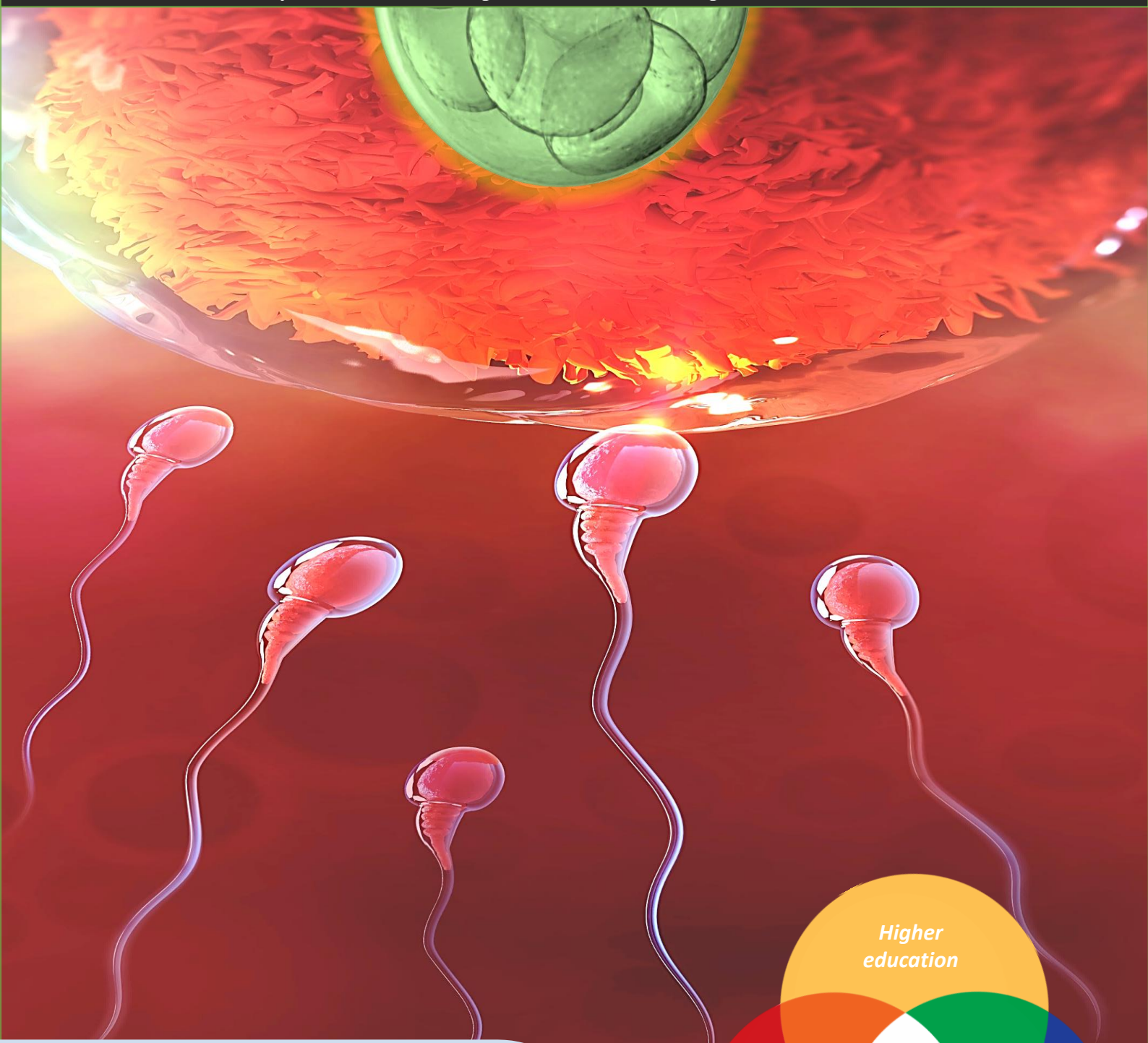
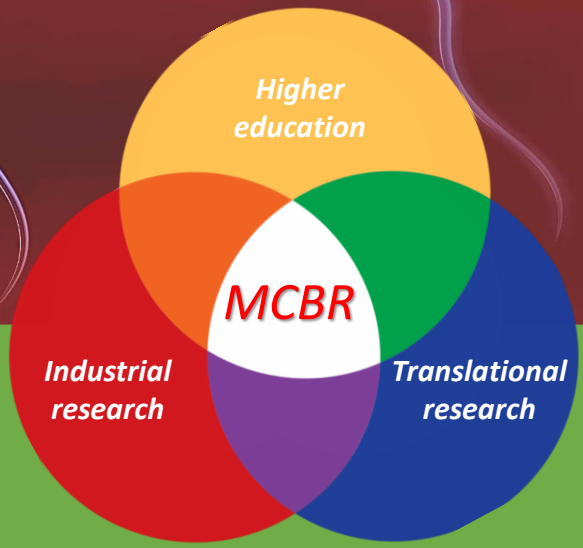
Vol: 6 April-June, 2023

