DEVELOPING MICROPHYSIOLOGICAL SYSTEMS IN INDIA

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1. PREFACE

This report has been prepared to understand the current status quo of research in India in the fields of microphysiological systems, including *in vitro* 3D organs and organ-on-chip models. This information should help scientists in this field, currently working in silos, to assess the area and form collaborations; regulators to evaluate and bring about policy changes to develop and establish India as a major player in this highly promising field of human-relevant methodologies in basic and clinical research.

The Introduction section addresses the limitations of animal models and the need to develop human-relevant model systems for basic and clinical research. The third section assesses the potential of organoids to represent the genetic landscape of human tissues and considers the evidence of their status as a better model system for drug testing. The fourth section discusses how funding and regulation are shaping the development of human-relevant model systems globally, and contrasts it to the situation in India where this is still an untapped domain. While India remains a nascent player, many academic institutes and industries are beginning to develop various tissue organoids and organ-on-chip models for basic and clinical research. The fifth section lists the major academic and private players in India who are working in this field along with their domains of specialization. Section 6 provides an approximate cost estimate of the tools and infrastructure to perform this research in India. In the end, Sections 7 and 8 identify the challenges and provide recommendations in terms of regulation, funding, innovation required to develop organoids and organ-on-chip as human-relevant model systems in India.

The information for research, costs, and infrastructure was collected by personal communication with scientists and targeted Pubmed search. The funding information was obtained from the online database and official websites of the Department of Biotechnology (DBT), Biotechnology Industry Research Assistance Council (BIRAC), Council of Scientific and Industrial Research (CSIR), Science and Engineering Research Board (SERB), and Indian Council for Medical Research (ICMR).

We would like to thank Dr. N. Madhusudhana Rao (CEO, Atal Incubation Centre - Centre for Cellular and Molecular Biology), Dr. Catherine Willett (Senior Director, Science & Regulatory Affairs, Humane Society International), Dr. Hari Rao (Scientist, Centre for DND Fingerprinting and Diagnostics) for reviewing and providing very insightful comments on the white paper. While we understand that the information represented in this report may not be exhaustive, it is nevertheless instructive of the state of science in this field in India.

2. INTRODUCTION

Animal models have been extensively used to gain insights into human biology owing to their genetic, anatomical, and physiological similarities [1]. However, it is increasingly becoming clear that, despite the similarities, animal models cannot depict all the facets of the severity, phenotype and genetic basis of a human disease [2].

In most cases, diseases which occur in humans do not naturally occur in animal models. Thus, to understand disease biology, researchers often artificially induce disease in an animal model. However, the induced disease in animals often shows different clinical and physiological manifestations compared to humans [3]. For example, amyotrophic lateral sclerosis (ALS) disease involves deterioration of neurons innervating the muscles in humans. The mouse model of ALS disease expresses a mutant form of RNA binding protein TDP43; however, this model shows mild and non-progressive disease involving minimal muscle wasting and reduction in lifespan.

Additionally, these animals die of acute bowel obstruction, rather than progressive muscle atrophy [4]. Thus, the use of this disease model to test for drug targets that provide survival benefits would prove to be irrelevant in humans.

The gene expression profile of animal models is also drastically different from humans. A recent study found that out of 15,000 genes, nearly 5000 genes are either over or under expressed in humans compared to mice [5]. Also the genome responses and major signalling pathways in mouse models were found to be poorly correlated with the humans during various states of inflammation, a common biological response during disease and infection states [6].

The search for therapeutic targets also relies on testing a large number of prospective compounds. The use of animal models to conduct such large-scale therapeutic screening involves prolonged duration and high costs [7]. Currently, the average cost of producing a drug can range from 1–4 billion dollars and can take around 10 years [8] .
Currently, 92% of drug targets that pass the preclinical stages fail during the clinical trials [9]. Primary reasons for the attrition include lack of efficacy (55%) and unforeseen toxicity (28%) [10,11].

[1] How closely related are humans to apes and other animals? How do scientists measure that? Are humans related to plants at all? (2000, October 23). Scientific American. https://www.scientificamerican.com/article/how-closely-related-are-h/
[2] van der Worp HB, Howells DW, Sena ES, Porritt MJ, Rewell S, O'Collins V, et al. (2010) Can Animal Models of Disease Reliably Inform Human Studies? PLoS Med 7(3): e1000245. https://doi.org/10.1371/journal.pmed.1000245
[3] Perrin S. Preclinical research: Make mouse studies work. Nature. 2014 Mar 27;507(7493):423-5. doi: 10.1038/507423a.
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[7] Giacomotto J, Ségalat L. High-throughput screening and small animal models, where are we? Br J Pharmacol. 2010 May;160(2):204-16. doi: 10.1111/j.1476-5381.2010.00725.x
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The situation is similarly poor in India, where more than 200 compounds entered preclinical and clinical stage development in the last two decades; however, only one reached the market [12].

Thus, there has been a drive among the global science communityto work towards more relevant and human-biology based models of diseases for drug discovery and research.

Until recently, there were a lack of suitable non-animal-based methods that could mimic human biology. Adherent 2D cell cultures (that attach to culture flask or petri dish), a commonly used in vitro tool, are easy to maintain, but the cells grow as flat monolayers, losing their three dimensional structure.

Fig 1: Key Terms

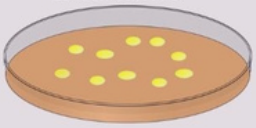
Types of in vitro model systems

2 D cell culture: In 2D cultures, cells grow as flattened monolayers, adhered to the surface or culture flasks or petri dishes. While 2D cultures are simple, lost-cost, and require low maintainance, the cells do not maintain their natural shape, structure, or cell-cell and cell-extracellular interactions.

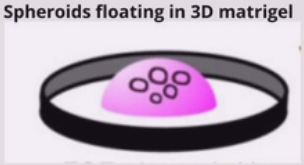
Organoids: Organoids are miniaturised, self-organising 3D versions of cultured cells that are created from primary or induced pluripotent stem cells by differentiating the cells and growing them in culture conditions that discourage attachment to the plate, resulting in spheroids. Organiods or uniform size can be generated that mimic aspects of the differentiated. tissue or organ.

Organ-on-chip and Microphysiological systems: A microphysiological systems are micron-scaled constructs that consist of interconnected multicellular architecture that can mimic tissue-tissue interfaces and cellular microenvironment.

2D cell culture

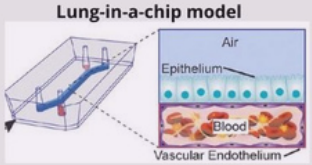


Spheroids floating in 3D matrigel



(Miller et al. 2019)

Lung-in-a-chip model



(Elveflow.com)

Types of cell sources

Cell lines: Cell lines are indefinitely proliferating cells established from a various organs and sources used in basic research. For example, one such line is Human Embryonic Kidney (HEK-293) cell lines.

Primary cells: Primary cells are extracted and isolated directly from tissues. They have a finite expansion capability and lifespan.

Induced pluripotent cells (iPSCs): These are adult cells that has been genetically reprogrammed to an embryonic-like state by expressing certain genes and chemical factors.

[12] Differding E. The Drug Discovery and Development Industry in India-Two Decades of Proprietary Small-Molecule R&D. ChemMedChem. 2017 Jun 7;12(11):786-818. doi: 10.1002/cmdc.201700043.

Cells grown in two dimensions also fail to exhibit many of the spatial and temporal dynamics that cells show and encounter inside the body. However, recent developments in cell culture technology and bio-engineering, including 3D organoids and organ-on-chip models, are providing human-derived, three-dimensional model systems that can begin to replace animal models in various areas of basic and clinical research (Fig. 1).

