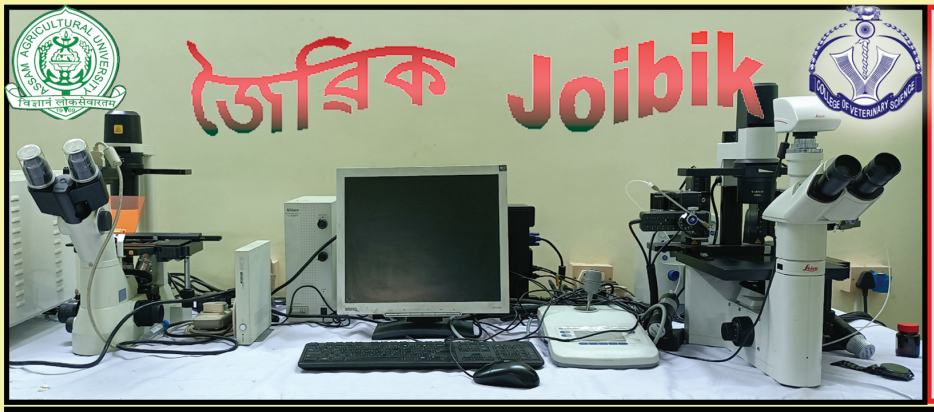

In this Issue

Three-Dimensional (3D) Cell Culture: Advances, Applications, and Future Directions.

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Introduction

Cell culture techniques have long been fundamental in biology and medicine. Traditionally, the majority of in vitro studies utilized two-dimensional (2D) cultures, creating a flat monolayer of cells. However, these methods belied the intricate physiological environments that cells experience in vivo, leading to misrepresented cellular behaviors, drug responses, and disease mechanisms.

Three-dimensional (3D) cell culture systems have emerged as a significant innovation in biological research, offering physiologically relevant environments that closely mimic in vivo conditions. The 3D cell culture marks a paradigm shift, allowing cells to grow and interact in a more naturalistic architecture. This method offers enhanced cell-cell and cell-matrix interactions, facilitating improved mimicry of tissue structure and function. As a consequence, 3D cell culture systems have significant implications for cancer research, drug discovery, toxicology, and tissue engineering.

Types of Three-Dimensional Cell Culture Systems

1. Spheroids

Spheroids are aggregates of cells that form spherical structures when cultured in non-adherent conditions. They can be generated using several methods, including:

- Hanging Drop Technique: Cells are suspended in drops of media hanging from a culture dish lid, allowing them to aggregate under gravity.
- Low-Attachment Plates: Plates made from materials that prevent cell adhesion encourage the formation of spheroids.
- Forced Suspension Culture: Systems like spinner flasks stir the cell-containing solution, promoting aggregation.

Spheroids exhibit gradients of nutrients and oxygen within their core, similar to solid tumors, making them invaluable for cancer research.

2. Organ-on-a-Chip Technologies

These microfluidic devices incorporate living cells in a precise, engineered microenvironment, showcasing in vivo-like tissue structures and functions. Their design allows for the manipulation of different cell types, fluid flow, and mechanical stimuli, enabling studies on organ interactions, disease modeling, and drug response.

3. Hydrogel-Based Cultures

Hydrogels, such as collagen, alginate, and Matrigel, serve as hydrating matrices that can be tailored in terms of stiffness and biochemical composition, providing a supportive scaffold for cell growth. Recent advancements in hybrid hydrogels incorporate responsive materials that allow for real-time modulation of their properties based on cellular activity.

4. Bioprinting

This novel approach utilizes 3D printing technologies to layer cells and biomaterials, allowing the construction of complex tissue structures. Utilizing bioinks, which can include living cells, scaffolds, and nutrients, bioprinting has paved the way for creating patient-specific tissues and organ models.

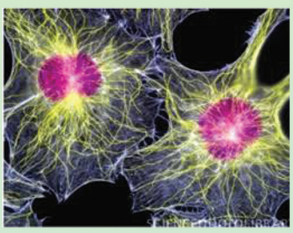
5. Decellularized ECM

Decellularized extracellular matrices (ECM) are derived from tissues that have had their cellular components removed, leaving behind a native scaffold. These scaffolds maintain biochemical and biomechanical properties and provide a more authentic microenvironment for cell growth and differentiation.

