J Biosci (2020)45:144
DOI: 10.1007/s12038-020-00112-8

Perspectives

The need to develop a framework for human-relevant research in India: Towards better disease models and drug discovery

SURAT PARVATAM[1]*, SHAM BHARADWAJ[1], VEGESNA RADHA[2] and MADHUSUDHANA RAO[3]

[1]*Centre for Predictive Human Model Systems, Atal Incubation Centre-CCMB, Hyderabad 500 039, India*

[2]*CSIR-Centre for Cellular and Molecular Biology, Hyderabad 500 007, India*

[3]*Atal Incubation Centre-CCMB, Hyderabad 500 039, India*

**Corresponding author (Email, suratsaravanan@gmail.com)*

MS received 30 April 2020; accepted 1 October 2020

The low translational efficiency of animal models to humans, and the development of new-age methodologies that are human-cell based, is fuelling a paradigm change across the globe. In this perspectives paper, we discuss the current state of research, funding, and regulation in these 21st century technologies, including organoids and organ-on-chip in India. Recently, a road-map was drawn by Indian Council for Medical Research (ICMR) regarding alternatives to animals in research in India and it also held a special session in January 2018 to discuss latest developments in new human-relevant model systems. We document the regulatory and research landscape in this field in India. We also discuss the challenges present in this field which include lack of training and skills to handle embryonic or induced pluripotent stem cell (iPSC) lines, funding limitations, lack of domestic production of reagents leading to elevated costs, and lack of infrastructure, such as microfabrication facilities. In the end, we provide recommendations to enable innovation and application of human-relevant methodologies to develop India as a key player in this arena globally.

Keywords. Disease models; drug discovery; framework in India; human-relevant research; organoids; organ-on-chip; policy

1. Growing shift towards human-relevant research

Animal models have played critical roles in understanding human biology, and this has been primarily driven by the fact that humans share a high degree of similarity with various animals (Bernhard 2000). For example, protein-coding regions of mouse and human genome are 85% similar. Also, it is easy to test the functionality of various genes in the model organisms by increasing, decreasing, or silencing the gene expression using various genetic tools. These advantages are the basis of regulations currently being followed to test all drugs in two animal models prior to clinical trials. However, it is increasingly emerging that the animal models cannot capture severity, and phenotype, of a human disease in its entirety (van der Worp *et al.* 2010). Also, the use of animal models to conduct such large scale therapeutic screening involves prolonged duration, high costs (Giacomotto and Laurent 2010), and trauma to animals.

Animals do not naturally develop many of the human diseases and researchers need to artificially induce the disease before studying them. However, the induced disease state in animals may not be physiologically similar to a human disease (Perrin 2014). For example, Duchenne muscle dystrophy (DMD) is the most common muscular dystrophy with an incidence of one in 5000 male live births. It is caused by mutation in dystrophin gene and leads to progressive and severe muscle wasting in humans. However, mutation of

Published online: 18 November 2020

dystrophin gene in mice causes only mild and non-progressive disease with minimal muscle wasting and reduction of life span (McGreevy *et al.* 2015). Similarly, cystic fibrosis, a disease caused by a mutation in cystic fibrosis transmembrane conductance regulator (CFTR), is one of the most common genetic disorders with an incidence of one in 2500 live births in the Caucasian population. However, mice models of the diseases do not show spontaneous bronchitis and lung disease that are typical hallmarks in human cystic fibrosis condition (Egan 2009). Around 150 drugs that successfully treated sepsis-like condition in mice failed in human clinical trials (Rittirsch *et al.* 2007).

The immune system and stress response pathways appear to be more divergent in humans compared to murine models. To understand the differences in immune response between mice and humans, a study took blood samples and looked at the gene expression profiles during traumatic injuries, burns, and bacterial infection. Similar groups of genes were involved in burn, trauma, and infection in humans; however, distinct sets of genes are employed in mice to fight against different types of stress (Seok *et al.* 2013). Mice are also more resilient to inflammation compared to humans. While the lethal dose of endotoxin is 5–25 mg/kg in mice, <30 ng/kg can cause shock in humans (Sauter and Wolfensberger 1980.).