Biotheracues

Quarterly Newsletter of Manipal Centre for Biotherapeutics Research, MAHE





MANIPAL
ACADEMY of HIGHER EDUCATION
(Institution of Eminence Deemed to be University)
INSPIRED BY LIFE

Contents

Message from the Chief Editor

Dear friends and well-wishers of MCBR,

I am pleased to present you with the sixth volume of *Biotheracues*.

The second quarter of this year was both eventful and productive in terms of research outcomes.

The current issue of *the newsletter* is going to provide us with the different aspects of academic advancement made by MCBR; MCBR filed its first patent during this period. We were visited by multiple international delegates from top ranked universities of Europe and the USA. The inauguration of the workshop on "3D-Bioprinting for Biomedical Applications" under the convenorship of Dr. Kirthanashri was one of the momentous events that happened during this period. Professionals from different MAHE campuses and from organizations like Tata Steel participated in this workshop. Besides that, multiple webinars and invited talks were conducted during this quarter. Students and faculties across the MAHE institutes took part in those.

Your feedback and suggestions are always welcome to enhance the quality of our newsletter.

Warm regards.

Dr. Souvik Dey

Inauguration of Workshop on "3D Bioprinting for Biomedical Applications"

Lt Gen (Dr) M D Venkatesh, the Vice Chancellor of Manipal Academy of Higher Education (MAHE) in Manipal, inaugurated a three-day hands-on workshop on "3D bioprinting for Biomedical Applications" at the Manipal Centre for Biotherapeutics Research (MCBR), MAHE. This workshop, the first of its kind in Manipal, aims to train participants in generating the G code, designing 3D structures, and using printing software for 3D bioprinting.

During the event, Lt Gen (Dr) M D Venkatesh highlighted the significance of 3D bioprinting in translational research, emphasizing its potential benefits for various medical fields such as orthopedics, dentistry, and dermatology. He expressed optimism that 3D bioprinting advancements would contribute to improved patient care, envisioning the future printing of organs like the esophagus, trachea, and liver. Lt Gen (Dr) M D Venkatesh praised the collaboration between MAHE and industry partner "***Alfatek Systems***" in organizing the workshop.

The chief guest of the function, **Dr. Sharath K Rao,** Pro Vice-Chancellor of Health Sciences at MAHE and a renowned orthopedic surgeon, shared his experiences using 3D printed bone implants in orthopedic applications.

He expressed his hope that 3D bioprinting would have wide-ranging applications in the medical field and even in the food industry, mentioning the growing industry of 3D-printed meat.

Mr. Sumant Bhutoria, the proprietor of Alfatek Systems in Kolkata and the convenor of the workshop, highlighted the importance of 3D bioprinting and its healthcare applications. Dr. Anil Kumar, a Scientist G from the Sree Chitra Tirunal Institute of Medical Science & Technology in Trivandrum, participated as one of the resource persons, while other resource persons from Switzerland and Finland delivered online talks.

Dr. Raviraja NS, Professor, and Coordinator of MCBR, welcomed the gathering and explained the research focus areas at MCBR.

Dr. S V Kirthanashri, associate professor at MCBR and the workshop convenor, provided a brief introduction to the workshop. Around 10 participants from leading biopharmaceutical companies and research institutions are taking part in the workshop. **Dr. Raghavendra Upadhaya**, assistant professor at MCBR and workshop co-convenor, delivered the vote of thanks, and **Ms. Jahnavy Joshi,** DST Inspire fellow and Research Scholar, served as the anchor for the inauguration program.