6. Organoids

Organoids are three-dimensional structures that are derived from stem cells and mimic the architecture and function of specific organs. They are created in vitro and can recapitulate some of the complex tissue structures and functions of real organs. Organoids can be generated from various types of cells, including pluripotent stem cells,

(Continued on Page 2)



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tissue-specific stem cells, or even primary cells from actual organs. Organoids have a high degree of similarity to their parental cells that replicate and simulate their unique biological characteristics. Additionally, organoids are able to self-renew and self-organize, contain various cell types, perform some specific functions, and form spatial structures similar to those of in vivo organs. Organoids are used in various fields, including disease modeling, drug testing, regenerative medicine, and personalized medicine.

Recent Advancements in 3D Cell Culture Technologies

1. Mechanobiology in 3D Culture

Recent studies highlight the importance of mechanical forces within 3D cultures, demonstrating that cell shape, stiffness, and matrix architecture markedly influence cellular behavior. For instance, modifying the rigidity of hydrogels can affect cell proliferation, migration, and differentiation.

2. High-Content Screening in 3D Models

Advancements in imaging technologies and automated analysis allow for high-content screening of 3D cultures. These techniques enable researchers to assess multiple phenotypic and functional endpoints simultaneously, enhancing the throughput of drug discovery processes.

3. Integration of Induced Pluripotent Stem Cells (iPSCs)

The use of iPSCs in 3D culture systems allows for patient-specific modeling of diseases and drug testing. These stem cells can differentiate into a variety of cell types, providing a rich resource for studying human-specific responses in a 3D context.

4. Incorporation of Immune Cells

The development of 3D co-culture systems that include immune cells provides a comprehensive model for studying tumor-immune interactions, systemic diseases, and inflammatory responses. These models enhance our understanding of immune modulation in various diseases.

Applications of 3D Cell Culture Systems

1. Cancer Research

3D cell culture models, such as organoids and spheroids, play a crucial role in cancer research by mimicking tumor architecture and heterogeneity. They allow for:

- **Studying Tumor Microenvironments:** The spatial organization within 3D cultures allows for the investigation of cellular interactions and biology in a way that 2D cultures cannot.
- **Drug Testing:** 3D models can assess drug efficacy and resistance mechanisms, providing a more accurate prediction of clinical outcomes.

2. Drug Discovery and Development

3D cell cultures improve the translational relevance of preclinical drug testing:

- **Predictive Toxicology:** By mimicking organ-specific environments, 3D cultures enhance the accuracy of toxicity testing, leading to better predictions of adverse drug reactions.
- **High-Throughput Screening:** Automated systems developed for 3D cultures have streamlined the process of evaluating thousands of compounds for promising therapeutics.

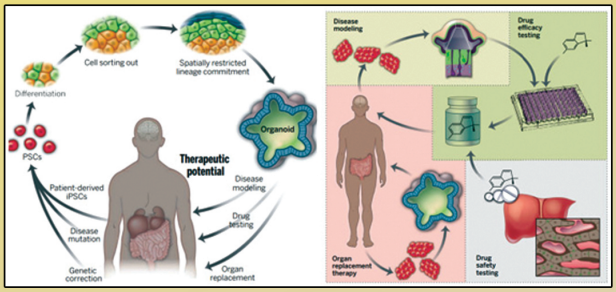

Fig. A schematic diagram used in the treatment of diseases after organogenesis using an organoid model developed by extracting patient-derived cells (PDCs).
Image Source: (Zhao Z. et al., 2022)
Nature Reviews Methods Primers, 2 (1) , art. no. 94

3. Tissue Engineering and Regenerative Medicine

3D cell culture technology has significant implications for tissue engineering:

- Scaffold Development: Biodegradable scaffolds can serve as platforms for cell attachment and proliferation, leading to the development of functional tissues for transplantation.
- Cell-Laden Hydrogels: Cells embedded in hydrogels can facilitate tissue regeneration through controlled release of growth factors and proteins.

4. Organoid Systems for Disease Modeling

Organoids derived from human stem cells provide a powerful platform for modeling various diseases, including cystic fibrosis, diabetes, and neurodegenerative diseases. They allow for personalized medicine approaches, where patient-specific organoids can be used for drug testing.

Challenges in 3D Cell Culture Systems

Despite their significant advantages, several challenges exist:

1. Standardization and Reproducibility

The lack of standardized protocols can lead to variability in 3D culture results. Establishing guidelines and benchmarks for various 3D techniques is essential for reproducibility and comparability across studies.