There are also species-specific differences in how drugs are absorbed, distributed, metabolized, and transported. Cytochrome P (CYP) is one the most important class of drug-metabolising enzymes and differences in the CYP isoforms between species is a major reason for differences in drug metabolism (Martignoni *et al.* 2006).

One of the most common reason for failure of clinical trials is lack of efficacy, followed by toxicity (Kondo and Masahiro 2019). A study that looked at the concordance of adverse drug reactions (ADRs) for 142 approved drugs found that musculoskeletal, respiratory and neurological ADRs showed a concordance of less than 30 per cent in terms of adverse reactions between mice models and humans (Clark and Steger-Hartmann 2018). These issues have led to a high rate of clinical failure when translating the drug targets from model organisms to humans. The efficiency of the pharmaceutical R&D is calculated using two parameters: success rates and time spent during the developmental stages. 92% of drug targets that pass the preclinical stage fail during the subsequent stages of phase I, II, and III (Plenge *et al.* 2013) clinical trials. The situation is equally dire in India, where drug development research has resulted in more than 200 compounds that have entered preclinical and clinical stage development; but none have reached the global markets in the last two decades (Differding 2017). The high failure rate during trials has been attributed to low precision in identifying potential target molecules during the early stages of drug discovery and development, off-target effects, and toxicity (Mohs and Greig 2017).

Currently, the average cost of producing a drug can range from $1–4 billion (Prasad and Mailankody 2017; Herper 2019). Due to the mounting costs and low efficacy, most of the major pharma companies in India have exited the drug discovery field (Differding 2017). This suggests the need for models that can predict human toxicity and drug responses better.

2. Organoids as a model system in drug discovery and research

Organoids are mini tissues grown *in vitro* in 3D cultures that mimic organization and function of tissues/organs in the body. These are generally grown from stem cells, or from tissue derived primary cells. Human organoids have been used to model various human diseases, including cystic fibrosis (Dekkers *et al.* 2013), microencephaly (Lancaster *et al.* 2013), autism (Mariani *et al.* 2015) etc to understand disease mechanisms and establish platforms for screening drugs (Lancaster and Meritxell 2019). One of the first evidences of the link between Zika virus and microencephaly was found using brain organoids (Li *et al.* 2016). Apart from disease biology, organoids are a great tool to study and understand human organogenesis and development (Sriram *et al.* 2020). For example, brain organoids can recapitulate various brain regions, such as forebrain, mid-brain, hindbrain, and the presence of different structures within an organoid leads to a more complex structure relatable to the human brain (Lancaster *et al.* 2017).

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 (Vaira *et al.* 2010). In one study, patient-derived organoids (PDO) were created from 9 patients with pancreatic duct adenocarcinoma and used to test various treatment possibilities. The PDOs were sensitive to some drugs but not others: five patients treated with drugs to which the PDOs 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 (Tiriac *et al.* 2018).

Similarly, engineered heart (Eder *et al.* 2016), liver (Kostadinova *et al.* 2013), intestine, kidney, pancreas (Saito *et al.* 2019), and other cancer (Saito *et al.* 2019; van de Wetering *et al.* 2015) organoids have been used as model systems to screen and test the efficacy of drug candidates for cardiovascular diseases, hepatotoxicity, various cancers, etc. The three-dimensional culture systems of liver can maintain liver function for a duration of three months, respond to inflammation, and detect drug induced toxicity, making them an attractive system to screen drugs for toxicity (Meng 2010).

Before an orally administered drug enters the systemic circulation, it is absorbed through the intestinal wall and transported to the liver. The dynamics of the adsorption, distributions, transport, and elimination (ADME) determines the overall effect of the drug exerted on the body. Few studies are beginning to evaluate the pharmacokinetic-pharmacodynamic properties in organoids. A recent study generated intestinal organoids from human induced pluripotent stem cells (hiPSCs) and showed that these organoids expressed drug transporters, efflux transport activity and induction of CYP3A4, a drug metabolising enzyme expressed in intestine (Onozato *et al.* 2018). They show that these intestinal organoids could be used for pharmacokinetic evaluation during the drug development process. In another study, the authors performed metabolic imaging of breast cancer organoids to assess drug response (Walsh *et al.* 2016). While these results indicate the potential of organoids for conducting PK/PD studies during drug development, their effectiveness with respect to various aspects of ADME still needs to be established.