ACTIVITIES AT MCBR

MCBR faculties with the participants during the workshop on 3D bioprinting

MCBR organized an OPEN HOUSE on 27th May from 9.30 am to 12.30 pm. Prospective students and researchers from various academic institutions visited and explored the facility, and interacted with faculties.

INVITED TALKS

Dr. S Varadharajan, Coordinator, IPTTO, MAHE, presented a talk on the topic, titled "A Detailed Insight Into Patenting Process" on 16th June 2023. He answered to various queries posed by the faculties and researchers of MCBR about the patent filing process. Later he was felicitated by Prof. Raviraja NS, Coordinator, MCBR.

Dr. Abhishek Singh, Assistant Professor, Amity Institute of Neuropsychology & Neuroscience, Amity University, Uttar Pradesh delivered an online talk on the topic titled, "Autophagy as a Promising Therapeutic Target for Neuroprotection" on 2nd June 2023. It was followed by a Q&A session between Dr. Singh and MAHE faculties.

Dr. Manu M Joseph, Senior Research Associate, Inter-University Centre for Genomics & Gene Technology (IU-CGGT), University of Kerala, presented a virtual talk on the topic titled, "Exploring New Frontiers in Biomedicine: Unveiling the Impact of My Scientific Research" on 9th June 2023.

Dr. Raghu Ramanathan, Postdoctoral Fellow, Department of Medicine-Gastroenterology division, University of Missouri, Columbia, USA, delivered a virtual talk on the topic titled, "Low dose thyroid hormone improves mitochondrial function and ameliorates diet-induced non-alcoholic fatty liver disease in mice" on 10th June 2023.

INSTITUTIONAL WEBINAR

Dr. Souvik Dey, DBT-Ramalingaswami Fellow and Assistant Professor-Research, MCBR, MAHE presented an online talk on the topic, "*Glycogen Synthase Kinase 3 alpha – the Master Regulatory Kinase in Male Reproduction*" on 12th May 2023.

Research Progress

Publications:

- Indrashis Bhattacharya#, **Souvik Dey#** and Arnab Banerjee. Revisiting the Gonadotropic Regulation of Mammalian Spermatogenesis: Evolving Lessons During the Past Decade. ***Frontiers in Endocrinology***. 2023. (doi: 10.3389/fendo.2023.1110572). (**IF: 6.05**). #Equal contributor.

- Pawan Kumar Gupta, P. Shivashankar, M. Rajkumar, Subhendu S. Mahapatra, Sanjay C. Desai, Anita Dhar, Vinay Krishna, **N. S. Raviraja**, Samatha Bhat, Pachaiyappan Viswanathan, Suresh Kannan, Jijy Abraham, Hema Boggarapu, M. S. Manjuprasad and K. Udaykumar. Label extension, single-arm, phase III study shows efficacy and safety of stempeucel® in patients with critical limb ischemia due to atherosclerotic peripheral arterial disease. Gupta et al. ***Stem Cell Research & Therapy.*** 2023, https://doi.org/10.1186/s13287-023-03292-w (**IF: 8.079**)

- Kodali M, Madhu LN, Reger RL, Milutinovic B, **Upadhya R**, Attaluri S, Shuai B, Shankar G and Shetty AK. A single intranasal dose of human mesenchymal stem cell-derived extracellular vesicles after traumatic brain injury eases neurogenesis decline, synapse loss, and BDNF-ERK-CREB signaling. ***Front. Mol. Neurosci***. 2023. doi: 10.3389/fnmol.2023.1185883 **(IF: 5.13)**

- Meghana Kasturi, Mrunmayi Gadre, Vidhi Mathur, Varadharajan S, **Kirthanashri S V (2023)**. Three-Dimensional bioprinting for Hepatic Tissue Engineering: From In Vitro Models to Clinical Applications. ***Tissue Engineering and Regenerative Medicine*** **(IF: 4.45**)

- Meghana Kasturi and **Kirthanashri S V**. Effect of Decellularization Using Sodium Dodecyl Sulfate on Glycosaminoglycans Content In Liver. ***Regenerative Medicine***. 2023. DOI: 10.2217/rme-2023-0050 (**IF: 3.8**)

Patent applied:

- Title: Decellularized esophagus as an ink for 3D printed tubular scaffold; Contributors: Vidhi Mathur, **S V Kirthanashri & Raviraja NS**. Application No. 202341042840.00. Date of filing: 26-Jun-2023

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A single intranasal dose of human mesenchymal stem cell-derived extracellular vesicles after traumatic brain injury eases neurogenesis decline, synapse loss, and BDNF-ERK-CREB signaling

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A single intranasal dose of human mesenchymal stem cell-derived extracellular vesicles after traumatic brain injury eases neurogenesis decline, synapse loss, and BDNF-ERK-CREB signaling

It has been reported that an optimal intranasal (IN) dose of human mesenchymal stem cell-derived extracellular vesicles (hMSC-EVs), administered 90 minutes after traumatic brain injury (TBI), prevents the progression of acute neuroinflammation into chronic neuroinflammation, thereby reducing long-term cognitive and mood impairments. This study examined whether hMSC-EV treatment following TBI can prevent hippocampal neurogenesis decline and synapse loss during the chronic phase of TBI. At 90 minutes post-traumatic brain injury (TBI), C57BL6 mice with unilateral controlled cortical impact injury (CCI) received a single intravenous (IV) administration of various doses of EVs or the vehicle. At 2 months post-TBI, quantification of neurogenesis in the subgranular zone-granule cell layer (SGZ-GCL) using 5'-bromodeoxyuridine and neuron-specific nuclear antigen double labeling revealed decreased neurogenesis in TBI mice receiving vehicle. incorporation into neurons and microglia in both the injured and uninjured hemispheres. However, the extent of neurogenesis in TBI rodents receiving EVs (12.8 and 25.6 109 EVs) was comparable to that of naive controls. When doublecortin-positive newly generated neurons in the SGZ-GCL were measured 3 months after TBI, a similar trend of decreased neurogenesis was observed. The aforementioned concentrations of EVs treatment reduced the loss of pre- and post-synaptic marker proteins in the hippocampus and somatosensory cortex following TBI. Furthermore, at 48 h post-treatment, brain-derived neurotrophic factor (BDNF), phosphorylated extracellular signal-regulated kinase 1/2 (p-ERK1/2), and phosphorylated cyclic AMP response-element binding protein (p-CREB) levels were decreased in TBI mice receiving the vehicle but were closer to nave control levels in TBI mice receiving above doses of hMSC-EVs. Notably, the improved BDNF concentration observed in the acute phase of TBI rodents receiving hMSC-EVs was maintained in the chronic phase. Thus, a single intravenous (IV) dose of hMSC-EVs administered 90 minutes after TBI can mitigate TBI-induced decreases in BDNF-ERK-CREB signaling, hippocampal neurogenesis, and synapses.

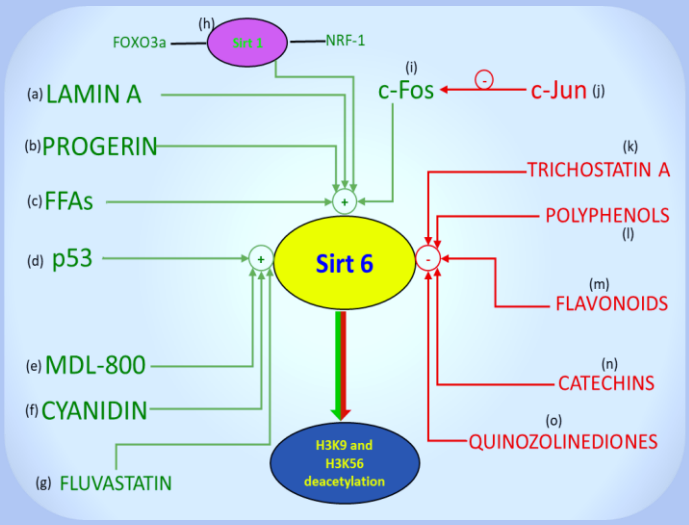
Sirt6 - A Key Target in Aging and Metabolic Disorders

Liston Augustine D'Souza *, Research Intern, MCBR, MAHE*

Sirtuins are NAD+-dependent epigenetic regulators that alter histone acetylation/deacetylation and are involved in regulating cellular processes such as cellular stress, inflammation, insulin resistance, chromatin silencing, cell cycle regulation, insulin resistance, transcription, and apoptosis. Among various sirtuin homologs, Sirt6 is an essential nuclear sirtuin. In recent years Sirt6 has surfaced to play an important role in regulating the pathophysiology of diabetes, cardiovascular disease, lipid disorder, neurodegenerative disease, cancer, and aging and as a therapeutic target in the treatment of these conditions.