Organoids are miniaturized, self-organizing 3D versions of organs created from differentiated adult stem cells, induced pluripotent stem cells (IPSCs), or from differentiated primary cells (Fig. 2) [13,14]. The cells are grown in culture conditions that discourage attachment to the plate, resulting in the formation of spheroids. The resulting organoids can then be used to understand human disease biology or test for specific drug responses. Organoids can also be created from stem cells extracted from patients providing disease models that can be then used to create personalised drugs or treatment programs. They can also be used to modify an ongoing treatment strategy based on acquired resistance to drugs [15,16]. For example, in a study, gastrointestinal cancer patient-derived organoids (PDO) were created from patient samples before, during, and after treatment with Regorafenib drug and the drug prescription was changed based on changes in sensitivity towards various drugs over time [17].

Organ-on-a-chip models are microfluidic cell culture devices that consist of separate tissue and vascular compartments. These devices can recapitulate the tissue-tissue interaction, micro-environmental cues, and vasculature observed in vivo [18].

However, these systems are not currently devoid of limitations. Simple organoids at present lack vasculature, immune system, microbiota, and micro-environment (Fig. 2). These limitations can limit the biological functionality of organoids, so that most only partially mimic tissue or organs. In addition, complex tissue-tissue interactions cannot yet be addressed.

Whole animals are also used to understand the route of administration of drugs, pharmacokinetics-pharmacodynamics (PK-PD) studies of drugs. While organoids or organ-on-chip may not provide answers for many of these issues yet, species-specific differences can have a huge impact on these parameters. Thus, developing a robust human-relevant model which addresses these limitations is an area of intense investigation. Scientists are currently trying to create organoids with functional vascular systems and different cell types (including immune cells) to overcome these limitations [19].

[13] Jeppesen M, Hagel G, Glenthoj A, *et al*. Short-term spheroid culture of primary colorectal cancer cells as an in vitro model for personalizing cancer medicine. PLoS One. 2017;12(9):e0183074. Published 2017 Sep 6. doi:10.1371/journal.pone.0183074
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[17] Vlachogiannis G, Hedayat S, Vatsiou A, Jamin Y, Fernández-Mateos J, *et.al*. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. Science. 2018 Feb 23;359(6378):920-926. doi: 10.1126/science.aao2774
[18] Ingber DE. Developmentally inspired human 'organs on chips'. Development. 2018 May 18;145(16):dev156125. doi: 10.1242/dev.156125.

Fig 2: Benefits and Limitations of Animal and Organoid Models

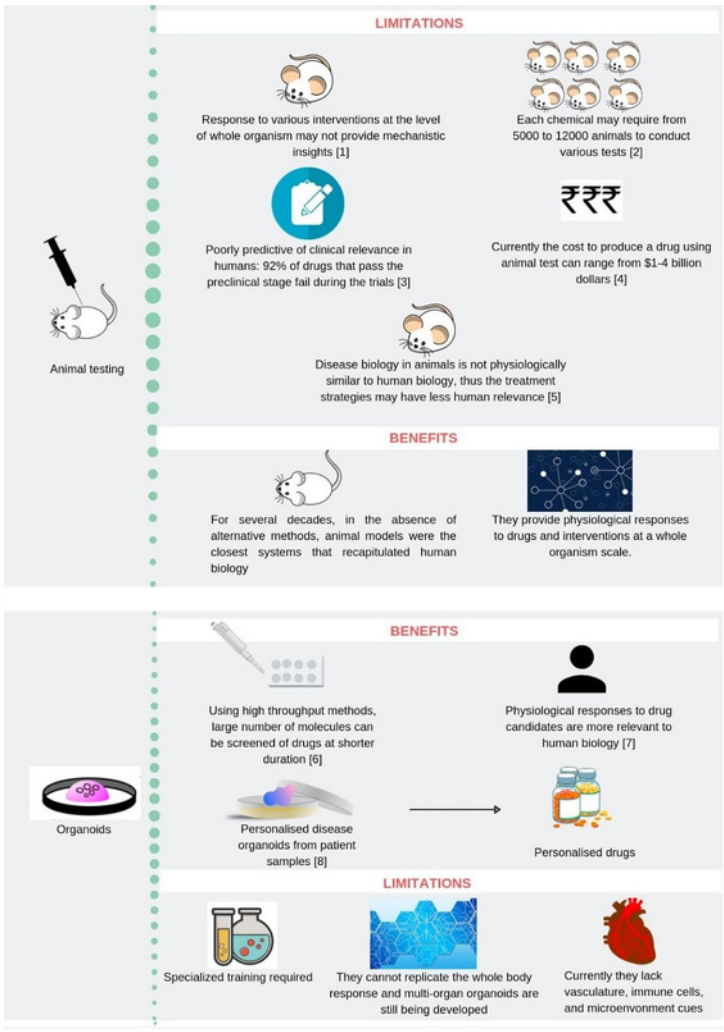
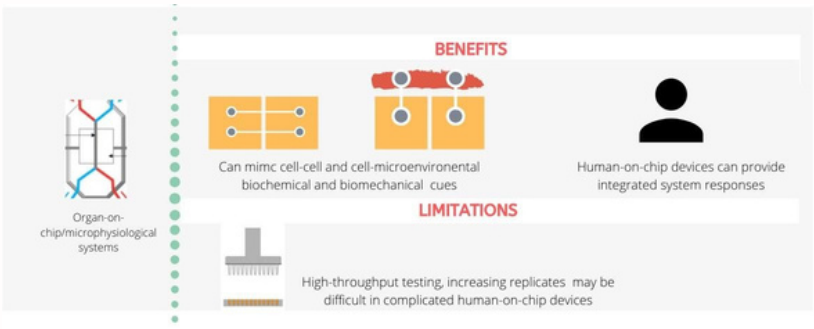


Fig 3: Benefits and Limitations of Organ-on-a-chip models



Similarly, several projects are in the pipeline to develop and integrate a multi-organ organoid/human-on-chip that can encapsulate the full complexity of human biology [20].

However, with the increase in their levels of complexity, it may become more difficult to perform large-scale high-throughput studies (Fig. 3)

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[19] Cakir, B., Xiang, Y., Tanaka, Y. et al. Engineering of human brain organoids with a functional vascular-like system. Nat Methods 16, 1169–1175 (2019). https://doi.org/10.1038/s41592-019-0586-5

[20] Koike, H., Iwasawa, K., Ouchi, R. *et al.* Modelling human hepato-biliary- pancreatic organogenesis from the foregut–midgut boundary. Nature 574, 112–116 (2019) doi:10.1038/s41586-019-1598-0).

3. Organoids, Tissue chips and MPS to study Drug discovery and Human development

One of the most common reasons for failure of clinical trials is lack of efficacy, followed by toxicity [21]. A study that looked at the concordance of adverse drug reactions (ADR) for 142 approved drugs found that musculoskeletal, respiratory and neurological ADRs showed a concordance of less than 30% in terms of adverse reactions between mice models and humans [22]. This suggests the need for models that can better predict human toxicity and drug responses.

Human organoids have been used to model various human diseases, including cystic fibrosis [23], microcephaly [24], autism [24], etc., to understand disease mechanisms and establish platforms for screening drugs [25]. One of the first demonstrations of the link between Zika virus and microcephaly was found using brain organoids [26].

Apart from disease biology, organoids are a great tool to study and understand human organogenesis and development. For example, brain organoids can recapitulate various brain regions, such as the forebrain, midbrain, and hindbrain [27].

