined small molecules for islet protecting activity against hypoxia, inflammatory cytokines and during cryopreservation of islets
3. To examine potential of human adipose derived stem cells against insulin resistance and placental stem cells for wound healing
4. To examine potential of DPSCs for dopamine secreting neural cell differentiation
5. To develop simple test for detecting genetic stability of MSCs

WORK DONE:

We showed for that first time that MSCs derived from mouse pancreatic tissue have the potential to generate functional islets (J CB 2012). Our data revealed that DPSCs could differentiate into functional islets which reverses experimental diabetes in mice upon transplantation (Cytotherapy 2013). DPSCs were shown to differentiate into dopamine secreting neurons (JCP 2014). We demonstrated for the first time that MSCs are genetically stable by estimating nuclear blebs and micronuclei (Cytotherapy 2015 JCP2015) and can be employed for testing genotoxicity of compounds (Mutagenesis 2015).A cocktail of small molecules has been developed that protests pancreatic isles and MSC derived ICAs against cryopreservation (JOP 2014,RDS 2014,) hypoxia (JCB 2016, Europian J Pharmacology 2016) Conditioned media from adipose tissue derived MSC have been shown to decrease insulin resistance in in vitro models (JCB 2016)

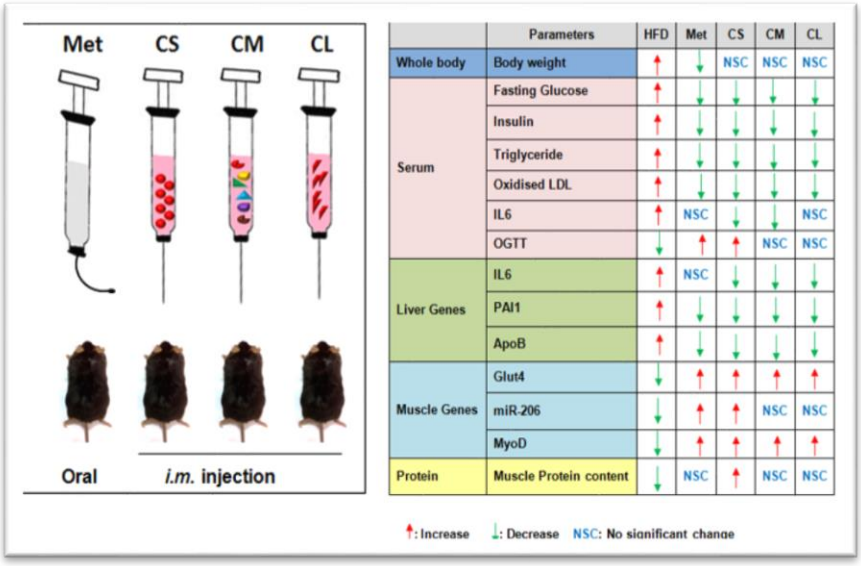


Fig: Met: Metformin, CS: Cell Suspension, CM: Conditioned Media, CL: Cell Lysate, LDL: Low density lipoprotein, IL6: Interleukin-6, OGTT: Oral glucose tolerance test, PAI1: Plasminogen activating Inhibitor1, ApoB: Apolipoprotein B, Glut4: Glucose transporter-4, miR-206: Micro RNA-206, MyoD: Myogenic Differentiation 1.

FUTURE PLANS

1. To explore potential of intramuscular islet transplantation in diabetes reversal
2. To examine whether ADMSC injections could ameliorate insulin resistance in high fat diet induced obese mice

LIST OF STUDENTS

1. Rajarshi Pal: 2012
2. Mohammad Kanafi: 2014
3. Shikha Sharma: 2014
4. Renjitha: 2015
5. Murali Mamidi: 2016
6. Suja Ann Mathew- 2018
7. Nitya Shree- 2018
8. Bhawna Chandravanshi: 2018

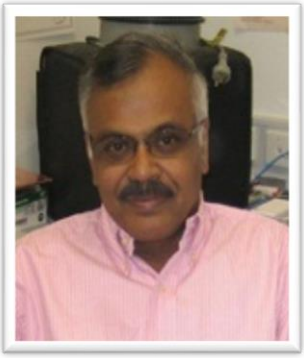
HONORS AND AWARDS

1. Dr TMA Pai Endowment Chair for Islet engineering & Banking during 2013-14, 2015-16
2. Dr C M Habibullah Oration Award conferred during Annual Meeting of the Society for Regenerative Medicine & Tissue Engineering on 31st January 2014
3. Appointed as Honorary Visiting Professor at the University of Colombo, Sri Lanka