2. Scalability

Many 3D models are challenging to scale up for high-throughput applications. Innovative strategies are needed to create scalable systems that maintain the integrity of 3D cultures.

3. Complexity of Tissue Architecture

While 3D models aim to replicate the complexity of tissues, achieving true physiological relevance remains a challenge. Researchers are continually working to develop more sophisticated models that account for the various cell types, ECM components, and biochemical factors present in vivo.

4. Cost and Accessibility

The materials, technologies, and equipment required for 3D cell culture can be expensive and less accessible,

especially for smaller research institutions. Developing cost-effective alternatives will be critical for wider adoption.

Future Directions

The future of 3D cell culture will likely involve the following advancements:

1. Multi-Organ Systems

Developing multi-organ systems will provide insights into systemic interactions and enhance the modeling of diseases that involve multiple organs, such as diabetes and cardiovascular diseases.

2. 3D Bioprinting of Functional Organs

Progress in bioprinting will advance the field toward creating functional organs that can potentially be used for transplantation, disease modeling, and drug screening.

3. Enhanced Computational Modeling

Integrating computational modeling and machine learning with 3D cell culture can offer predictive insights into cellular behavior, disease progression, and treatment outcomes.

4. Personalized Medicine

Leveraging patient-derived cells in 3D cultures will enhance personalized medicine approaches, enabling tailored treatments based on individual cellular responses and genetic makeup.

Conclusion

Three-dimensional cell culture systems have transformed the landscape of biological research, providing valuable insights into cellular behavior, disease mechanisms, and drug responses. As technology continues to advance and overcome the existing challenges, 3D cell culture models will play an increasingly critical role in enhancing our understanding of human and animal biology and improving therapeutic strategies.

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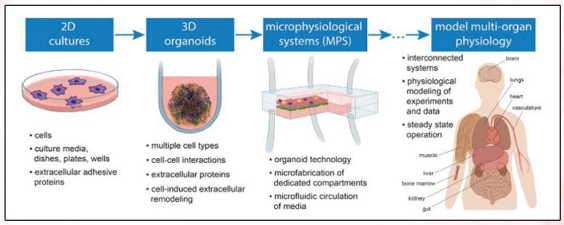

Fig. Microphysiological systems (MPS) as advanced platforms to model cellular properties in vitro.Culturing cells in 3D as advanced platforms to model physiological properties in vitro.

Source: https://doi.org/10.1002/cpt.1458

International Research Highlights

- **A gel has been invented to stabilize bioactive protein during transportation:**

  Transportation of protein keeping its biological activity intact is difficult as it exposes the protein to environmental odds often denaturing/degrading the protein. To solve this problem a group of scientist form Department of Chemistry, University of Glasgow, Glasgow, UK (Bianco et al. 2024) have developed a hydrogel that has the ability to stabilize protein up to 50 °C. This method of protecting protein from thermal degradation has the potential to revolutionize the method of transportation of bioactive protein by lowering the cost of transportation by many-fold.

  Source: https://doi.org/10.1038/s41586-024-07580-0

- **A humanized mouse that mounts mature class-switched, hypermutated and neutralizing antibody responses:**

  Scientists from The Antibody Laboratory, Department of Microbiology, Immunology & Molecular Genetics, The University of Texas Long School of Medicine, USA constructed a humanized (THX) mouse which mimics a fully developed and functional human immune system. This was to overcome limitations of currently available in vivo human models for various immunology research purposes. THX mice reconstitute a human lymphoid and myeloid immune system, including marginal zone B cells, germinal center B cells, follicular helper T cells and neutrophils, and develop well-formed lymph nodes and intestinal lymphoid tissue, including Peyer's patches, and human thymic epithelial cells. These mice have diverse human B cell and T cell antigen receptor repertoires and can mount mature T cell-dependent and T cell-independent antibody responses, entailing somatic hypermutation, class-switch recombination, and plasma cell and memory B cell differentiation.