Organoid culture of tumor cells preserves the three dimensional tissue architecture,cell viability, pathway activity, and gene expression profile compared to human tumor samples, indicating their potential as a platform to test drugs (Fig. 4) [28].

In one study, patient-derived organoids (PDO) were created from nine patients with pancreatic duct adenocarcinoma and then used to test various treatment possibilities. The PDO were sensitive to some drugs but not others: five patients treated with drugs to which the PDO were sensitive had a progression-free survival (PFS) of 332 days compared with the expected PFS of 180 days, showing the clinical relevance of these models (Fig. 5) [29].

Similarly, engineered heart [30], liver [31], intestine [32], kidney [33], pancreas, and other cancer [34,35] organoids have been used as model systems to screen and test the efficacy of drug candidates for cardiovascular diseases, hepatotoxicity, cancer, etc. The three-dimensional culture systems of the liver can maintain function for a duration of three months, and respond to inflammation – this makes them an attractive candidate to screen for and detect drug-induced toxicity [36].

[21] Dekkers JF, Wiegerinck CL, de Jonge HR, Bronsveld I, Janssens HM, *et.al*. A functional CFTR assay using primary cystic fibrosis intestinal organoids. Nat Med. 2013 Jul;19(7):939-45. doi: 10.1038/nm.3201.[22] Lancaster, M. A. et al. Cerebral organoids model human brain development and micro- cephaly. Nature 501, 373–379 (2013).
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[26] Lancaster, M., Corsini, N., Wolfinger, S. et al. Guided self-organization and cortical plate formation in human brain organoids. Nat Biotechnol 35, 659–666 (2017). https://doi.org/10.1038/nbt.3906

Fig. 4: Comparison of organoids and patient tissue samples

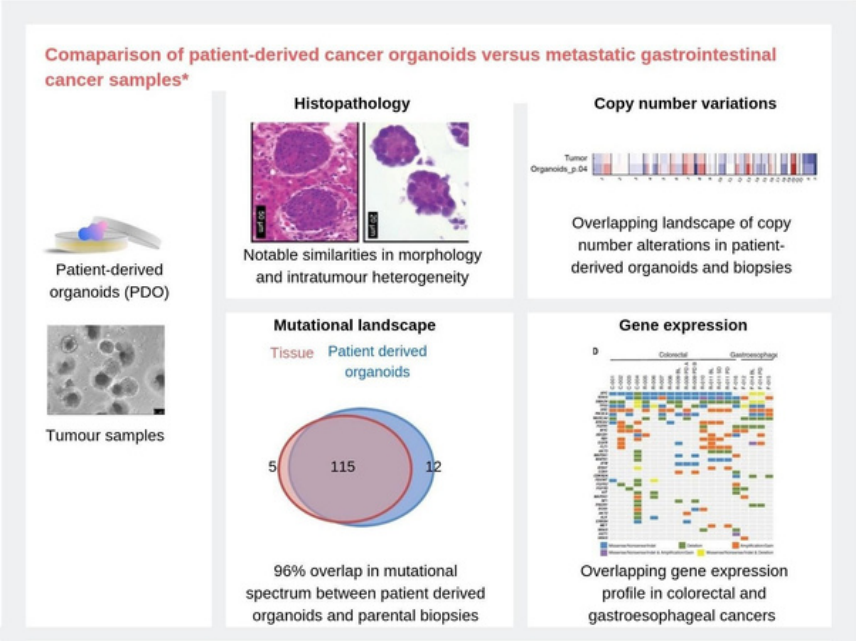
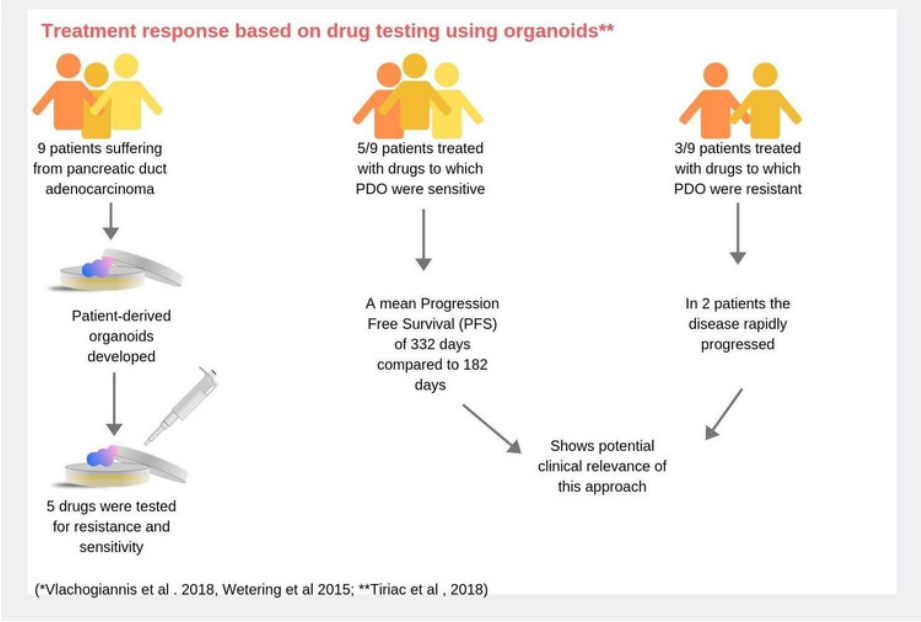


Fig. 5: Treatment response based on organoid-based drug testing



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4. GLOBAL PUSH TOWARDS NON-ANIMAL AND HUMAN- RELEVANT MODEL SYSTEMS

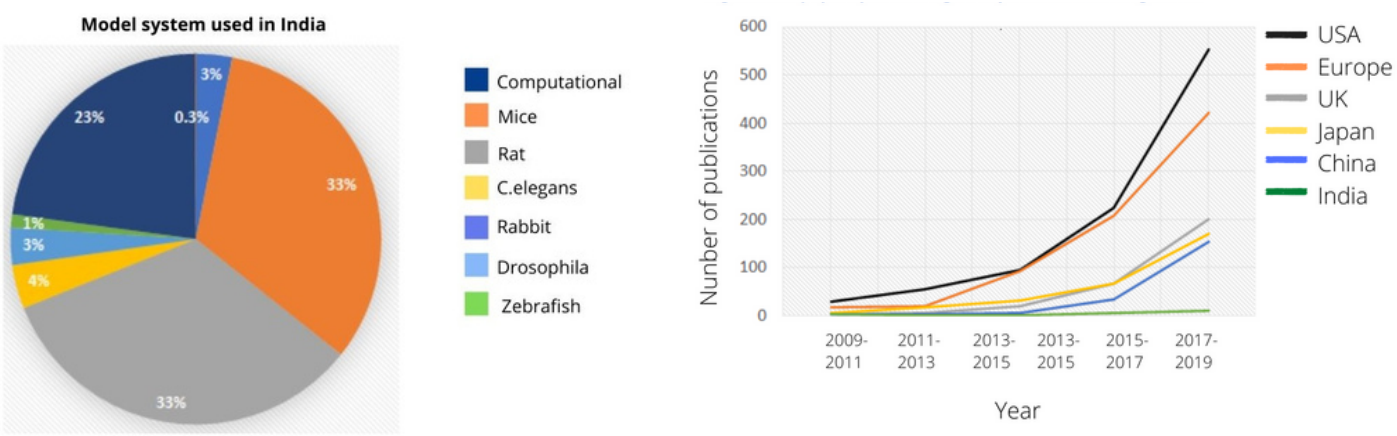
To explore complex questions related to drug toxicity, disease development, and human biology, the U.S., U.K., and European Union member nations among others have recognized the potential of organoids/ organ-on-chip technologies. In the Indian context, the field of non-animal model systems is still developing (Fig. 6A).

