Vol: 8 October - December 2023

Botheracues

Quarterly Newsletter of Manipal Centre for Biotherapeutics Research

Higher education



MCBR

Industrial

research

Translational research

Patrons

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Dr. Sharath Kumar Rao, Pro Vice-Chancellor, Health Sciences, MAHE

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Biotheracues -

Message from the Chief Editor



Dear friends and well-wishers of MCBR,

I am delighted to present you with the eighth volume of *Biotheracues*.

The last quarter was arguably the most eventful and busiest one since the inception of this center. We not only celebrated our second establishment day and first-ever annual 'sports day', but also inaugurated new laboratory facilities at MCBR, and co-hosted a 7-day handson workshop under the DBT-STUTI scheme.

The second batch of students of MSc in Biotherapeutics by Research finished their firstsemester curriculum in December and now are ready for their internship in different biopharma companies and research labs across India.

The faculty strength at MCBR also increased during this quarter with the joining of Dr. Abhayraj S. Joshi. I must thank him for significantly contributing to this edition of Biotheracues as an associate editor. We also inaugurated the 'National Fellowship Training Club' to guide our masters and newly joined doctoral students for national-level PhD fellowship examinations. We are expecting a new batch of Ph.D. students in the upcoming quarter.

As always, we hope to receive your feedback to make this newsletter a better read for all of you.

Warm regards.

Dr. Souvik Dey

Second Establishment Day Manipal Centre for Biotherapeutics Research, MAHE, Manipal



Manipal Centre for Biotherapeutics Research (MCBR, MAHE) celebrated its <u>2nd Establishment Day</u> on 16th October 2023. MAHE Pro-Chancellor Dr. H. S. Ballal was the president of the function, and MAHE Vice-Chancellor Lt. Gen. (Dr.) M. D. Venkatesh was the chief guest. Provice chancellor of Health Sciences Dr. Sharath K. Rao and Pro-vice chancellor of Strategy and Planning Dr N. N. Sharma were the guests of honour. MAHE Leadership appreciated the impressive progress made by the MCBR family in the last two years under the leadership of its Coordinator Prof. (Dr.) Raviraja N. S.



Inauguration of DST-STUTU ICT Workshop on "Technical Approaches in the Purification and Characterization of Biomolecules"







Technical

Approaches

Involved in the

Purification and

Characterization

Biomolecules

Od - 10 December, 2023
MHE, Manipal, Karnataka

7-days hands-on workshop, jointly hosted by the Manipal Centre for Biotherapeutics Research (MCBR) and the Manipal College of Pharmaceutical Sciences (MCOPS) at Manipal Academy of Higher Education (MAHE), Manipal, on <u>"Technical Approaches Involved in the</u> <u>Purification and Characterization of Biomolecules"</u> was inaugurated by the MAHE Vice Chancellor, Lt. Gen. (Dr.) M. D. Venkatesh, on 4th December 2023, at the Dr. PGR Conference Hall, MCOPS, MAHE Manipal. Prof. (Dr.) Raviraja N. S., the chairperson of the organizing committee, welcomed the gathering, and Dr. Souvik Dey, the organizing secretary, gave the preamble of this workshop. Dr. Vadiraja Bhat, the business development manager for Country Biopharma, and scientist from Agilent Technologies, Bengaluru, India was the chief guest for this event. The inaugural ceremony was presided over by Lt. Gen. (Dr.) M. D. Venkatesh, the Vice-Chancellor of Manipal Academy of Higher Education, Manipal. Prof. Dr. Mallikarjuna Rao, the principal of MCOPS, proposed the vote of thanks. This workshop was focused on handson activities encompassing recombinant protein purification, antibody characterization, and mass-spectrometry-based biochemical profiling. Proceedings of DST-STUTU ICT Workshop on "Technical Approaches in the Purification and Characterization of Biomolecules"













During this 7-day hands-on workshop, participants underwent rigorous theoretical as well as practical training. Every day, the morning session was dedicated to lectures from distinguished and well-experienced speakers from academia and the biopharma industries; whereas the afternoon session was spent entirely in labs for hands-on training on the latest edition of high-end instruments. On the final day, for the Valedictory session, Dr. B. S. Satish Rao (Director, Research, Directorate of Research, MAHE, Manipal, and Director, Manipal School of Life Sciences, MAHE, Manipal) was invited as chief guest and Dr. C. Mallikarjuna Rao (Principal, Manipal College of Pharmaceutical Sciences, MAHE, Manipal) attended the ceremony as the *Guest of Honor*. After listening to the heartfelt gratitude expressed by several participants for providing them with this scientific opportunity and looking at their bond of friendship formed over the period, we could say the workshop was a great success!