  Source:- https://doi.org/10.1038/s41590-024-01880-3

- **FDA approved a ground-breaking CRISPR treatment for beta thalassemia.**

  Vertex Pharmaceuticals and CRISPR Therapeutics have scored an FDA approval for their gene-editing therapy "Casgevy (exa-cel)" to treat transfusion-dependent beta thalassemia (TDT). The approval comes less than six weeks after the U.S. regulator signed off on Casgevy to treat patients with sickle cell disease (SCD). The companies have opened nine authorized treatment centers (ATCs) to administer

Casgevy to patients. Currently, the approval covers Transfusion Dependent Thalassemia patients ages 12 and older who need regular transfusions. Vertex and CRISPR charge $2.2 million for the one-time treatment. A lifetime of treatment of transfusion-dependent beta thalassemia is estimated to exceed $5 million in USA.

Source: https://www.fiercepharma.com/pharma/vertex-crispr-win-early-fda-nod-gene-therapy-casgevy-treat-beta-thalassemia

- **Gilead Science Inc. announces a new drug for HIV-AIDS:**

An US based Biotechnology company, Gilead Sciences has published the results of advanced stage clinical trial of a new drug molecule Lenacapavir for treatment of HIV-AIDS. Compared to the existing drugs. Efficacy of Lenacapavir is found to be much higher and only two injections per year is sufficient to keep patients virus free (non-infective). So, this molecule may be the next wonder molecule against AIDS.

Source: https://www.unaids.org/en

- **Modified mosquitoes to combat Dengue in Brazil**

The World Mosquito Program has released mosquitoes infected with Wolbachia in five municipalities of Brazil: Rio de Janeiro, Niterói, Belo Horizonte, Campo Grande, and Petrolina. There they breed with wild mosquitoes. Wild females that mate with Wolbachia-infected males produce eggs that don't hatch. Wolbachia-infected females produce offspring that are also infected. Over time, the bacteria spread throughout the population. Niterói is the first Brazilian city which have fully protected with Wolbachia method.

Sanctioning of research projects under "DBT-ALSBT Hub

A total of 10 research proposals in different areas of biotechnology and allied sciences were sanctioned out of total 54 proposals received from young researchers below 45 years of age across the North Eastern Region. A total amount of Rs. 20.66 lakhs as the first-year grant and Rs 49.693 lakhs as second year grant were sanctioned for the research projects of 2-year duration. The beneficiaries included researchers from The Assam Royal Global University, Guwahati; CSIR-NEIST Jorhat; College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl; College of Veterinary Sciences and Animal Husbandry Central Agricultural University (I), Nagaland; Dibrugarh University; College of Veterinary Science, AAU, Khanapara; Lakhimpur College of Veterinary Science, Joyhing, AAU

ALSBT Hub Micro grant (Phase 1) interim review meeting for monitoring 2 years of progress.

Dr. Ratan Kumar Choudhary delivering his online lecture on 3rd June, 2024

Patent received by Dr. Debajit Borah & his team including Dr. Probodh Borah, DR (Vety.) and co researchers.

Visit of ALSBT Hub by Dr. Mohan Lal Brahma, Ex. Vice Chancellor of Bodoland University.

National Research Highlights

- **HILLCHOL-Oral Cholera Vaccine:** Bharat Biotech International Limited (BBIL) in collaboration with Hilleman Laboratories, and the University of Gothenburg and Gotovax AB have launch the Oral Cholera Vaccine (OCV) 'HILLCHOL'. It is a novel single-strain OCV which is administered orally on Day 0 and Day 14, and it is recommended for individuals older than one year. Bharat Biotech is expected to produce about 200 million doses annually to meet the global deficit.

Source: https:www.bharatbiotech.compress_ Releases.html

- **The clinical trials of Spanish tuberculosis vaccine:** The phase-3 clinical trial of Spanish tuberculosis vaccine 'MTBVAC' has stated in India by Bharat Biotech in collaboration with Biofabri to evaluate the safety, immunogenicity and efficacy of the vaccine. It is the first live attenuated vaccine derived from Mycobacterium tuberculosis which was isolated from a human source. MTBVAC has been developed for new-borns, adults and adolescents and the duration of immunity is expected to be longer than the available BCG vaccine.

Source: https://www.bharatbiotech.com bharat_news.html

- **3D printed knee meniscus:** Researchers from Indian Institute of Technology Guwahati (IITG) and West Bengal University of Animal and Fishery Sciences, Kolkata, have collaboratively customized 3D bio-printed knee meniscus. The researchers have formulated the bio-ink with silk fibroin methacrylate, gelatin methacrylate, and polyethylene glycol dimethacrylate. This innovative approach will be helpful in managing knee injury patients with specific manner and with minimum invasive surgical

procedures.