3. Organ-on-a-chip system in drug discovery and research

Organs-on-a-chip are memory chip-sized microfluidic devices that have hollow channels lined with specific cells of a human tissue (Sosa-Hernández *et al.* 2018). These cells are interfaced with vasculature or endothelial cells to recapitulate the tissue-tissue interactions. Additionally, mechanical forces can be applied to these devices to recapitulate the physical cues present *in vivo*, such as breathing motions or peristalsis (Ingber 2018). These engineered micro-fluidic devices have been created for several human organs, such as lung, intestine, kidney, skin, blood–brain barrier, etc. (Qirui *et al.* 2020).

Recently, organs-on-chip are emerging as a potential solution to understand *in vivo* pharmacokinetic and pharmacodynamic processes. A multi-organ chip hosting four human organ equivalents was designed using reconstructed 3D small intestine; skin biopsy for oral absorption; 3D liver equivalent; and kidney proximal tubule compartment to study the PK/PD dynamics of drugs (Maschmeyer *et al.* 2015). Additionally, a peristaltic micropump ensured pulsatile flow of media which interconnected the four tissues and excretion of fluid *via* the kidney chamber. Following the administration of the drug to this system, it was distributed to the liver compartment, followed by skin and kidney. The authors propose this system could be used for ADME profiling of drug candidates for over 28 days. Another such multi-type cell microfluidic device was developed to understand the drug absorption and metabolism profile *in vitro* where Caco-2, HepG2, and U251 cells were co-cultured in different chambers to mimic intestine, liver, and glioblastoma cells (Jie *et al.* 2016). Drug cytotoxicity assays were performed using this system suggesting its application for high throughput drug target screening.

4. Current limitations in the organoid/organ-on-chip field

Despite their high potential, organoids/organ-on-chip are not devoid of limitations. Achieving *in vivo* cellular complexity, geometry, tissue growth, and function are some of the current challenges in organoids. Simple organoids do not possess a vasculature, immune cells, or a microenvironment. The gene expression profile of organoids is often fetal in nature (Luo *et al.* 2016); however, studies show that extending the time spent in the culture can lead to improved maturation of the tissues (Spence *et al.* 2011; Takasato *et al.* 2015). But the lack of vasculature leads to low nutrient and oxygen availability which further impedes their scale of growth. These limitations are being overcome by either grafting the organoids into the vascular bed of animal models or engineering organoids with functional vascular-like systems to promote their growth and function (Cakir *et al.* 2019).

Reproducibility is a major issue where under the same experimental conditions, the sample yields of organoids may not provide similar organoid size, architecture, shape, cellular composition etc. Reproducibility in PDO's has also been difficult to achieve. Also, some tissues have not been amenable to develop organoids.

While organ-on-chip field has progressed greatly in the last decade, there are still certain challenges to

achieve a more physiologically relevant system, especially to conduct PK/PD studies (Lee *et al.* 2019). Intestinal wall needs to be reproduced with four layers, including mucosa, sub-mucosa, muscular layer, and serosa as these layers have implications in the absorption of drugs. Integration of microbiota and scalability is another challenge (May *et al.* 2017). Incorporation of biomaterials for the fabrication of microfluidic systems is another exciting area of research as polydimethylsiloxane (PDMS) can adsorb hydrophobic molecules and alter absorption profile of drugs (Zhang *et al.* 2018).

These microfluidic devices are micro engineered usually in the microfabrication facility. The availability of microfabrication facilities may also be a significant deterrent (further discussed in section 8).