LIST OF 5 SIGNIFICANT PUBLICATIONS

1. Chandravanshi B, Bhonde RR. Shielding Engineered Islets with Mesenchymal Stem Cells Enhance Survival Under Hypoxia. J Cell Biochem. 2017 Jan 18. doi: 10.1002/jcb.25885. [Epub ahead of print]
2. Shree N, Bhonde RR. Can yoga therapy stimulate stem cell trafficking from bone marrow? J Ayurveda Integr Med. 2016 Jul - Sep;7(3):181-184. doi: 10.1016/j.jaim.2016.07.003. Epub 2016 Sep 17.
3. Shree N, Bhonde RR. Metformin preconditioned adipose derived mesenchymal stem cells is a better option for the reversal of diabetes upon transplantation. Biomed Pharmacother. 2016 Dec;84:1662-1667. doi: 10.1016/j.biopha.2016.10.086. Epub 2016 Nov 11.
4. Shree N, Bhonde RR. Conditioned Media From Adipose Tissue Derived Mesenchymal Stem Cells Reverse Insulin Resistance in Cellular Models. J Cell Biochem. 2016 Oct 28. doi: 10.1002/jcb.25777. [Epub ahead of print]

5. Hassan S, Bhat A, Bhonde RR, Lone MA. Fighting Diabetes: Lessons from Xenotransplantation and Nanomedicine. Curr Pharm Des. 2016;22(11):1494-505.



ARUNASALAM DHARMARAJAN

Professor Arun Dharmarajan was educated at the University of Madras, India and the University of Western Australia, Perth, Australia (PhD;1985). He did his postdoctoral training at Johns Hopkins University School of Medicine, Baltimore, USA. On completion of his postdoctoral training, he was appointed as an instructor in the department of Gynecology and Obstetrics and subsequently was promoted to Assistant and Associate Professor in the same department. Professor Dharmarajan, moved back to UWA late 1994 having spent 10 years at Johns Hopkins, Baltimore USA. He has been a Professor in the School of Anatomy and Human Biology, University of Western Australia, Perth since 2001. Currently Professor Dharmarajan is at the Faculty of Health Sciences in a role as a Co-ordinator of South Asia Research Initiatives for the School of Biomedical Sciences. He is also associated with MIRM as an Adjunct Faculty from Jan, 2018 till date.

BACKGROUND AND OBJECTIVES

Professor Dharmarajan has carried out pioneering studies in apoptosis (Physiological Cell Death) and cell signaling mechanisms in several endocrine Organs such as corpus luteum, placenta, uterus, and ovary etc. His laboratory has cloned several apoptosis-associated genes. In late 1990, Professor Dharmarajan along with Professor Bob Friis, University of Bern, Switzerland discovered Secreted Frizzled Related Protein-4 (sFRP4), a wnt anatagonist. Professor Dharmarajan's group has published extensively on the role of sFRP4 and apoptosis and cancer and more recently its role in blocking angiogenesis. He has US, India and Australia patent for this work. His group also has a shown that sFRP4 can cause the cancerous ovarian cells to become more sensitive to chemotherapy treatments in laboratory studies. Professor Dharmarajan's current research interest is cancer stem cells and ways to specifically target cancer stem cells.

LIST OF 5 SIGNIFICANT PUBLICATIONS

1. Regan, S. L. P., P. G. Knight, J. L. Yovich, Y. Leung, F. Arfuso, and A. Dharmarajan. 2018. "Granulosa cell apoptosis in the ovarian follicle-A changing view." Frontiers In Endocrinology 9 (MAR)
2. Ko, Y. A., M. F. B. Jamaluddin, M. Adebayo, P. Bajwa, R. J. Scott, A. M. Dharmarajan, P. Nahar, and P. S. Tanwar. 2018. "Extracellular matrix (ECM) activates ÃŸ-catenin signaling in uterine fibroids." Reproduction 155 (1): 61-71.
3. Deshmukh, A., F. Arfuso, P. Newsholme, and A. Dharmarajan. 2018. "Regulation of cancer stem cell metabolism by secreted frizzled-related protein 4 (SFRP4)." Cancers 10 (2)
4. Brook, N., E. Brook, A. Dharmarajan, C. R. Dass, and A. Chan. 2018. "Breast cancer bone metastases: pathogenesis and therapeutic targets." International Journal Of Biochemistry And Cell Biology 96: 63-78.
5. Visweswaran, M., F. Arfuso, R. J. Dilley, P. Newsholme, and A. Dharmarajan. 2018. "The inhibitory influence of adipose tissue-derived mesenchymal stem cell environment and Wnt antagonism on breast tumour cell lines." International Journal Of Biochemistry And Cell Biology 95: 63-72.