Source: https://indiabioscience.org/news/2024/3d printing-with-silk-to-treat-knee-injuries

- **BioE3 policy of India:** The Government of India has introduced a new biotechnology policy called as 'BioE3' (Biotechnology for Economy, Environment and Employment). This policy aims to tackle food, energy and climate challenges through bio-manufacturing from medicines to bio-plastics. Moreover, this policy also aims to boost innovation and entrepreneurship, increase GDP, create new job opportunity, and strengthen sustainable development.

  Source: https://bmi.dbtindia.gov.in/

- **Indigenous Monkey pox detection kit:**

  Monkeypox is another emerging threat to the Humanity and the WHO has declared it as public health emergency of International concern. Recently, Siemens Healthcare Private Limited has got clearance from the Central Drugs Standard Control Organisation (CDSCO) to manufacture and commercialize the Monkeypox detection Real Time -PCR kit **IMDX Monkeypox Detection RT-PCR** Assay as an initiative of **'Make in India'**

Research and Achievement of ALSBT Hub during 2023-2024

- Biosurfactant, namely rhamnolipid from Pseudomonas aeruginosa and lipopeptide from Bacillus licheniformis has been studied for antifungal efficacy against dermatophytes Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum canis and Microsporum gypseum and was found that both types of biosurfactant exhibited effective antifungal and antibiofilm activities against the dermatophytes.
- In the study of MRSA from various sources, we found that expression of cfr gene, which imparts resistance through the ribosomal modifications, are absent in the isolated MRSA strain. Presence of cfr gene related resistance would have made the isolates resistant against chloramphenicol, clindamycin and erythromycin.
- In the study of MRSA from various sources, we found that expression of msrA and msrB gene, which impart resistance through efflux mediated mechanism and expression of ermC gene, which imparts resistance through ribosomal methylation, were found prevailing against macrolide, lincosamide and streptogramin.
- A patent has been granted entitled "A process for producing banana beer and a composition for the same". This research was carried out under the DBT Microgrant project (99/DRV/341/2022–23/ Microgrants/5032–40, Dated: 31/10/2022).
- The state of the art facilities generated though the Biotech Hub had helped to receive extramural grant of 17 research projects, sanctioned by ASTEC, Assam, Bodoland Pig Mission (BPM), IBSD, ICMR and DBT to the core faculties of Animal Biotechnology Department amounting around 3 crore rupees.
- Bodoland Pig Mission (BPM), BTR, has signs MOU with Directorate of Research, AAU (Vety.), Khanapara for providing diagnostic and advisory services based on the facilities created at ALSBT Hub.
- A patent has been applied entitle "A Nutraceutical Supplement for the Prevention and Improvement and the Method of Preparation Thereof" Application No: 202431068762 , Date: 11/09/2024 to Dr. Luit Barkalita based on the research work completed using ALSBT Hub facilities.
- Porcine circovirus PCV-1 and PCV-2 were detected in aborted fetus, stomach and intestinal content of porcine. Pseudo rabies virus (PRV) was detected from brain sample of porcine origin. Mycoplasma was detected in large intestine and heart samples of deceased pig. PPV5 was detected in porcine blood sample.
- Porcine adenovirus (PAV) was detected in rectal swab samples. Sequencing results revealed that, the tested adenovirus is belonging to the porcine adenovirus 3.
- After bioinformatics analysis, PCV2 ORF2 gene have been codon optimized and synthesized commercially. ORF2 gene has been subcloned into pcDNA3.4 vector. Transfection of pcDNA3.4-ORF2 plasmids in Vero Cell line for development of stable cell lines.
- Development of a serum bank and a virus repository with the well-characterized field viral isolates for future diagnostics and vaccine development programs is underway. A total of 88 serum samples have been stored till now.
- Dr. Pankaj Deka, Co-coordinator of ALSBT Hub has received ISVIB Mid Career Scientist Award-2024 in recognition of research work on "Development of classical swine fever bait vaccine and evaluation of its immunogenic potential in Pigs" in the field of Veterinary Immunology and Biotechnology. (Dated 26th -29th September, 2024, held at Madras Veterinary College, Chennai).
- Molecular characterization of canine distemper virus (CDV) field isolates based on H and F gene/amino acid sequences showed a distinct genetic lineage well separated from the other Indian strains as well as reference CDV strains from other countries.
- RT-PCR confirmation of clinical CDV infection in around 17% vaccinated dogs revealed a concerned about the emergence of new CDV variants or failure of the existing vaccine which are mostly based on the imported viral strains.
- Two stable Cell Lines i.e. Vero-CDV-H and Vero-CDV-F have been developed for constitutive secreted expression of canine distemper virus H and F protein, respectively, for use as diagnostic ELISA antigen and development of monoclonal antibodies.