The functions of Sirt6 are diversified. On activation, Sirt6 acts on various substrates that contribute to alleviating many pathological processes. The table below describes the action of Sirt6 in aging and metabolic disorders. Sirt6 delays aging by stabilizing telomeres and inhibiting IGF-1. In cardiac disorders, they improve heart glucose metabolism, protecting from heart failure and hypertrophy. They also have a protective action on neurodegenerative diseases by acting on GSK3α/β that decreases tau protein accumulation. It also affects the metabolism of lipids and glucose through its action on various other substrates.

Having a plethora of biological activities, Sirt6 makes an attractive molecule for researchers to develop effective therapeutics. Sirt6 is activated in calorie restriction via NAD+, but there are various other modulators that modulate its action. The figure below depicts the action of various modulators in either activation or inhibition of Sirt6.



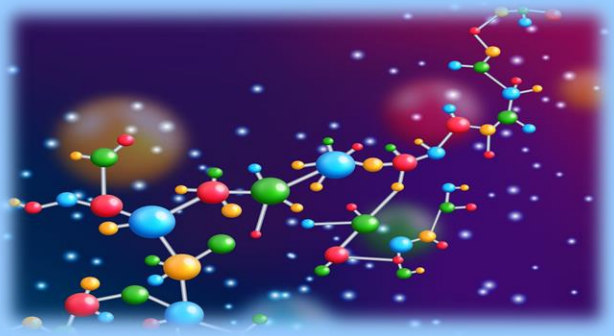
DISEASE	TARGET	EFFECT	References
Aging	H3K9ac	Telomere stability, DNA damage response	(Khan R. I. et al., 2018)
	H3K56ac	Telomere stability, DNA damage response	(Khan R. I. et al., 2018)
	H3K18ac	Heterochromatin silencing	(Khan R. I. et al., 2018)
	IGF-1	Reduction in somatotropic axis	(Mao et al., 2018)
Cardiac disorder	PDK4	Improve cardiac glucose metabolism	(Khan D. et al., 2018)
	IL1, NF-KB	Inhibit activation of pro-inflammatory cytokines responsible for atherosclerosis; Prevent endothelial damage	(Vitiello et al., 2017)
	ICAM-1, PAI-1	Protect endothelial damage	(Guo et al., 2019) (D'Onofrio et al., 2017)
	IGF	Prevent heart failure and cardiac hypertrophy	(Sundaresan et al., 2012)
	Nmnat-II	Activates Nmnat-II, prevent cardiac hypertrophy	(Cai et al., 2012)
Neurodegenerative disease	WRN	Maintains genomic stability and telomeric length	(Khan R. I. et al., 2018)
	GSK3α/β	Decreased tau protein activation	(Tang, 2019)
	Aβ42	Prevents DNA damage	(Jung et al., 2016)
Lipid metabolism	SREBP	Suppresses LDL-cholesterol synthesis	(Kuang et al., 2018) (Tao et al., 2013b)
	CK2	Facilitates adipogenesis	(Kuang et al., 2018) (Chen et al., 2017)
	PGC1α	Increased Brown adipose tissue thermogenesis and expression of thermogenesis genes.	(Kuang et al., 2018) (Yao et al., 2017)
	PCSK9	Decreased LDL cholesterol levels	(Kuang et al., 2018) (Tao et al., 2013a)
Diabetes	TXNIP	Increased glucose stimulated insulin secretion	(Qin et al., 2018)
	FOXO1	Reduces expression of gluconeogenic genes	(Kuang et al., 2018)
	GLUT1, LDH & PDK1	Promote glycolysis	(Hu et al., 2006) (Zhong et al., 2010)
	GCN5 & PGC1α	suppress hepatic gluconeogenesis	(Dominy et al., 2012)

Despite discovering various Sirt6 modulators that act directly or indirectly, further specific modulators could generate therapeutic targets that will enhance current therapy. Due to its diversified effects, targeting Sirt6 poses both challenges as well as a ray of hope for extensive studies to understand their precise mechanism and functioning, further as a potential therapeutic target that can enhance or even substitute the existing lines of therapy.