Globally, there has been a great push towards developing non-animal methodologies with respect to regulation and funding (Fig. 7). Efforts from countries like the USA, United Kingdom, Canada and several other countries of the European Union (EU) and the Indian scenario are discussed below.

UNIKED KINGDOM AND EUROPEAN UNION

European Innovative Medicines Initiative (IMI) is a 2 billion euro private-public partnership between the European Commission and European Federation of Pharmaceutical Industry Associations which aims to replace animals with advanced in vitro and in silico models. IMI initiatives include VAC2VAC, which aims to validate human and veterinary vaccines using non-animal testing methods, and STEMBANCC, which intends to set up a biobank to provide well characterizedpatient-derived Induced Pluripotent Stem Cells (IPSCs) along with proof-of-concept studies to treat various disorders.

Fig 6A: Papers published in India using various model systems in the past decade
6B: Number of papers published globally in the field of organoids



(Parvatam *et al.* 2020)

EU-TOXRISK, is another program for toxicology testing and risk assessment using advancements in cell biology, -omics, systems biology, and computational methods to provide a human cell-based assessment of chemical hazards and associated risks.

NC3Rs is a UK-based scientific organisation that provides a £10 million funding annually to support research, open innovation, and commercialization of 3R (refine, reduce, replace) technologies. Regulatory incentives within the EU include the directives on the Protection of Animals Used for Scientific Measures; Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH); and the Cosmetics Directives which strongly recommend the use of non-animal methodologies [37,38,39]. The European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) is an established organization committed to the scientific development and validation of NAMs.

USA

The Tissue Chip for Drug Screening Program is a $142 million project whose aim is to develop 3D platforms to test drug toxicity. As a part of the program, the National Institute of Health (NIH) initiated funding to 13 projects to develop 3D platforms using living cells and tissues. In 2018, a 5-year grant provided funding to address Type-2-diabetes through the development of tissue chip systems mimicking the condition. Efforts are also being made to fund two Tissue Chip Testing Centers and a Database Center. Researchers in the U.S. are also coming together to create an integrated human body-on-a-chip.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is a permanent committee of the National Institute of Environmental Health Sciences (NIEHS). It has representation from 16 federal agencies and aims to promote regulatory acceptance of non-animal toxicology and safety tests. In 2018, it published a strategic roadmap document to establish non-animal testing methods and evaluate the safety of chemicals and medical products. Along with the NIEHS, the U.S. EPA, and FDA have also published roadmap documents [40, 41].

The U.S. Environmental Protection Agency (U.S. EPA), in a milestone announcement, said that it would stop conducting and funding all mammalian studies by 2035 and reduce funding directed for animal studies by 30% by 2025. For its part, the U.S. Government Accountability Office concluded that federal agencies including the

[37] Directive 2010/63/EU of the European Parliment and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union, L 276/33 - L 276/79
[38] European Commission Environment Directorate General - REACH in brief (Oct 2007).
https://ec.europa.eu/environment/chemicals/reach/pdf/publications/2007_02_reach_in_brief.pdf
[39] Regulation (EC) No 1223/2009 of the European parliament and of the council of 3oth November 2009 on cosmetic products. Official Journal of the European Union, L 136/3 - L 136/279
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https://www.gov.uk/government/publications/non-animal-technologies-in-the-uk-a-roadmap-strategy-and-vision

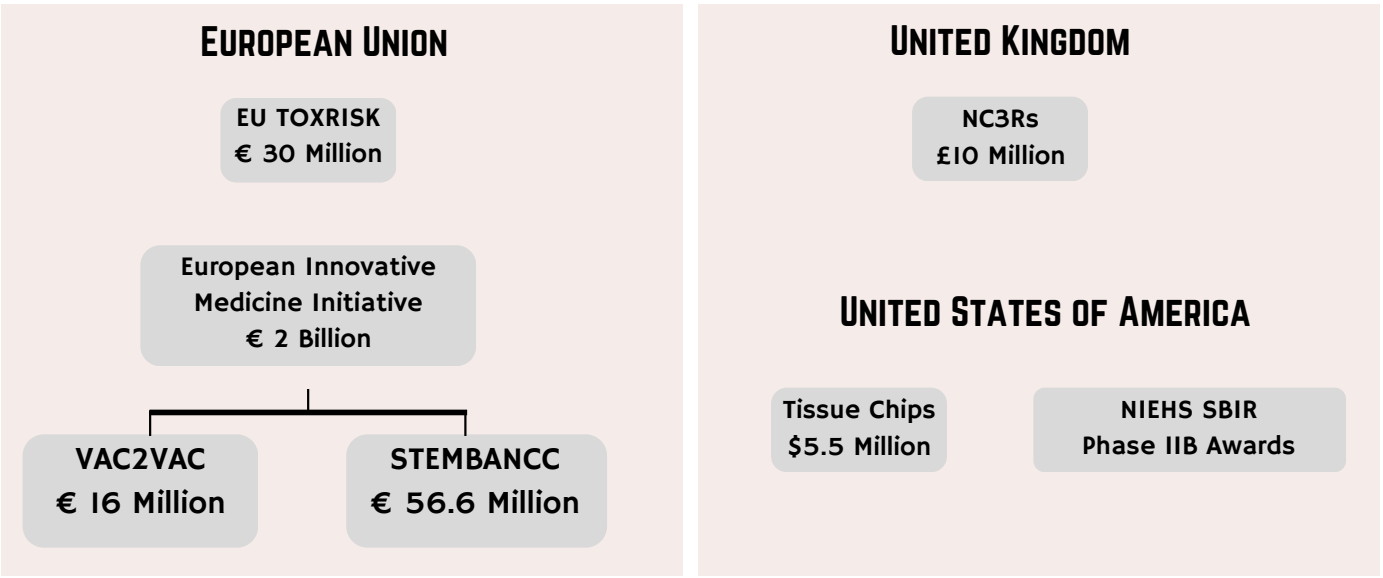

Fig 7: Global Funding Initiatives in non-animal and human relevant research

Department of Health and Human Services (HHS), U.S. Department of Agriculture (USDA), and Environmental Protection Agency (EPA) should independently assess efforts to develop and promote non-animal research in the U.S. [42, 43]

CANADA

The University of Windsor (Canada) established a Canadian Centre for Alternatives to Animal Methods in 2017 to develop, validate, and promote non-animal methodologies in toxicity testing and biomedical research.

All of the above initiatives reflect the global push towards realizing the need to develop non-animal methodologies in basic and clinical research.

INDIA

Mice and rat models currently represent the most widely used model systems used in India, followed by computational methods (Fig 6A). Fig. 8 shows the laws and regulations in India with respect to animal use. A notable step taken by the University Grants Commission (UGC) and Ministry of Health and Family Welfare (MoHFW) in 2012 was to discontinue the use of animals in undergraduate level educational institutions. Also in 2013, cosmetic testing on animals was banned. In 2014, India was the first south Asian country to ban the import of cosmetics that were tested on animals. In 2016, the 'Draize' test was banned by the MoHFW which was used to detect eye and skin irritation in rabbits. Another amendment in 2015 was passed to spare the repeat animal testing of chemicals/drugs in India.