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ACTIVITIES AT MCBR





Inauguration of new laboratories at MCBR

This year, MCBR inaugurated four new labs established on the 2nd Floor of the building:

- 1. 3D Bioprinting & Cell Culture Lab,
- 2. Tissue Handling & Processing Lab,
- 3. Biochemistry Lab, and
- 4. General R&D Lab-3

Dr. Sharath K. Rao – Pro-vice Chancellor, Health Sciences, MAHE and Mr. C. G. Muthana – Chief Operating Officer, Operations, MAHE were the chief guests. Mr. Khilesh A. Surana – Deputy Director, Projects was the guest of honour. New labs will meet the growing needs of MCBR and they will contribute significantly to the glory of MCBR in future.





Inauguration of National Research Fellowship" training club

MCBR, MAHE, inaugurates "National Research Fellowship" training club, marking a significant step towards research career development. The MCBR Lecture Hall was buzzed with enthusiasm and anticipation on the 15th December 2023, as the curtains rose on the inauguration of the new club dedicated to "National Research Fellowship Training." Mr. Satish Pai, Head of HR at MAHE, Manipal, graced this occasion as the Chief Guest.





Faculty recruitment

MCBR proudly welcomes **Dr. Abhayraj S. Joshi** as Assistant Professor, effective from 9th October 2023. Dr Abhayraj Joshi secured his Ph.D. from Indian Institute of Technology Kanpur (IIT-Kanpur) and completed his Postdoctoral training at Technical University of Denmark (DTU). His areas of expertise include Nanomedicine, Neurodegenerative diseases therapy, Cancer therapy.

Biotheracues .



RESEARCH PROGRESS

Publications:

 Alok Ghosh Chaudhuri, Saptadip Samanta, Monalisha Dey, Raviraja N S, <u>Souvik Dey</u>*. Role of Alpha-fetoprotein in Pathogenesis of Cancer. *Journal of Environmental Pathology, Toxicology and Oncology*. (Accepted for publication; Article ID: JEP(T)-49145); (Q3_IF: 2.4).
 *Corresponding author.

RESEARC

- Alotaibi A*, Gadekar VP*, Gundla PS, Mandarthi S, Ravi S, Mallya D, Thungekar A, Lavanya BV, Bhagavath AK, Cordero MW, Pitkaniemi J, Raviraja NS, Bepari A, Hebbar P., A comprehensive analysis of mRNA expression profiles of Esophageal Squamous Cell Carcinoma reveals downregulation of Desmoglein 1 and crucial genomic targets. Cancer Biomarkers, 2023, 38(4):465-487. doi: 10.3233/CBM-230145. (Q2_IF: 3.8)
- Prachi Agarwal, Akankshya Kar, Kirthanashri S V, Subhas C Kundu, Sonia Kapoor. Silk protein based smart hydrogels for biomedical applications. Silk-Based Biomaterials for Tissue Engineering, Regenerative and Precision Medicine. Editors: Subhas C. Kundu, Rui L. Reis. Elsevier. ISBN: 97803239601752022.

PATENT

Patents applied:



Online talks:

- Dr. Maloy Ghosh, Chief Scientific Officer (CSO) of Zumutor Biologics, Bengalore delivered an online talk on the "Outlook for the Immunotherapy of Cancer and Vision of Zumutor Biologics" on 14th October 2023. He also gave his opinions about current scenario of immunotherapy and had an excellent discussion with MCBR faculty, research staff, and M. Sc. students.
- Dr. Sarita Aryal, a Postdoctoral Fellow from Dana-Farber Cancer Institute, Harvard Medical School, Boston, USA interacted with M.Sc. first-semester students and presented her research work titled "Biophysical studies of the αβ T-cell receptors (TCRs) to develop TCRbased vaccines" on 27th November 2023.
- Dr. Kanupriya Singh, a Scientist from Immuneel Therapeutics Pvt. Ltd., Bangalore delivered an online talk on "Development of allogenic platform for the generation of CAR $\gamma\delta$ T-Cells for the immunotherapy of the solid cancers" on 13th December 2023.