5. Regulatory push towards non-animal models

5.1 *Global*

Various countries, including US, UK, and the European Union are recognizing the potential of organoids and organ-on-chip technologies to investigate questions related to drug toxicity, disease development, and human biology. The organoid research has increased exponentially globally in the last decade (figure 1). Apart from the research, there has been a concomitant increase in regulation and funding to develop various non-animal methodologies globally (figure 2). The European Innovative Medicines Initiative (IMI) is a € 5.3 billion private–public partnership between European Commission and European Federation of Pharmaceutical Industry Associations which has recognized 'replacing animals with better *in vitro, in silico* models' as one of its key focus areas. Some of the initiatives of IMI that involve the development and application of non-animal methodologies include VAC2VAC and STEMBANCC. VAC2VAC aims to develop and validate non-animal testing methods for human and veterinary vaccines. STEMBANCC program intends to provide well characterized patient-derived induced pluripotent cells via a biobank. It also aims to show proof-of-concept for using induced pluripotent stem cells (iPSCs) to treat various disorders.

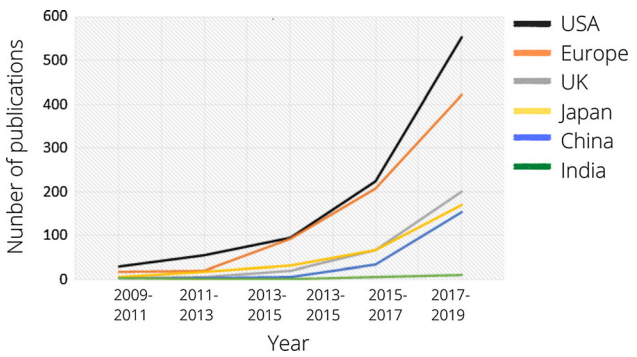

Figure 1. Number of papers published globally in the field of organoids.

EU-TOXRISK is a € 30 million European flagship program for toxicology testing and risk assessment for 21st century. It plans to integrate various advancements in cell biology, omics technologies, systems biology, and computational modelling to provide a human-cell based assessment of chemical hazards and risks.

Initiatives in US include $142 million-dollar Tissue Chip Program which aims to develop 3D platforms to test drug toxicity. Under this initiative, NIH funded 13 projects in 2017 to develop 3D chips using living cells and tissues that can represent human organ systems. In 2018, it issued 5-year-awards towards developing tissue chip systems that could replicate type-2-diabetes; and funded three Tissue Chip Testing Centre (TCTC) to test and validate tissue chip platforms and a Microphysiology Systems Data Center to create a database of experimental data generated on diverse microphysiological systems organ models. Scientists are now collaborating to combine various organ chips to develop an integrated system or human body-on-a-chip.

Apart from funding, various regulatory measures have stimulated the development of non-animal methodologies (NAM) globally. In the EU, the Directive on the Protection of Animals Used for Scientific Measures, REACH, and the Cosmetics Directives strongly encourage the use of non-animal methodologies (Directive 2014; Regulation (EC) No 1223/2009). The EU has also formally established an organization EURL ECVAM, the European Union Reference Laboratory for Alternatives to Animal Testing, to scientifically develop and validate NAMs. The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is a permanent committee of the National Institute of Environmental Health Sciences in US. ICCVAM has representatives from 16 federal and research agencies and it promotes the regulatory acceptance of non-animal toxicology and safety tests. In 2018, ICCVAM published a strategic roadmap to establish non-animal testing methods to evaluate the safety of chemicals and medical products. US EPA, FDA and NIEHS have also developed roadmaps to develop non-animal technologies (Casey 2017; Biotechnology and Biological Sciences Research Council 2017).