In 2017, the Ministry of Agriculture revised its pesticide testing regulations to recognise modern, human cell-based methodologies. The Central Insecticides Board Registration Committee (CIBRC) under the Ministry of Agriculture released a guidance document on the Toxicology for Registration of Chemical Pesticides in India in 2018.

[42] U.S. EPA to eliminate all mammal testing by 2035. (2019, September 11). Science | AAAS. https://www.sciencemag.org/news/2019/09/us-epa-eliminate-all-mammal-testing-2035
[43] Animal Use in Research: Federal Agencies Should Assess and Report on Their Efforts to Develop and Promote Alternatives. (2020, June 23). Https://Www.Gao.Gov/. https://www.gao.gov/products/gao-19-629

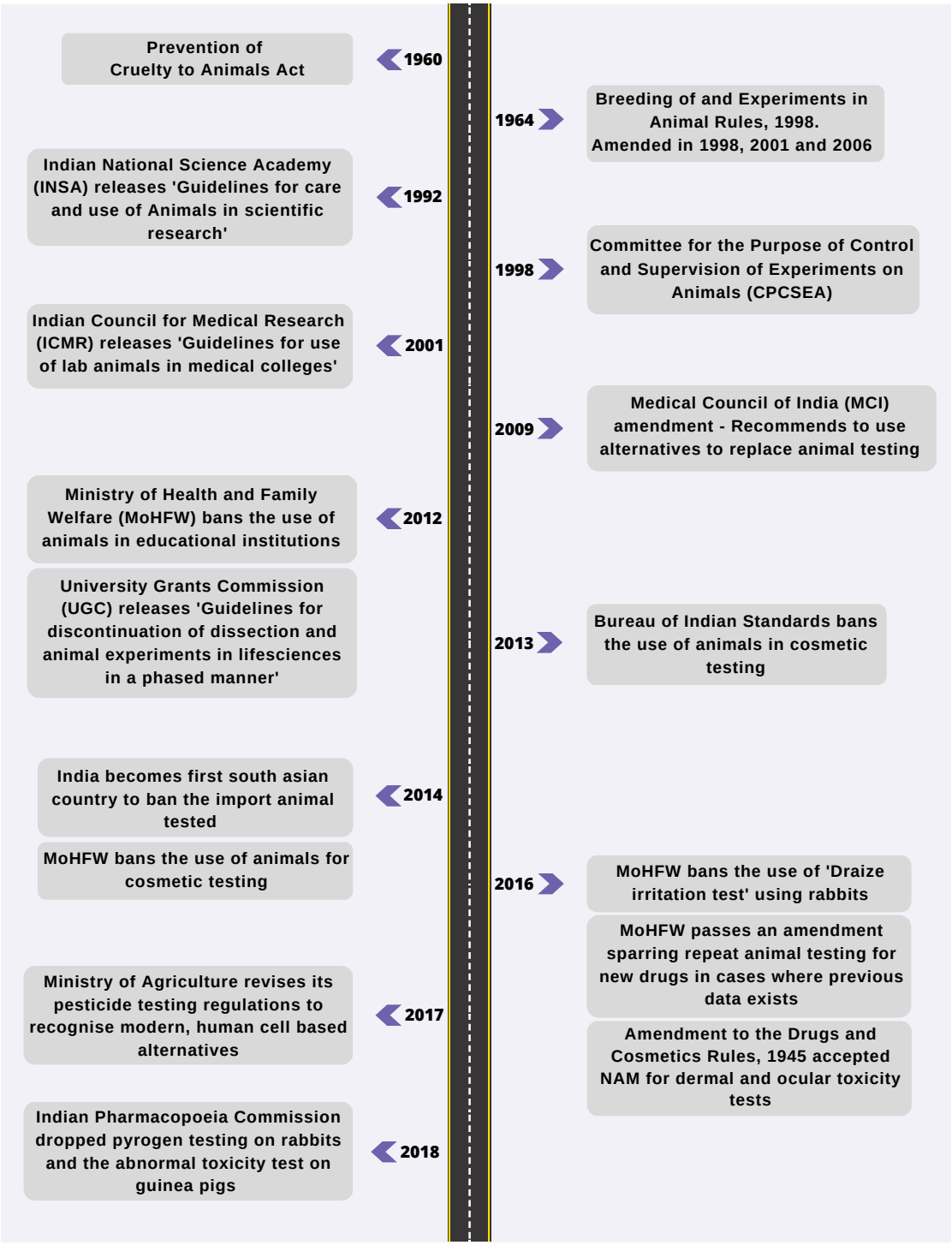

Fig 8. Laws and regulations in India with respect to animal use along with use of NAM's in research

The Department of Biotechnology (Ministry of Science and Technology, India) in collaboration with the Indian Council of Medical Research (ICMR) has formulated the "National Guidelines for Stem Cell Research (NGSCR)" [44]. The guidelines include the permissible, restrictive, prohibitive and regulation in various areas related to stem cell research.

Despite some of the regulations passed in this area in the last decade in India, the potential of non-animal and human-relevant model systems is still unrealised and strong regulatory and research measures are required if India is to become a major player in this field.

[44] Geeta Jotwani. (2017, October). National Guidelines for Stem Cell Research. Dbtindia.Gov.In. https://dbtindia.gov.in/sites/default/files/National_Guidelines_StemCellResearch-2017.pdf

5. Organoid, Tissue chip and MPS research in India - Key academic and industry players

While organoid research in India is still in its infancy, many research institutes and private companies are beginning to explore this field (Fig. 9). This section explores some of the current research and key academic groups working in this area in India. The full list of all research labs and companies working in this space in India is listed in Annexure* Table 1 and Table 2.

A research team at L V Prasad Eye Hospital has devised a simple and efficient culture method to create complex three-dimensional corneal organoids using both human embryonic stem cells and induced pluripotent stem cells [45].
The Indian Institute of Science, in collaboration with the University of Pune, recently reported the creation of breast cancer organoids to predict therapeutic responses and for personalised chemotherapy [46].

In a collaborative study [47], the metastatic condition of the lung tumor was recreated by building a primitive lung-in-a-dish in a research lab in the University of Pune. This model was then tested against current chemotherapy agents, suggesting its potential role in personalised medicine.

Eyestem, a cell-therapy company, is currently involved in creating 3D retinal organoids using human iPSCs. These retinoids will be used to study disease biology and for drug screening. Eyestem is also generating transplantable retinal photoreceptor cells from iPSCs to replace damaged or dysfunctional photoreceptors. The company plans to conduct GLP toxicology studies using the 3D retinal structures.

Pandorum Technologies Pvt. Ltd., a biotechnology company, is working on human liver organoids. These organoids are currently scaled at 10,000–100,000 cells and have a cell viability of two to four weeks. This allows a longer time exposure for preclinical drug screening, including drug induced liver toxicity and drug metabolism studies. These are currently being optimised for modelling liver diseases, such as non-alcoholic steatohepatitis and liver-stage malaria.

Reagene Lifesciences Pvt. Ltd. is creating an integrated 3D cell system to study diseases and human biology using a novel methodology. The company is also planning to conduct clinical trials-on-a-dish using cells from patient samples to reduce the cost and human resources used for conducting trials. This would also ease the process of personalised screening and response to drugs.