(Website: https://staffportal.curtin.edu.au/staff/profile/view/A.Dharmarajan)

KAUSHIK CHATTERJEE

Dr. Kaushik is an Associate Professor at Indian Institute of Science with a Ph.D. in Bioengineering from Pennsylvania State University, an M.S. in Materials Science and Engineering from the University of Virginia and B.E. in Metallurgical Engineering, Bengal Engineering College

BACKGROUND AND OBJECTIVES

Our research work is in the area of bioengineering. It is interdisciplinary in nature and typically involves the application of advanced materials technologies to address biomedical problems. We use physical cues from engineered biomaterials to influence biological responses including bacterial and human stem cell responses on biomaterial surfaces and in 3D tissue scaffolds.

The current research activities in our group can be broadly divided into the following major areas:

- ✓ Scaffolds for tissue engineering: We fabricate 3D scaffolds from novel biodegradable polymers and polymer nanocomposites incorporating different types of nanomaterials to promote tissue generation in the scaffolds.
- ✓ Engineering organotypic tissue models: We are studying human cells in 3D scaffolds toward engineering tissues in vitro that mimic the cell response in vivo to facilitate the study of cell biology and drug screening in tissue-like environments including breast tumor, intestinal and cardiac tissues.
- ✓ Ti-alloys for medical implants: We are developing surface engineering techniques for conventional biomedical alloys and novel alloys for use in the next-generation of orthopedic implants with enhanced mechanical and biological performances.

LIST OF 5 SIGNIFICANT PUBLICATIONS

- ✓ S. Nilawar, Q. Dasgupta, G. Madras, **K. Chatterjee**: "Degradable poly(ester amide)s from olive oil for biomedical applications" Emergent Materials 2019: in–press [Invited article]
- ✓ V. Agarwal, N. Vargehese, S. Dasgupta, A.K. Sood, **K. Chatterjee**: "Engineering a 3D MoS2 foam using keratin exfoliated nanosheets" Chemical Engineering Journal 2019, 374: 254–262
- ✓ J. Hasan, A. Roy, **K. Chatterjee**, P.K.D.V. Yarlagadda: "Mimicking insect wings: The roadmap to bio-inspiration" ACS Biomaterials Science and Engineering 2019: in–press
- ✓ S. Acharya, S. Bahl, S.S. Dabas, S. Hassan, V. Gopal, A.G. Panicker, G. Manivasagam, S. Suwas, **K. Chatterjee**: "Role of aging induced α precipitation on the mechanical and tribocorrosive performance of a β Ti-Nb-Ta-O orthopedic alloy" Materials Science and Engineering C 2019, 103: 109755
- ✓ B. Nayak, G.M. Balachander, S. Manjunath, A. Rangarajan, **K. Chatterjee**: "Tissue mimetic 3D scaffold for breast tumor-derived organoid culture toward personalized chemotherapy" Colloids and Surfaces B: Biointerfaces 2019, 180: 334–343

(Website: https://sites.google.com/site/iiscbiomaterials/home)

STEM CELL CULTURE AND RESEARCH FACILITY FOR 8 INDEPENDENT GROUPS: We have an advanced cell culture facility that is used for both educational and research purposes. It is currently being used for studying human and mouse pluripotent stem cells, adult stem cells and various other animal cell types.

CELL CULTURE FACILITY

- Class 2 Biological safety cabinets MSC Advantage from Thermo Electron.
- CO_2 Incubators Heraeus Heracell 240 from Thermo Electron.
- Refrigerated and Non refrigerated Heraeus Centrifuges from thermo electron.
- Fridges, Freezers and Liquid Nitrogen tanks for sample storage and cell banking.
- Stocks of Basic consumables like culture media, sera, growth factors, analytical and research reagents, and cell culture dishes.
- There is also a separate autoclave system for sterilization.

INSTRUMENTATION FOR MOLECULES & GENE ANALYSIS

- PCR thermocyclers: Gene Amp PCR System 9700, Applied biosystems, Veriti 96 well thermocycler, Applied biosystems, Step one plus and 7500 Real Time PCR System, Applied biosystems
- Spectrophotometers: Optizen 2120UV Plus, Nanodrop, Perkin Elmer multimode Plate Reader Ensight

MICROBIOLOGY FACILITY: We have a microbiological facility for Recombinant DNA technology related work and cloning experiments.