References:

1. Raj S, Dsouza LA, Singh SP and Kanwal A (2020) Sirt6 Deacetylase: A Potential Key Regulator in the Prevention of Obesity, Diabetes and Neurodegenerative Disease. *Front. Pharmacol. 11:598326. doi: 10.3389/fphar.2020.598326.*

1. Kanwal A, Dsouza LA (2019) Sirtuins and diabetes: optimizing sweetness in the blood. *Transl. med. Commun 4:3. doi.org/10.1186/s41231-019-0034-7*

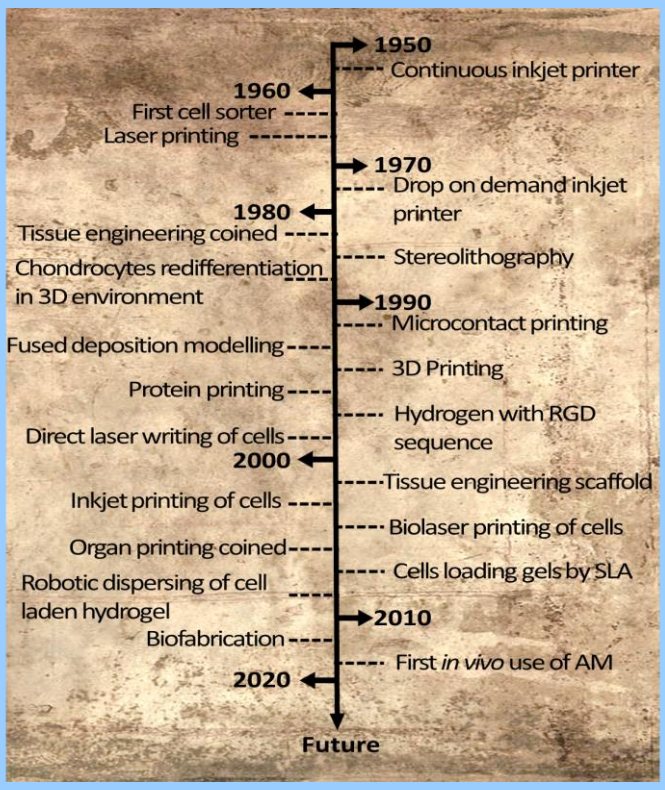


A Brief History on the Development of Tissue Engineering

Mrunmayi Gadre, *Dr. TMA Pai Scholar, MCBR, MAHE*

Tissue engineering is a technique that involves the use of biomaterials to fabricate 3D platforms for in vitro culture. The recent advances in the field of tissue engineering which have empowered researchers to dive deeper into the advancement of various techniques and one such technique is 3D bioprinting. Although the technology is wide spreading recently it is a very old technology aging almost more than 70 years old. This technology has now been gradually applied in various fields like drug discovery, patient-specific repair and regeneration, and various other therapies like transplantations.

Early 1980s and 1990s was the era when Charles Hull first discovered stereolithography which allowed lead to creation of models in multiple layer by using resin. During this time there were assumptions that such developing technology can have direct impact for developing the modern science and it's medicinal applications. There were also other developments in the production of consumables for 3D printing focusing majorly on hydrogels. Later, in 1996, Dr. Gabor Forgacs who was then the founder of Organovo, now a leading company studied the cell behaviour in order to combine it with the materials for developing spatial structures. In 2000, University of Wake forest first created the synthetic organ using the spatial scaffold design. Another project led by Professor Anthony Atala developed a prototype of a 3D kidney which astonished the public during his TED talk. In 2003, a leading scientist from the university of EL Paso, Thomas Boland developed his own device which is the first ancestor of currently used bioprinters. In 2004, Dr. Forgacs made his debut with his own bioprinter, it was a first of it's kind and helped in the 3D direct biodegradation where the live cells could be printed without the need of scaffold. In 2009 the technology was then applied by the ORGANOVO to generate NOVOGEN MMX and within few months' time, biodegradable blood vessel was bioprinted. Following years major development took place where the scientific research projects gave out results in developing structures such as baptismal tissue in 2012, liver in 2012, tissues with the blood-borne network in 2014 and the heart valve in 2016. In 2015, a leading Swedish company, CELLINK engineered their own bioprinting machine named INKREDIBLE costing $ 5,000. In the near future there would be increasing number of application of the bioprinting technology leaving behind the illusions of organ production. According to the leading scientist this technology can also become the standard equipment in hospitals as well as major laboratories and factories.