[45] Susaimanickam, J et al. Generating minicorneal organoids from human induced pluripotent stem cells. Development 2017 144: 2338-2351; doi: 10.1242/dev.143040
[46] Nayak B, Balachander GM, Manjunath S, Rangarajan A, Chatterjee K. Tissue mimetic 3D scaffold for breast tumor-derived organoid culture toward personalized chemotherapy. Colloids Surf B Biointerfaces. 2019 Aug 1;180:334-343. doi: 10.1016/j.colsurfb.2019.04.056.
[47] Ramamoorthy P, Thomas SM, Kaushik G, Subramaniam D, Chastain KM, *et.al.* Metastatic Tumor-in-a-Dish, a Novel Multicellular Organoid to Study Lung Colonization and Predict Therapeutic Response. Cancer Res. 2019 Apr 1;79(7):1681-1695. doi: 10.1158/0008-5472.CAN-18-2602. Epub 2019 Jan 23.

*Annexure tables are based on information collected in 2019. In 2023, CPHMS created an updated database of academic labs and companies working on MPS in India which includes 70+ members. To access this database, please go here.

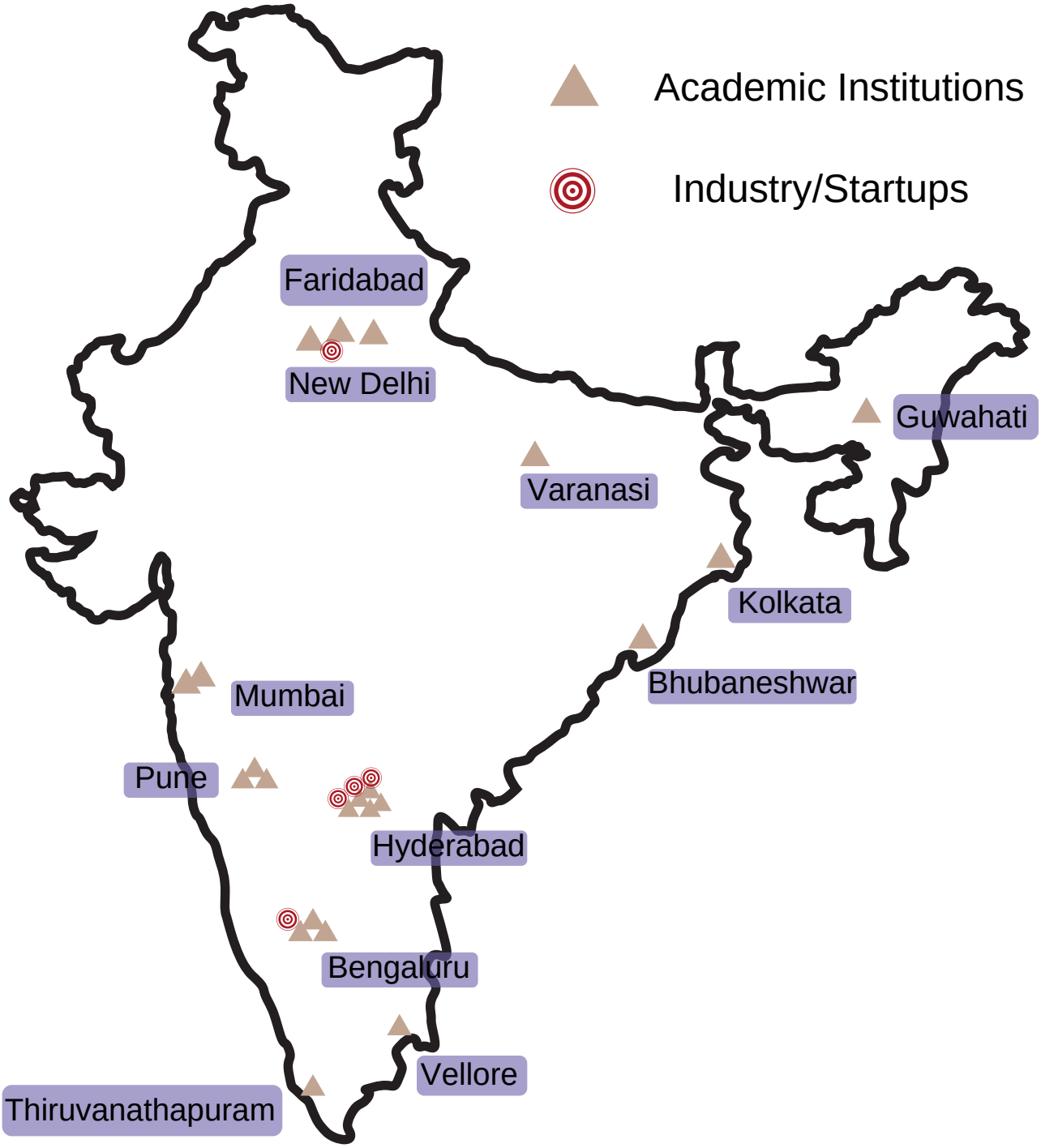

Fig 9: Location-wise academic groups and companies in India working on organoid/organ-on-chip research*

*This map is based on information collected in 2019. In 2023, CPHMS created an updated database of academic labs and companies working on MPS in India which includes 70+ members. To access this database, please go here.

6. Infrastructure and tools

6.1 Organoids

Many of the commonly used instruments for performing organoid experiments overlap with those used for general cell culture experiments (Annexure, Table 3). However, a segregated space to work on human cell lines is critical to avoid accidental contamination. Three dimensional scaffolds have been developed and are being used to optimize the growth of organoids and to control all geometrical, biomechanical, and biochemical properties. While the use of scaffolds can reduce the[47] variations in the 3D self-organisation process, the use of 3D printers to create scaffolds can increase costs (Annexure, Table 3).

Another technology at hand is the bioreactor, a 3D cell culture platform that can ensure a dynamic flow of media around cells. This can create physiologically relevant gradients of nutrients and waste products between the surface and the core of the organoid. Bioreactors help to address the issue of nutrient availability in a growing organoid and help in the long-term growth of cultures; thus, their use may become critical as the scale of the 3D tissue structure increases.

Visualising the three-dimensional structure of organoids is presently done by sectioning the tissue and/or requires the use of confocal microscopy. While live imaging of the organoids has been achieved elsewhere, it is presently not being done in India.

The basic infrastructure costs for conducting experiments in 3D models are summarised in Annexure, Table 3.

At present, the cost of a single vial of growth factor used to differentiate the stem cells towards specific lineages during the process of creating organoids may cost between 20,000–25,000 rupees. On an average, the total cost of consumable reagents used for organoid cultures can range from 25–30 lakhs/year.

6.2 Organ-on-chip

For designing a tissue chip, a microfabrication facility is required. Currently, there are limited academic institutions that have this facility in India. Some of the institutes with this facility include C-CAMP (National Centre for Biological Sciences, Bangalore), IIT Bombay, IIT Kanpur, Indian Institute of Sciences, Bangalore, IIT Madras and Central Mechanical Engineering Research Institute (Kolkata). A basic knowledge of engineering is also required for designing the chip.

7. UNMET NEEDS AND CHALLENGES

7.1 FUNDING IN INDIA

Stem cell research and regenerative medicine has been recognised as one of the priority areas in the biomedical research division of the Department of Biotechnology (DBT) in India. DBT has a task force on Bio-engineering, and some of the labs in India working on bioengineered 3D organs have received funding under this umbrella.

Apart from the funding opportunities provided under this program, DBT also occasionally releases special call for grants which can offer funding opportunities. These include “Call for proposals on organ development and organ regeneration” and “Call for proposal on artificial organ development”. However, the funding amounts awarded under these grants (30–50 lakhs/year) are currently limited.

The DBT grant amounts in the field of stem cell and specifically 3D model system and organ-on-chip projects in the last few years are mentioned in Fig 10. There are no specific funds provided by Council of Scientific and Industrial Research (CSIR), Science and Engineering Research Board (SERB) and DST towards these areas.