Prof. (Dr.) Raviraja, the Coordinator of MCBR, was selected as the **Dr. TMA Pai Endowment Chair,** in *Stem Cell Research and Therapeutics,* on October 31, 2023, for the next one year. MCBR faculty members and research scholars celebrated this prestigious achievement of Dr. Raviraja.

Dr. Abhishek Kumar Singh, an Professor, Associate MCBR, MAHE, Manipal, delivered an oral presentation "Chlorogenic acid ameliorates titled oxidative stress and neurotoxicity by amyloid-beta through induced activation of autophagy and pro-survival signalling pathways" at the 42nd Annual Conference of Society of Toxicology -India (STOX 2023). The conference was organized by the Department of Zoology, University of Calicut, Kerala and it was held in Kerala, between 23rd to 25th November 2023..





Dr. Souvik Dey, DBT - Ramalingaswami Fellow and Assistant Professor, MCBR, MAHE, Manipal, was selected as <u>Review</u> <u>Editor on the **Editorial Board** of Molecular</u> <u>and Cellular Reproduction</u> (specialty section of *Frontiers in Cell and Developmental Biology*) in November 2023.

Dr. Souvik Dey presented a poster titled "<u>Role of FTO in Spermatogenesis</u>" at the '92nd Annual Meet of The Society of Biological Chemists hosted by BITS Pilani'. The conference was held at BITS-Pilani, K. K. Birla Goa Campus, Goa between 18th to 20th December 2023.

ARTICLE UNDER FOCUS

Cancer Biomarkers 38 (2023) 465–487 DOI 10.3233/CBM-230145 IOS Press

IOS Press



A comprehensive analysis of mRNA expression profiles of Esophageal Squamous Cell Carcinoma reveals downregulation of Desmoglein 1 and crucial genomic targets

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Esophageal Squamous Cell Carcinoma (ESCC) is a specific kind of esophageal cancer that develops in the squamous cells of the esophagus. Just 19% of people diagnosed with ESCC have a 5-year survival rate. This underscores the need to identify biomarkers with high confidence that can aid in the early diagnosis, prognosis, and identification of potential therapeutic targets for the cure of ESCC. The authors of this published article conducted a meta-analysis of 10 mRNA datasets and successfully identified genes that were consistently disrupted in all of the experiments. In addition, the authors utilized ESCC ATLAS to separate 'core' genes and examine the effects of primary gene perturbations. They next investigated relationships between genes and dysregulated molecular signaling networks. Through the integration of toxicogenomics data, the scientists found the gene connections with environmental exposures, trace elements, chemical carcinogens, and therapeutic compounds. In addition, they inferred the clinical results of potential genes by doing survival analysis on the ESCC dataset from The Cancer Genome Atlas. Through this study, they have successfully identified 237 previously recognized and 18 newly discovered disrupted candidate genes. Desmoglein 1 (DSG1) is a gene that was noticeably reduced (Fold Change = -1.89, p-value = 8.2e-06) in ESCC across six distinct datasets. The authors also identified 31 'core' genes that include genetic variations or are regulated by epigenetic alterations. They also discovered important biological pathways through the interaction of these genes in gene-gene networks. The functional enrichment analysis revealed substantial dysregulation of biological processes and pathways.



The CRISPR/Cas9 System Delivered by Extracellular Vesicles

Mr. Ramnarayan Bhat, Dr. TMA Pai Scholar, MCBR, MAHE

The tiny vesicles, previously disregarded as 'cellular debris' have emerged as pivotal components of wide array of bio therapeutic applications. As scientific exploration progressed, the significance of extracellular vesicles, as vital agents orchestrating diverse physiological functions, both normal and disease related has been unveiled. Simultaneously as they are involved in various physiological functions such as immune response, tissue repair and cell growth the value of EVs in the field diagnostics, therapeutics and theragnostics, has been uncovered. In addition to this recent advancement have shown them as vectors to deliver genes and biomolecules.

The CRISPR/Cas system was initially identified as bacterial and archaeal defensive mechanisms against foreign nucleic acids. The simpler type II CRISPR/Cas9 system, particularly using a single Cas9 protein aided by a synthetic guide RNA (sgRNA) formed from crRNA and trans-activating crRNA (tra crRNA), has become widely applied in genome editing across various fields such as basic biology, biomedicine, and agriculture research. CRISPR has enabled efficient editing of human cells in culture and is being potentially utilised as therapeutic tool for treating human diseases (1). However, CRISPR-Cas9 has to be delivered to the site action in in vivo system to bring out efficient outcome. Conventionally Adenoviruses have been used as in vivo gene delivery and were also used to deliver CRISPR/Cas9(2). However, limited viral genomic DNA packaging capacity, requirement of neutralizing antibodies against AAV capsids, and immunogenicity limits the utilisation of these systems for development of therapeutics(3,4).