IMAGING FACILITY: We have an imaging facility with high quality fluorescent, Phase contrast and stereo microscopes.

- Fluorescence Microscopes
- Nikon Eclipse TE2000 U with a Qicam Fast 1394 digital camera.

- Nikon Eclipse 80*i* with a Qicam Fast 1394 digital camera.
- Olympus 1X73 with QiClick cooled camera

  Inverted microscopes:
- Nikon eclipse TE 2000-S microscope with a Retiga 2000R digital camera.
- Nikon eclipse TE 2000-S microscope with a Qicam Fast 1394 digital camera

  Stereo microscopes:
- Nikon SMZ 1500 stereo microscopes

FLOW CYTOMETRY FACILITY:

- Becton Dickenson FACS Calibur.

LIBRARY:

Our library has a print and electronic collection of latest editions of over 500 books. We have over 15 independent terminals for electronic access to subscribed journals including **Stem Cells, Regenerative Medicine, Stem Cell and Translational Medicine, Cytotherapy** etc. The library is supplemented with Wi-Fi internet, audio visual facilities printers and scanners.

STUDENTS ALUMNI

Name: Joseph Mathew K.
Batch: 2007-2009
Current position & organization:
PhD Scholar, NCBS, TIFR

Name: Raghavan Vallur, PH.D
Batch: 2007-2009
Current position & organization: *Postdoctoral researcherAcademic Associate for Life Sciences at the Gateway Education, Sonipat, Delhi-NCR*

Name: Deepti Mathew
Batch: 2007-2009
Current position & organization:
Editor, FCB Health, New York

Name: Sardesai Varda Sandeep
Batch: 2008-2010
Current position & organization:
Research Assistant at St. Vincent's Centre for Applied Medical Research, Sydney, Australia

Name: S. Rangarajan, PH.D.

Batch: 2008-2010

Current position & organization:

Postdoctoral Research Fellow, University Health Network (UHN), Ontario, Toronto, Canada

Name: Sumit Rai, PH.D.

Batch: 2008-2010

Current position & organization: *Postdoctoral Fellow, Massachusetts General Hospital (MGH) and Harvard Medical School (HMS)*

Name: Gurrala Charan Thej

Batch: 2009-2011

Current position & organization:

Stempeutics Pvt. Ltd, India

Name: Kavina Ganapathy K. PH.D.

Batch: 2009-2011

Current position & organization: *Postdoctoral Fellow, National Center for Biological Sciences*

Name: Sudhanshu Shekhar, PH.D.

Batch: 2010-2012

Current position & organization: *Postdoctoral Scholar, Guldberg Musculoskeletal Research Laboratory, University of Oregon, Eugene, OR*

Name: Dhruv Raina

Batch: 2010-2012

Current position & organization: *Research Associate, Max Planck Institute of Molecular Physiology, Dortmund*

Name: Chaitra Venugopal

Batch: 2011-2013

Current position & organization:

PhD (2018), MIRM, MAHE

Name: Shalini Suresh

Batch: 2012-2014

Current position & organization: *PhD Student, Department of Tumor Immunology, ACTREC-TMC, Navi-Mumbai*

Name: Venkata Anudeep B

Batch: 2012-2014

Current position & organization: *PhD student, Department of Gene Therapy & Regenerative Medicine (GTRM), Free University of Brussels (VUB)*

Name: Shilpa Patil

Batch: 2012-2014

Current position & organization: *PhD student, Goettingen University*

Name: Dr Manoj B Bansode

Batch: 2012-2014

Current position & organization: *Manager, Laboratory Operations Curecells Stem Cell Banking & Research Pvt Ltd,Chinchwad, Pune, Maharashtra*

Name: Dr. Danish N

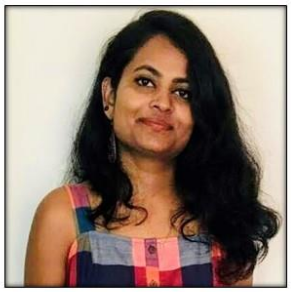
Batch: 2013-2014

Current position & organization: *Medical Superintendent, Fortis Memorial Research Institute*

Name: Ankita Arun Hiwale

Batch : 2013-2015

Current position & organisation : *Ph.D. Scholar, Dr.Praveen Kumar Vemula's lab Instem, Bangalore*