Life sciences start-ups in India are majorly funded by BIRAC (Biotechnology Industry Research Assistance Council), a public sector enterprise set up by DBT. Biotechnology Ignition Grant scheme (BIG) is one of the flagship programs of BIRAC which is one of the largest early-stage biotech funding programs in India. Under this grant, a maximum award of 50 lakhs may be provided for a period of 18 months for start-ups to upscale and validate the

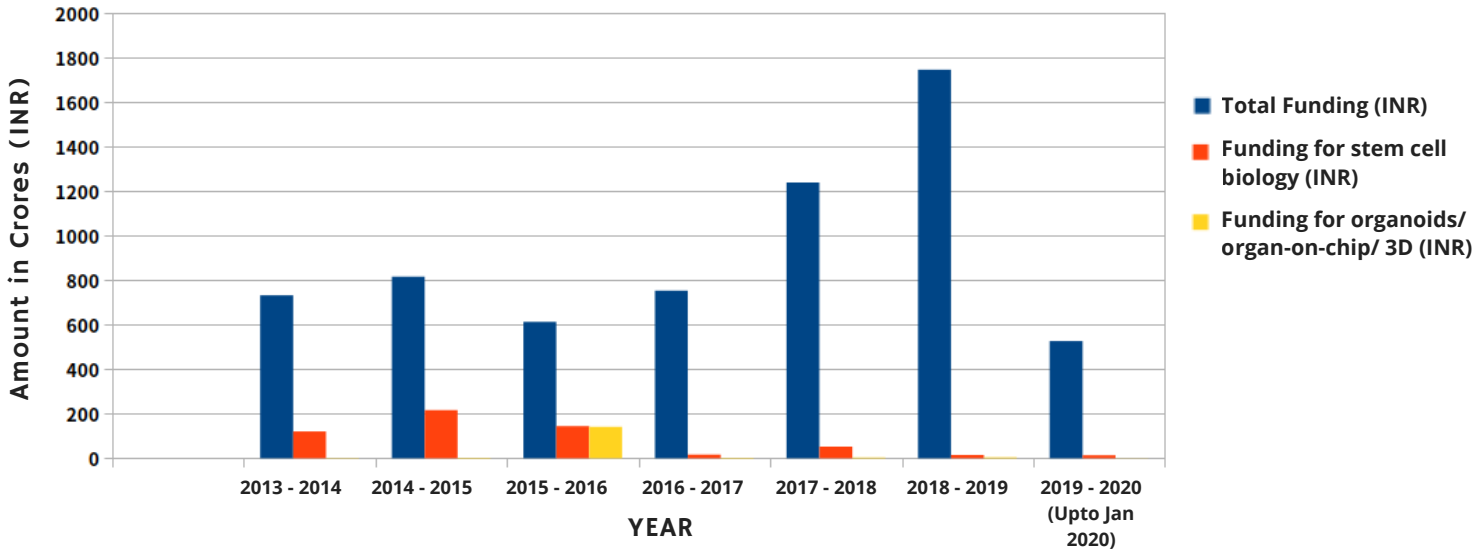

Fig 10. DBT Funding in stem cell/3D in vitro model research in India

Source of Funding	Number of groups that received funding
DBT BIRAC (Department of Biotechnology -Biotechnology Industry Research Assistance Council)	
BIG (Biotechnology Ignition Grant)	3
BIPP (Biotechnology Industry Partnership Program)	1
SBIRI (Small Business Innovation Research Initiative)	1
DBT India Alliance	2
DBT (Other)	6
DST (Department of Science and Technology)	
SERB (Science and Engineering Research Board)	1
Other	2
CSIR (Council for Scientific and Industrial Research)	1
Institutional funding	5
Private funding	6

Fig. 11: Source of funding for organoid/organ-on-chip project proposals
(Number of academic groups and startups interviewed - 26, a few groups received more than one type of funding)

proof-of-concept (Fig. 11). Similarly, various incubators across the country that house life science start-ups, including the ones working on microphysiological systems often provide seed funding.

7.2 Cost of consumables and tools

The high cost of bioreactors and 3D printers (to create scaffolds) may provide impediments in increasing the scale of organoid research in India. The import of reagents (to differentiate stem cells towards specific lineages) significantly increases the cost of performing these experiments.

Lack of sufficient microfabrication facilities in India is another crucial limiting factor in performing organ-on-a-chip experiments in India.

7.3 Reproducibility

Under the same experimental conditions, the sample yields of organoids may not provide similar organoid size, architecture, shape, cellular composition etc. and this is a critical parameter for therapeutic applications.

7.4 Lack of training and skill building in these areas

Currently India lacks skill building and training programs in these areas. There are two programs in India which train researchers in the handling, maintaining, and differentiating iPSCs. These include the “Human induced Pluripotent Stem Cell (iPSC) workshop” organised by the Centre for Stem Cell Research, CMC Vellore and “2019 CIRA-ADBS Training Program in Generation and Maintenance of Human Induced Pluripotent Stem cells” offered by InSTEM (NCBS, Bangalore).

However, these programs do not include creating, maintaining, and characterizing organoids or organ-on-chip systems. Also, there are no training or workshops on how to design microfabricated chips or conduct organ-on-chip experiments.

8. Tackling the challenges

8.1 Domestic production of reagents and tools

The cost of consumables required for research experiments such as reagents, etc., can be brought down several-fold when locally manufactured in India.

For organ-on-chip experiments, the micro fabricated chip is a substantial expense. While a chip designed in India can cost 1200–1500 rupees/chip, an imported chip may cost between 10,000–20,000 rupees/chip. Establishing more micro-fabrication facilities could reduce the cost to conduct organ-on-chip research in India.

8.2 Increasing the training and building expertise

Targeted workshops and training should be designed involving the creation, maintenance, and characterisation of organoids / in vitro 3D model systems. For instance, organ-on-chip experiments require training to design the micro- fabricated chip.

There is also a dearth of training for various techniques, such as imaging the 3D organoids or organ-on-chip. To train researchers/ students /scientists in India, three advanced microscopy workshops are held: the Bangalore Microscopy Course (NCBS, Bangalore); the Workshop on BioImaging/Advanced Light Microscopy (IISER, Pune); and Bioscopy 2019 (IISER Kolkata).

8.3 Increasing governmental and non-governmental funding

Some of the needed instruments, such as a 3D printer, bioreactor etc. and consumables can create a significant financial burden. Government and private funding opportunities in this space can help encourage more scientists to conduct research in this area.

8.4 Fostering collaborations to promote innovation

To bring these systems closer to human complexity, further research and innovation involving collaborative and multidisciplinary science are required. Dialogue between different fractions of science, academia and industry can be initiated by organising meetings, conferences, and symposiums to understand the cross-sector requirements.

8.5 Regulatory engagement

A constant engagement with the regulatory bodies and updating them of the advancements from the early stages can also help in fastening the process of regulatory acceptance as well as build more confidence, by creating awareness about the usage of non-animal methodologies.