Several Extracellular vesicle based systems have been developed to transfer ribo-nucleoprotein to the site of interest. Recently developed Nano vesicle based ribonucleo protein delivery system (NanoMedic), uses chemical induced dimerization to recruit Cas9 protein into EVs, followed by a viral packaging signal and two self-cleaving riboswitches for releasing sgRNA into these Nano vesicles. The delivery system was utilised to efficiently edit the genome in induced pluripotent stem cell, neurones and myoblasts with 90% efficiency in these 'hard to transfect' cell types. The systems utility in invivo genome editing was proved in exon skipping luciferase reporter mouse model and mdx mice model. The technology was tested for treating Duchenne muscular dystrophy (DMD), a muscular degenerative disease caused by X-linked gene mutation. Mutation leads to loss of dystrophin protein in skeletal and cardiac muscle cells causing loss in muscle stability and reduction in muscle mass. The exon skipping modifies the splicing pattern there by preserves the partial production of functional dystrophin. Single intramuscular injection of NanoMEDIC induces permanent genomic exon skipping indicating the efficiency of the system

in *in vivo* genome editing therapy of Duchenne muscular dystrophy (DMD)(5).

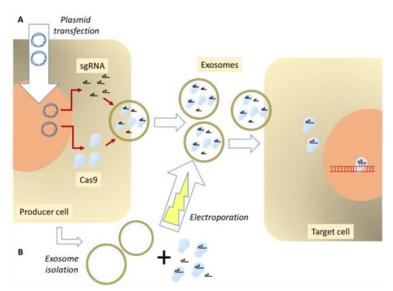


Figure: General routes for loading CRISPR/Cas components into Evs. (A) Endogenous loading: producer cells can be transfected with plasmids encoding the Cas9 protein and the sgRNA. (B) Exogenous loading CRISPR/Cas components into EVs (Horodecka and Düchler, 2021).

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2. Knott GJ, Doudna JA. CRISPR-Cas guides the future of genetic engineering. Science. 2018 Aug 31;361(6405):866–9.

3. Louis Jeune V, Joergensen JA, Hajjar RJ, Weber T. Preexisting anti-adeno-associated virus antibodies as a challenge in AAV gene therapy. Hum Gene Ther Methods. 2013 Apr;24(2):59-67. doi: 10.1089/hgtb.2012.243. Epub 2013 Apr 3. PMID: 23442094; PMCID: PMC3732124.

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5. Gee P, Lung MSY, Okuzaki Y, Sasakawa N, Iguchi T, Makita Y, et al. Extracellular nanovesicles for packaging of CRISPR-Cas9 protein and sgRNA to induce therapeutic exon skipping. Nat Commun. 2020 Mar 13;11(1):1334.

6. Horodecka K, Düchler M. CRISPR/Cas9: Principle, Applications, and Delivery through Extracellular Vesicles. Int J Mol Sci. 2021 Jan;22(11):6072.



Exploring the capabilities of biological robots

Mrs. Jahnavy Joshi, Research Scholar, MCBR, MAHE

Biological robots, or biobots are engineered multicellular structures now being explored to target disease site. Biobots started with a combination of supporting substances like hydrogels and living cells, and assembling them to form a kind of living structure. From there rose the invention of xenobots, which had only embryonic cells from frogs and are motile without any extrinsic force. A new study took this one step further by using human bronchial epithelial cells to form a spheroid with cilia. Culturing these cells in specific cell culture media assisted these cells to form spheroidal organoids with cilia facing outwards from the structure. A key point to note here is that in natural systems, the cilia faces towards the lumen, but these anthrobots have cilia facing outwards to assist their locomotion. This was done by tailoring the culture conditions, first by growing the cells on matrigel and then replacing the medium to water-based culture medium after dissolving the basement membrane. By day 7 of the culture the motile appendages, which in this case are cilia appeared. It was also observed that the viscosity of the cell environment along with the initial seeding density plays a key role in the self assembly of the cells to form anthrobots. Their movement was linear, curvilinear or circular and showed consistent pattern dependent on their developmental morphology. To analyse their in vitro action, human induced pluripotent stem cell derived neurons were used for a simple scratch assay wherein it was observed that these anthrobots can track and move along the scratch outline contributing to the scratch closure, but the mechanism still needs to be studied. The regenerative and delivery capabilities of these anthrobots can be explored in future for personalized medicine, but more study about their mechanisms needs to be done.