Name: M. David Luther

Batch : 2013-2015

Current position & organisation : *PhD. Scholar, SORM, MAHE*

Name: Vikrant R. Patil

Batch: 2014-2016

Current position & organisation : *Research Associate, Regenerative Medicine Laboratory/ Biotechnology & Bioinformatics Institute, Dr. D. Y. Patil, Pune*

Name: Akshata Malkood

Batch: 2015-2017

Current position & organisation: *Research Associate at GROW Lab, Narayana Nethralaya, Bangalore, India*

Name: Dr. Mata Sundeep

Batch: 2016-2018

Current position & organisation : *PhD. Scholar, MIRM, Bangalore*

Name: Nishtha Kusum Jain

Batch: 2016-2018

Current position & organisation : *M.Phil in Biophysics, NIMHANS*

GALLERY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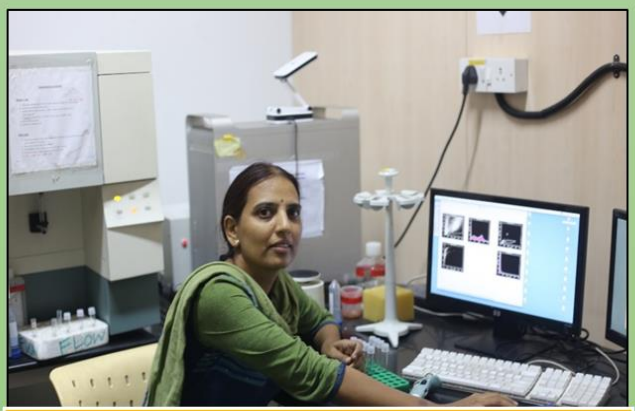
M.Sc. LABORATORIES
LIBRARY

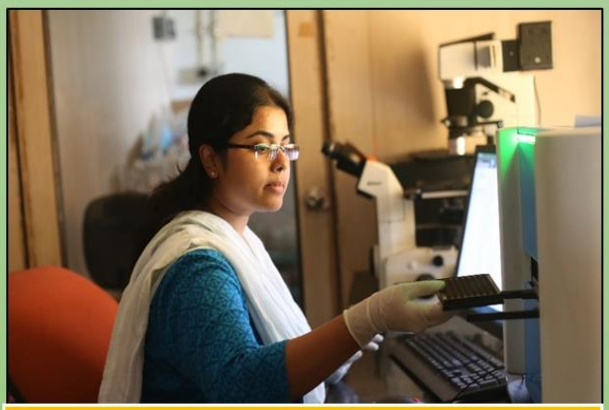
RESEARCH FACILITIES

JRF LABORATORY

CELL IMAGING FACILITY

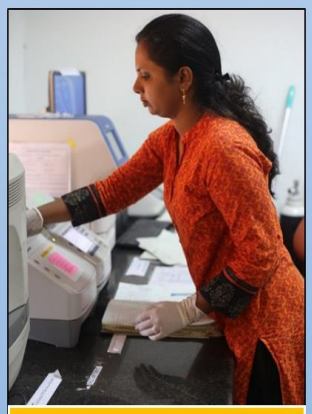


FLOW CYTOMETRY FACILITY



SPECTROMETRY FACILITY

FLUOROSCENCE IMAGING FACILITY



PCR FACILITY

CRYOPRESERVATION FACILITY

RESEARCH ACTIVITIES

NATIONAL SCIENCE DAY CELEBRATION QUIZ COMPITITION

NATIONAL STEM CELL CONFERENCE

INVITED GUEST LECTURES

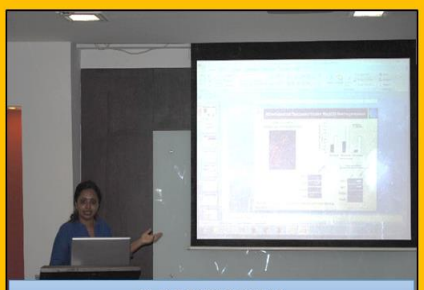
NATIONAL TECHNOLOGY DAY

TRAINING WORKSHOPS

VISITS BY INTERNATIONAL SCIENTISTS

LECTURE PRESENTATION BY SORM FACULTY

POSTER PRESENTATION BY SORM FACULTY



PhD DEFENCE VIVA

ACADEMIC ACTIVITIES

UNIVERSITY CONVOCATION AT MANIPAL

UGC MEETING

UGC INSPECTION

BOARD OF STUDIES MEETING

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Cover Page*:*

Differentiation of Embryonic Stem Cells to neural progenitors

(Shagufta Parveen, PhD)

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