Synthetic Embryo to Revolutionize *In Vitro Fertilization*

Scientists have achieved a groundbreaking development in human reproduction by successfully creating synthetic human embryos using stem cells. This achievement has the potential to revolutionize the current understanding of *In Vitro Fertilization* (IVF) in the field of human reproduction, as it eliminates the need for eggs and sperm in the creation of human embryos.

Although the full details of this remarkable work conducted at the Cambridge-Caltech lab are yet to be published in a journal paper, it was described during a plenary address at the annual meeting of the International Society for Stem Cell Research in Boston. Professor Magdalena Żernicka-Goetz, from the University of Cambridge and the California Institute of Technology, emphasized that this human model represents the first three-lineage human embryo model, specifically identifying amnion and germ cells, which are precursor cells of egg and sperm. The Guardian quoted her as stating that these synthetic embryos, created entirely from embryonic stem cells, are a remarkable achievement.

Synthetic embryos generated from stem cells lack a beating heart or the beginnings of a brain, but they do possess the cells that form the placenta, yolk sac, and the embryo itself. These synthetic embryos serve an important purpose in scientific research. Scientists believe that they could provide crucial insights into the biological causes of recurrent miscarriages, which affect an estimated 23 million pregnancies worldwide annually.

Moreover, they aim to gain a deeper understanding of the "black box" period of human development, which encompasses the duration between 16 or 17 days after fertilization and over a week after the free-floating embryo attaches to the uterine lining.

Currently, the legal limit for cultivating embryos in the laboratory is 14 days. Beyond that point, researchers rely on pregnancy scans and donated embryos for studying further development. By employing stem cells to accurately model normal human embryonic development, scientists hope to acquire extensive knowledge about the early stages of human development and identify potential abnormalities without the need for early-stage embryos in research. Robin Lovell-Badge, the head of stem cell biology and developmental genetics at the Francis Crick Institute in London, explained that this approach would enable researchers to gain valuable information about the initiation of development and potential issues that may arise.

In summary, the creation of synthetic human embryos using stem cells represents a significant breakthrough in the field of human reproduction. These embryos have the potential to provide insights into the causes of recurrent miscarriages and enhance scientific understanding of early human development without the necessity of using early-stage embryos in research.

Reference:

https://www.theguardian.com/science/2023/jun/14/synthetic-human-embryos-created-in-groundbreaki ng-advance

NOTABLE VISITORS

Dr. Steve Minchin, Associate Professor & Academic Director, from the University of Birmingham, UK visited MCBR to explore new collaborative areas for student exchange programs and research activities.

Prof. (Dr.) Sooryanarayana Varambally, Director of Translational Oncologic Pathology Research, from the University of Alabama, Birmingham, USA, visited MCBR to give his insights on his research and also to explore collaboration with MCBR

Dr. Shibashish Giri, Faculty at the University of Leipzig, Germany, and Chief Scientific Officer, AB Company, USA visited MCBR to share his research experience on the identification and reactivation of local stem cells of the skin, hair, liver, heart, and brain.

NOTABLE VISITORS

Dr. Gaurav Narula, Professor of Pediatric Oncology & Health Sciences, and **Dr. Albeena Nisar,** Scientific Officer D, CAR-T Cell Therapy Centre & cGMP Cell Therapy Unit Project, TATA Memorial Centre, Mumbai visited MCBR on 24th June 2023 to explore possibilities of setting up a CAR T cell manufacturing unit in MCBR facility.

FAREWELL

Ms. Meghana Kasthuri, a research intern under Dr. SV Kirthanashri was given a farewell by Prof. Raviraja NS and other MCBR members. She is going to the University of Michigan, Ann Arbor, MI, USA to pursue her doctoral research work. MCBR wishes her all the success in her life and career.

FUN MOMENTS

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

Ms. Meghana

17th April

Dr. Souvik

20th May

Dr. Ramya

26th June

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