Reference:

Gumuskaya, G., Srivastava, P., Cooper, B. G., Lesser, H., Semegran, B., Garnier, S., & Levin, M. (2022). Motile living biobots self-construct from adult human somatic progenitor seed cells. bioRxiv, 2022-08.

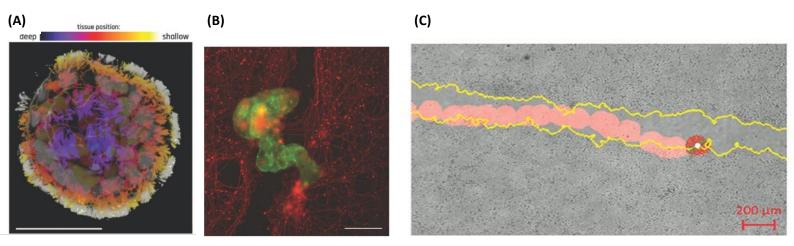


Figure: (A) Anthrobot immunostained with α-tubulin (cilia marker), ZO-1 (tight junction marker), and DAPI (nuclear stain), (B) Anthrobot immunostaining reveals sample bridge superbot (green) and the neuronal tissue (red), (C) Sample tracking output with scratch edge highlighted in yellow and bot path in red.

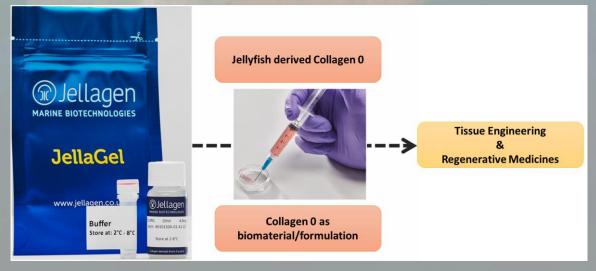
GLOBAL RESEARCH UPDATE

Dr. Abhayraj S Joshi, Assistant Professor, MCBR, MAHE

Jellyfish is one of the invertebrates that dates back to around 500 million years ago. A great deal of research is being carried out in order to understand the biology of jellyfish and their role in marine ecosystems. Recently, a collaborative offbeat research done by the National Physical Laboratory (NPL, UK) and biotech company Jellagen Ltd. using one species of jellyfish: Rhizostoma pulmo; also known commonly as Barrel Jellyfish, has opened doors for cell biologists, material scientists, bioengineers, formulation scientists, and biotechnologists. Their new research has revealed the potential of jellyfish collagen as a versatile biomaterial for medical applications and biological research. The human collagen (Types I, II, III, IV, and V) are inherent part of several tissues such as skin, tendon, blood vessels, organs, bone, cornea, cartilage, reticular fibers found in connective tissue of organs, basement membrane in various tissues, cell surfaces, cornea, hair, and placenta. Their research has confirmed that the collagen isolated from *Rhizostoma pulmo* has structural similarity with human collagen types I, II, III, and V which allows one to use it for several medical applications. As this collagen predates to the mammalian collagen, it has now been termed as Collagen zero (Collagen type 0). Due to its simple nature and ancient ancestry to mammalian collagen, it is biocompatible and safe. Unlike other collagens, due to its inherent anti-inflammatory properties, it has shown tremendous potential in chronic wound healing and tissue regeneration without scar formation. Furthermore, its formulations (such as scaffolds, fillers, hydrogels, pellets, sponges, films, beads, patches, and plugs) have shown promising results in cell and gene therapy, cardiovascular complications, orthopedic complications, bone tissue engineering, and cell culture. The research published in Materials Today Bio journal (Q1, IF 8.2) shows that the collagen matrix supports various human cells, including stem cells, immortalized cell lines, and primary cells. It also enables key biological functions like neuronal firing synchrony, stem cell differentiation, and cancer spheroid formation. Most human cells need extracellular matrices (ECM) for support, communication, and tissue/organ development. According to the research done by NPL and Jellagen Ltd., this revolutionary biomaterial can form a collagen matrix that supports cell adhesion, ECM secretion, cell migration, and cell survival just like its mammalian counterparts. As this ECM is obtained from Collagen type 0, it is termed as "ECM type 0". As it is being investigated thoroughly for its applications, several mysteries surrounding the jellyfish collagen will be unraveled. After solving these mysteries, this revolutionary biomaterial will certainly bring a paradigm shift in collagen chemistry as stated by Prof. Andrew Spragg, chief scientific officer (CSO) of the Jellagen Ltd. In the near future, it will become a revolutionary platform for tissue engineering and regenerative medicine.