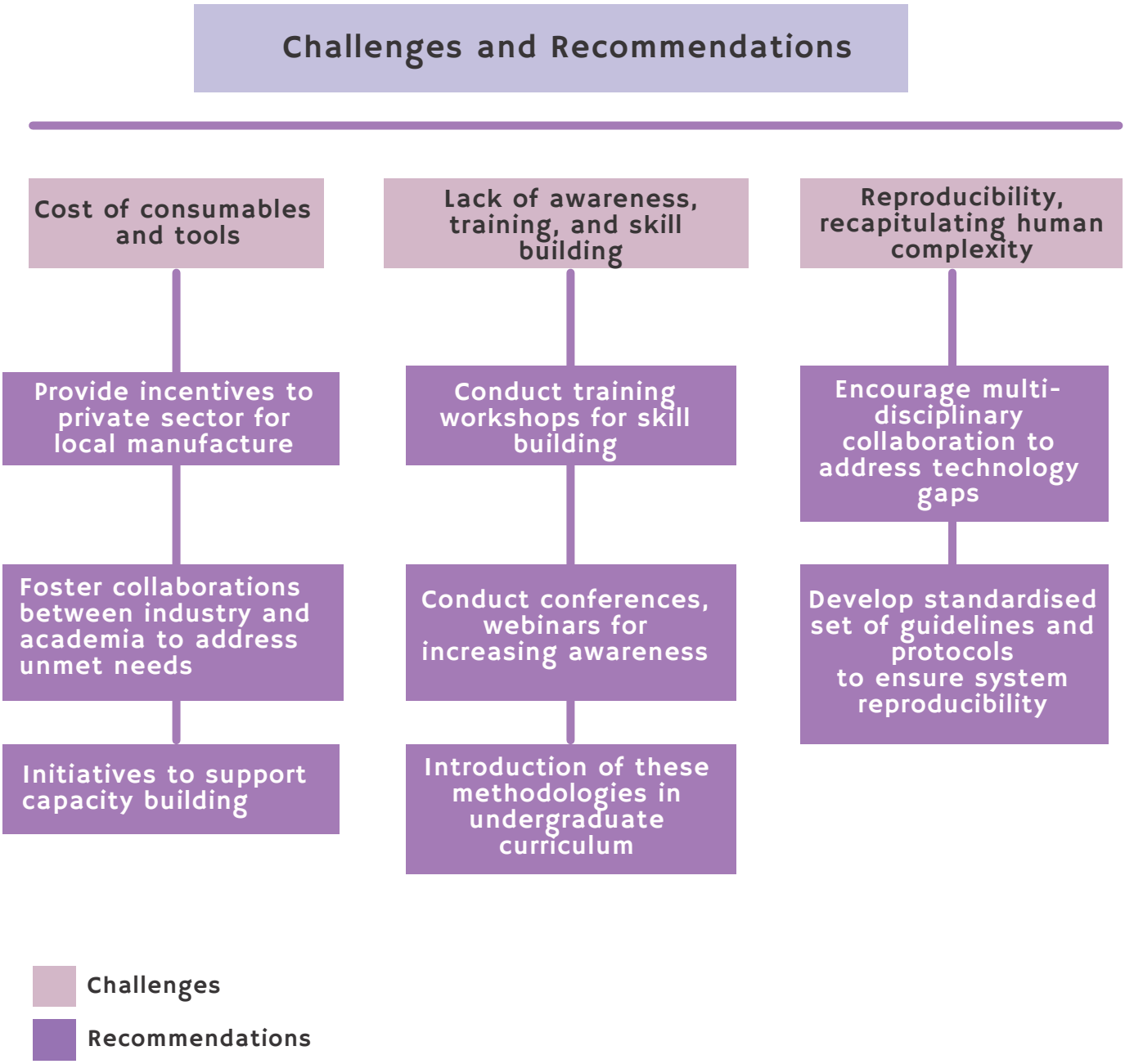

Fig 12. Challenges and recommendations for promoting human-relevant model systems in India

9. Conclusions: Way Forward

The move towards methods that are human-based will lead to a paradigm shift in how we investigate questions related to drug discovery and development. While several countries across the globe are taking significant steps in this direction, this field needs to develop further in India.

Recently, a Perspective paper on the need for alternatives to animals in the Indian context was published by ICMR. The paper emphasizes the need to promote top-down funding decisions towards human-based methods instead of newer animal models; creating avenues for national and international collaboration to create open access and high-quality data for these alternative methodologies. This knowledge base could fuel further understanding of human disease mechanisms. Another key factor is cross-talk between industry, academia, and government to develop innovations in this area. The paper also proposed setting up “Centres of Excellence” to conduct human-relevant research in India. As a first step, ICMR established the first ‘Centre of Excellence in Human-Pathway-Based Biomedicine and Risk Assessment’ facility in NARF-BR(Hyderabad)

A collaboration between Atal Incubation Centre-CCMB and Humane Society International-India has also led to the establishment of the "Centre for Predictive Human Model Systems". The Centre has been established with the objective of developing it as a scientific and policy think-tank to promote non-animal and human-relevant methodologies in India.

This white paper is an attempt to assess the current landscape in India in terms of microphysiological systems, identify the gaps, and provide recommendations for the same (Fig 12). These initiatives should help in the development of India as a key player in the innovation and application of human-relevant methodologies across the globe.

ANNEXURE

TABLE 1 - KEY ACADEMIC PLAYERS IN THE FIELD OF ORGANOID AND ORGAN-ON-CHIP IN INDIA*

SL. NO	NAME	LOCATION	AREA OF WORK
1	Indian Institute of Technology	Delhi	Dermal organoids
2	Indian Institute of Science	Bengaluru	Breast cancer organoids
3	InSTeM	Bengaluru	IPSc lines
4	Center for Cellular and Molecular Biology	Hyderabad	Hepatic, pancreatic, brain, trophoblast, neural cancer organoids
5	National Institute of Nutrition	Hyderabad	Umblical cord mesenchymal stem cells for drug testing
6	National Center for Cell Science	Pune	Liver organoids
7	Indian Institute of Technology	Guwhati	Liver organoids
8	Indian Institute of Technology	Hyderabad	3D printed corneas, organ-on-a-chip
9	Tata Memorial College	Mumbai	Cancer organoids
10	National Institute for Research in Reproductive Health	Mumbai	Placental organoids
11	University of Pune	Pune	Lung-on-a-dish, infection-on-a-chip
12	Indian Institute of Technology	Varanasi	Neuronal connections on a chip, liver-on-a chip
13	Indian Institute of Technology	Bombay	Tumour-on-a-chip skin-on-a-chip, lung-on-a-chip
14	Indian Institute of Chemical Technology	Hyderabad	retina-on-a-chip, skin-on-a-chip, placenta-on-a-chip,tumour-on-a-chip

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ANNEXURE

TABLE 2 - KEY INDUSTRY PLAYERS IN THE FIELD OF ORGANOID AND ORGAN-ON-CHIP IN INDIA*

SL. NO	NAME	LOCATION	AREA OF WORK
1	L V Prasad Eye Hospital	Hyderabad	Corneal organoids and 3D printed cornea
2	Sapien Biosciences	Hyderabad	Cancer organoids, 3D ex vivo skin cultures
3	Pandorum Technologies	Bengaluru	Liver organoids
4	Eyestem	Bengaluru	3D Retinal organoids
5	Reagene Lifesciences	Bengaluru	Integrated 3D system-on-a-chip, clinical trials-in-a-dish

TABLE 3 - INFRASTRUCTURE COSTS FOR CONDUCTING ORGANOID EXPERIMENTS

SL. NO	NAME OF THE INSTRUMENT	COST(INR LAKHS)
1	CO_2 incubator	3.2
2	Centrifuge	6.1
3	Freezers (-80 ^{o}C)	5.4
4	Microscopes (confocal/light)	2.6
5	Water bath	2.6
6	Laminar flow	1.5
7	Liquid nitrogen	0.6
8	Containers (20L)	2 - 5
9	3D printers, Bioreactors (optional)	20
10	Rotor shaker	0.2

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