Major Research Focus of ALSBT Hub, Assam

Viral pathogens associated with porcine reproductive disorders with special reference to Porcine Circovirus 2 (PCV2): Molecular characterization, and development of rapid diagnostics and new generation prophylactics .

MoU Signing between Directorate of Research (Veterinary), AAU, Khanapara and Bodoland Pig Mission, BTC, Assam

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Activities of ALSBT Hub during 2023-2024

Hands on training programmes organized during 2023-2024

Sl	Title of Training	Date	No	Level of participants
1	Advances in Animal Cell culture techniques and expression of heterologous protein in mammalian cell lines	20th to 26th June, 2024	13	MSc & Ph.D students and faculties

Outreach programme conducted during 2023-2024

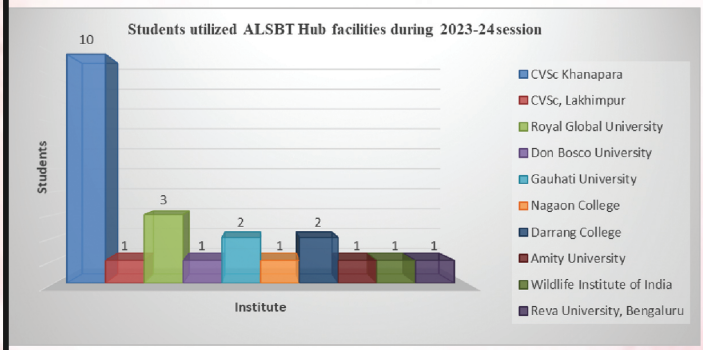
Sl	Title of Outreach Programme	Date	No	Level of participants
1.	"Scientific management and biosecurity measures in pig farming" at AAU–ZLR Station, Mandira, Kamrup, Assam.	6th June, 2024	35	Pig farmers and Entrepreneurs

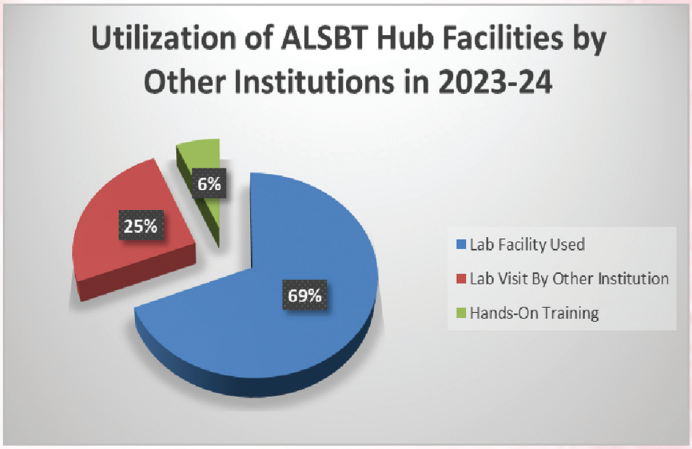
"Online Lecture Series" during 2023-24

`Sl	Date	Topic	Speaker
1.	19th July 2023	Biofilm and therapeutic failure in *Staphylococcus aureus* infections	**Dr. Fernanda R. Buzzola** Laboratorio de Patogenia, Bacteriana (PatoBac) Facultad de Medicina, Buenos Aires, Argentina
2	3rd June 2024	Stem cells: Enhancing lactation persistency and combating mastitis in bovine	**Dr. Ratan Kumar Choudhary** Assistant Professor, College of Animal Biotechnology, GADVASU, Punjab

Lab Visit and Demonstration of Molecular Techniques during 2023-24

Sl	Visitor	Date	Visitor no
1.	Internee student of National Forensic Science University.	6th November, 2023	10
2.	B.Sc. Botany students of Cotton University.	10thNovember, 2023	20
3.	MBBT student of Cotton University.	21stNovember, 2023	16
4.	B.Sc. student of Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar.	12th January, 2024	20





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