Currently, Centres for Alternatives to Animal Methods have been established in US, Canada, and

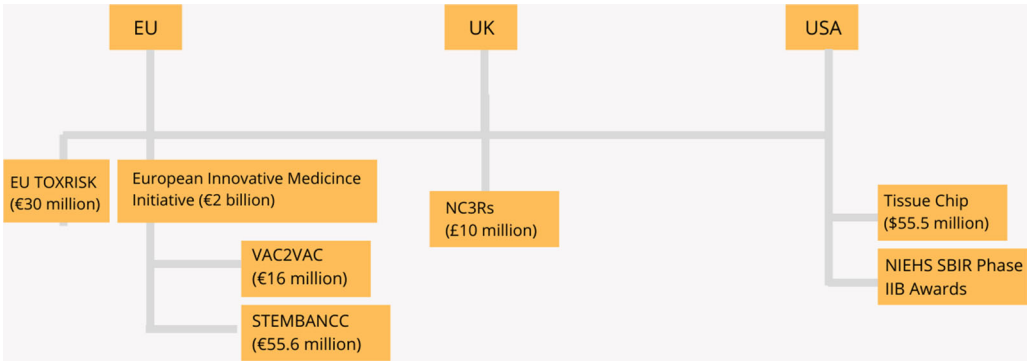

Figure 2. Global funding initiatives in non-animal research.

Europe to develop, validate, and promote non-animal methodologies in toxicity testing and biomedical research.

In a landmark move, the US Environmental Protection Agency (EPA) recently announced that it would reduce the funding of animal studies by 30 per cent by 2025, and eliminate all requests and funding for mammalian studies by 2035 (Grimm 2019). The US Government Accountable Office also recently held that federal agencies, including The Department of Health and Human Services (HHS),U.S. Department of Agriculture (USDA), and Environmental Protection Agency (EPA) should assess the efforts to develop and promote alternatives to animal research in US (U.S. GAO, 2019).

These initiatives strongly indicate the global push towards the development and use of non-animal and more human relevant model systems in basic and clinical research.

5.2 *India*

Some Indian laws and regulations regarding animal use and use of NAMs in research are listed in figure 3. In 2009, the Medical Council of India instructed the use of alternatives to animal experiments during undergraduate teaching. In 2012, University Grants Commission and Ministry of Health and Family Welfare (MoHFW) banned the use of animals in educational institutes. In 2014, India became the first south Asian country to ban the import of cosmetics that were tested on animals. In 2016, MoHFW banned the Draize test for eye irritation in rabbits, and additionally passed an amendment that sparred the repeat animal testing of chemicals/drug in India that had been previously tested abroad. In 2018, Central Insecticides Board Registration Committee (CIBRC) under the Ministry of Agriculture released the Guidance Document on Toxicology for Registration of Chemical Pesticides in India'. In the document, the Ministry of Agriculture revised its pesticide testing regulation to recognize human-cell based alternative testing methods to measure eye and skin irritation.

Recently, a perspective paper on the need for alternatives to animals in the Indian context was published by the Indian Council of Medical Research (ICMR) (Swaminathan *et al*. 2019). Apart from this, a roadmap has also been drawn by ICMR for Alternatives to Animals in Research in India. The ICMR also sponsored a special session on Alternatives to animal models at the International congress of Cell Biology held in Hyderabad, India in January 2018 where the latest developments in new model systems were discussed.

6. Market research and cost analysis

The market research in organoids predicts a high Compound Annual Growth Rate (CAGR) for the period 2019–2027 (Research Nestester 2019). The market share in terms of application is expected to be highest in domains of biomedical research, cancer research, and drug discovery. Both rapid rate of research and advancements in 3D cell culture methodologies are expected to propel the market growth of this methodology.

In a recent study, Thomas Hartung group at John Hopkins Centre for Alternatives to Animals (CAAT) estimated the economic landscape of various toxicity tests performed in animal models and found that cost per test ranged from $1200 (*in vivo* skin irritation test) to $4700 (skin sensitization) (Meigs *et al.* 2018. Madeline Lancaster, a pioneer in the field of brain organoids currently working at Medical Research Council (MRC) Laboratory of Molecular Biology in Cambridge, UK estimated the cost at roughly $150 per organoid experiment (excluding the equipment) (Chi 2015). However, the cost estimates might vary