References:

- 1. https://jellagen.co.uk/
- 2. Nilofar Faruqui *et al.*, Extracellular matrix type 0: From ancient collagen lineage to a versatile product pipeline JellaGel[™], Materials Today Bio, Volume 22, 100786, 2023.



NOTABLE VISITORS

Drugs Controller General of India (DCGI), **Dr. Rajeev Singh Raghuvanshi** briefly visited MCBR, MAHE on 19th November 2023 and interacted with faculty and researchers. He lauded the research focus and lab infrastructure of MCBR. We were proud to host the top drug regulator of India.





Mr. Ragupathi Bhat - an exmember of the Karnataka Legislative Assembly from the Udupi Assembly constituency visited MCBR, MAHE and its laboratory set up on 28th October 2023 and lauded the vision, mission, infrastructure, and progress of the MCBR. We sincerely thank him for his gracious visit. **Prof. Paul Stone, Dr. Shelini Surendran, Prof. Kamalan Jeevaratnam** – from University of Surrey, UK visited MCBR, MAHE on 16th October 2023 and interacted with MCBR faculty with an intention of scientific collaboration. They shared their research focus with MCBR and praised the MCBR for its vision and mission.



NOTABLE VISITORS

Mr. Maheshwer Peri (Chairman & Founder, Career360) and Mr. Dhruv Aggrawal, (Head of Client Delivery, Careers 360) visited MCBR, MAHE on 30th October 2023, and had a tour of the MCBR facility.





Manjunatha Dr M. V.. Professor at NIMHANS. Associate Bengaluru, and adjunct faculty at MCBR, MAHE, visited MCBR on 15th November 2023 and interacted with faculty, researchers and students. He shared his rich experience in Neurovirology and Vaccinology with us. Mr. Kamal Karanth, Co-founder, XPHENO also visited to explore collaboration with MAHE. We thank them both profusely.

Dr. Srinivas Sekallu from Anthem Biosciences visited MCBR, MAHE and its laboratory setup on 3rd November 2023 and interacted with MCBR faculty. He also shared his experience at Anthem Biosciences during the interaction. We thank him for this wonderful interaction.



NOTABLE VISITORS



Prof. Gouri Gargate from IIT Kharagpur visited MCBR on 17th November 2023 and interacted with MCBR faculty and MCBR research staff to share her views.

Prof. Kanneboyina Nagaraju, and Professor Dean of **Pharmaceutical Sciences** at Binghamton University, State University of New York visited MCBR on 8th December 2023 and MCBR faculty. interacted with During his brief visit he shared his experience in drug discovery and translational research in the fields of "Autoimmune and Genetic muscle diseases".





Dr. Rambabu Atluri, Founder and CEO of Elvikon India visited MCBR on 15th December 2023 to take a look at MCBR vision, mission, and research infrastructure. He praised MCBR efforts towards stem cell research and biotherapeutics discovery.

Celebrating last working day of first semester of second batch of M. Sc. Students before sending them to industry



On 9th December 2023, we celebrated last working day of the first semester of second batch of M.Sc. Students (2023-2025 batch) before sending them to the research companies for completing rest of their curriculum in the form of active participation in ongoing research project of the company. All the faculties interacted with students and celebration ended with the high tea. We wish you all the best for your research projects..!!





FUN MOMENTS

We celebrated Navaratri festival from 14th October 2023 to 22nd October 2023 by spending brief time for *Pooja* and *Bhajan* everyday.

















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FUN MOMENTS

We celebrated our very first "Annual Sports Day" on 28th October 2023 with various events.



As a service to the community and as a part of , MCBR staff and students did Kodi Bengre beach cleaning on 4th November 2023.



MCBR had cakes and claps for the birthdays of our research students and staff!







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MANIPAL CENTRE FOR BIOTHERAPEUTICS RESEARCH MANIPAL

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Par Jah





MCBR

(Institution of Eminence Deemed to be University)

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