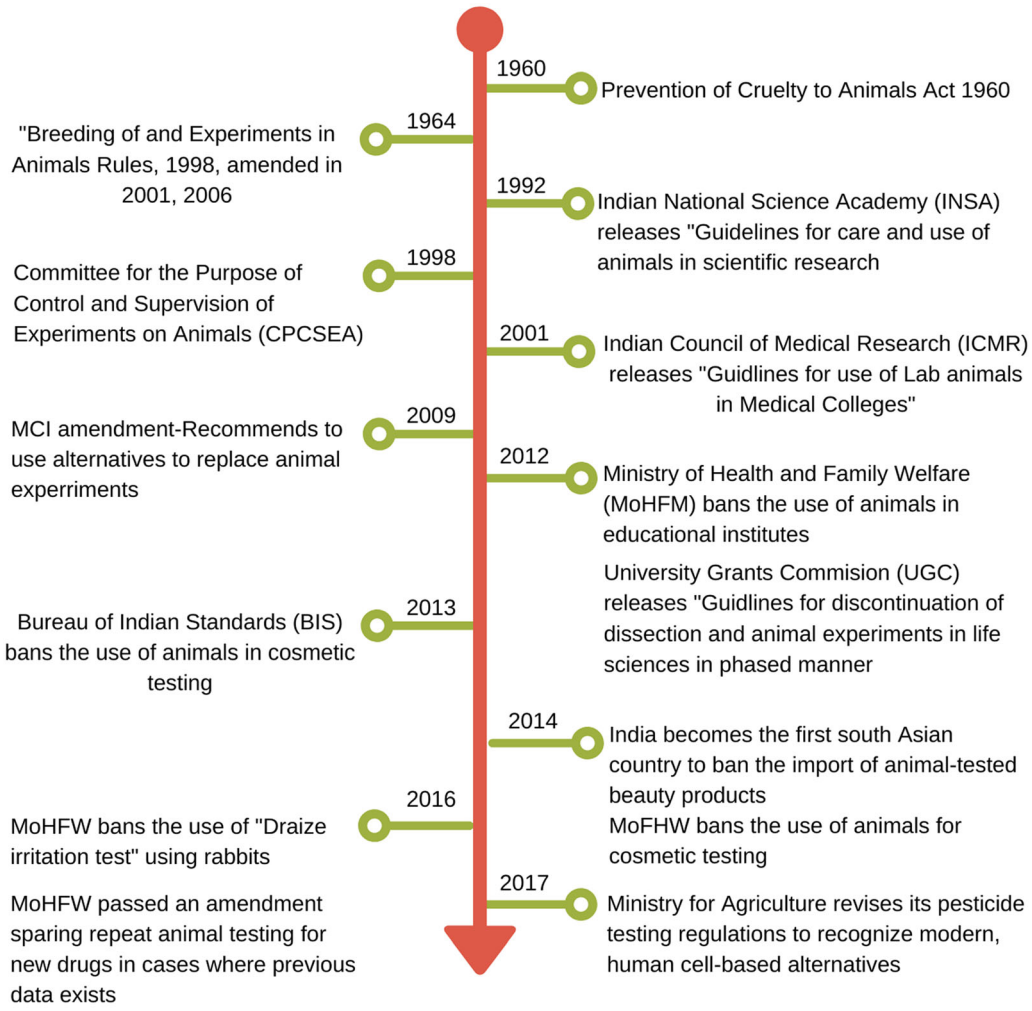

Figure 3. Indian laws and regulations to reduce animal use in research and toxicology in India.

depending on various parameters such as the scale of the experiment, type of organoid, how long the cultures have to be maintained etc.

Another study, estimated the impact of organ-on-chip models on the cost of different phases of pharmaceutical R & D, including target to hit, hit to lead, lead optimization, preclinical, Clinical Phase I, Phase II, Phase III trials (Franzen *et al.* 2019). The total reduction in cost was estimated to be 10–26%, and preclinical stages and lead optimisation stages were reported to be the two domains where cost reduction could be highest due to the greater predictability of these models.

7. Status of organoid/organ-on-chip research in India

As evident from the number of published papers, organoid research has exponentially increased globally in the last few decades; however, the exploration of non-animal alternate model systems in terms of research and regulation is still almost negligible in India. This is evident from number of papers published when compared to other countries (figure 1). An analysis of major model systems in India using advanced PubMed search revealed approximately two thirds of research uses animal models, either mouse or rats. Interestingly, around 23% of the research was carried out using computational or systems biology methods/tools/methodologies (figure 4). Computational methodologies are an emerging field in basic and drug research. A study showed that *in silico* drug trials had higher accuracy in predicting the drug-induced cardiotoxicity compared to animal models (Passini *et al.* 2017). Another recent study used machine learning approaches to detect new antibiotics with novel mechanisms of action (Stokes *et al.* 2020). As the model predicted molecular functions without the prior assumptions on drug action, it was able to detect

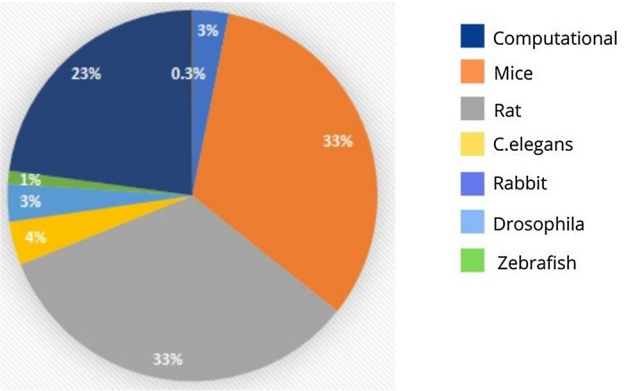

Figure 4. Papers published in India using various model systems in the past decade.

new patterns. These models serve to provide leads that will enable experimental testing on a reduced scale.

Many research institutes and private companies are beginning to establish and explore the field of organoids and organ-on-chip. Table 1 shows the academic institutions, and table 2 shows private institutes/start-ups in India that are currently working in this area. In most cases, the research is still in its infancy.

The funding opportunities in this area currently are still limited. An analysis of funding received by 26 government labs and start-ups in this area in India showed that DBT is the major funding body in this area. So, we analyzed the funding allocated by DBT in the area of organoids/organ-on-chip in the last decade, and funding to organoid/organ-on-chip receives around 0.2% of total DBT funding (table 3). We also looked at the number of projects funded by DBT in this area, and the number is still dismal (table 4) with around three organoids/organ-on-chip projects funded by DBT every year. However, most of the funding has been concentrated in the past 3–4 years, showing a positive inclination to recognize and support this branch of research.

Table 1. Academic institutions working in the field of organoids/organ-on-chip

Sl.No.	Institute	Location	Area of work
1.	IIT Delhi	Delhi	Dermal organoids
2.	IIT Guwahati	Guwahati	Liver organoids
3.	IIT Hyderabad	Hyderabad	3D printed corneas, Organ-on-chip
4.	IIT - BHU	Varanasi	Neuronal connections-on-chip, liver-on-chip
5.	IIT Bombay	Mumbai	Retina-on-a-chip, skin-on-a-chip, placenta-on-chip, tumour-on-a-chip
6.	CCMB	Hyderabad	Hepatic, pancreatic, brain, trophoblast, neural and cancer organoids
7.	NIN	Hyderabad	Umbilical cord mesenchymal stem cells for drug testing
8.	NCCS	Pune	Liver organoids
9.	SPPU	Pune	Lung-on-dish, Infection-on-chip
10.	Tata Memorial College	Kolkata	Cancer organoids
11.	NIRRH	Mumbai	Placental organoids
12.	ICT	Mumbai	Skin-on-chip, Lung-on-chip
13.	IISc	Bengaluru	Breast cancer organoids
14.	InSTEM	Bengaluru	IPSc lines

8. Issues plaguing this area in India

The cost of reagents to differentiate stem cells towards specific lineages is a significant deterrent. In this regard, it may be worthwhile to generate the required growth factors, and chemical entities in India. This will enable cost cutting as well as saving time on imports. While stem cell research and regenerative medicine has been recognized as one of the thrust areas in the biomedical research division of the Department of Biotechnology (DBT) in India, the funding in this area is still miniscule as shown in tables 3 and 4.

Lack of training is a major factor where most labs in India currently lack skills and training required to generate and handle embryonic stem cells (ES) or iPSC lines. It will help to identify 2–3 institutes that have successfully established organoid cultures to take up

Table 2. Key players among Industry in the field of organoid and organ-on-chip in India

Sl. No.	Name of the company	Location	Area of work
1.	L.V. Prasad Eye Hospital	Hyderabad	Corneal organoids, 3D printed cornea, Retinal Organoids
2.	Sapien Biosciences	Hyderabad	Cancer organoids, 3D *ex vivo* skin cultures
3.	Reagene Lifesciences	Hyderabad	Integrated 3D system-on-chip, Clinical trials-in-a-dish
4.	Pandorum Technologies	Bengaluru	Liver organoids
5.	Eyestem	Bengaluru	3D retinal organoids

Table 3. Number of projects funded by Department of Biotechnology (DBT) towards stem cell biology and organoid/organ-on-chip research

Sl. No	Year	Total funding (INR Crores)	Stem cell biology (INR Crores)	Organoids/ Organ-on-chip/3D (INR Crores)
1	2013–2014	732.74	120.54	13.49
2	2014–2015	816.5	216.31	1.94
3	2015–2016	613.48	144.21	141.36
4	2016–2017	753.62	16.32	2.01
5	2017–2018	1239.95	52.15	3.49
6	2018–2019	1747.44	14.8	5.77
7	2019–2020 (up to Jan 14, 2020)	527.09	13.34	0.42
8	Total funding 2013–2020 (INR)	6430.82	577.67	168.48

Table 4. Number of projects funded by Department of Biotechnology (DBT) towards stem cell biology and organoid/organ-on-chip research

Sl. No	Year	Total number of projects	Stem cell biology projects	Organoid/ organ-on-chip/3D projects
1	2013–2014	650	15	3
2	2014–2015	621	25	3
3	2015–2016	392	5	1
4	2016–2017	517	15	3
5	2017–2018	746	24	6
6	2018–2019	724	14	4
7	2019–2020 (up to Jan 14, 2020)	510	7	1
8	Total projects 2013–2020	4160	105	21

training. The Government can help in this regard by providing funds for this purpose.

For designing an organ-on-a-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, Central Mechanical Engineering Research Institute (Kolkata). While a chip designed in India can cost 1200–1500 rupees/chip, a chip imported from outside may cost between 10,000–20,000 rupees/chip. Establishing more microfabrication facilities in India can accessibility and reduce the cost to conduct organ-on-chip research in India. A basic knowledge of engineering is also required for designing the chip. Continuing cross talk between the cell biologists and engineers will enable achieving targets quicker.

9. Recommendations to develop the field in India

9.1 *Domestic production of reagents and tools*

When produced locally in India, the cost of consumables, such as reagents can be brought down several fold, significantly lowering the experimental costs. Establishing more micro fabrication facilities in India can reduce the cost to conduct organ-on-chip research in India where the microfabricated organ-on-chips are in many cases imported from outside. Creating biomaterial-based scaffolds is another area in bioengineering that can be, and needs to be developed in India.

9.2 *Increasing the training and building expertise in these areas*

Specific workshops and training modules need to be designed that involve creating, maintaining, and characterizing organoids or *in vitro* 3D model systems. For organ-on-chip experiments, training is required to design the microfabricated chip. There is also a lack of training for imaging the 3D organoids or organ-on-chip. In 2019, three advanced microscopy workshops were held in India: Bangalore Microscope Course (NCBS, Bangalore); Workshop on BioImaging: Advanced Light Microscopy (IISER, Pune); and Bioscopy 2019 (IISER Kolkata).

9.3 *Establishment of government bodies/committees to oversee development*

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is a permanent committee established by the National Institute of Environment Health Sciences (NIEHS, USA). Similar committee or an organization could be set-up in India

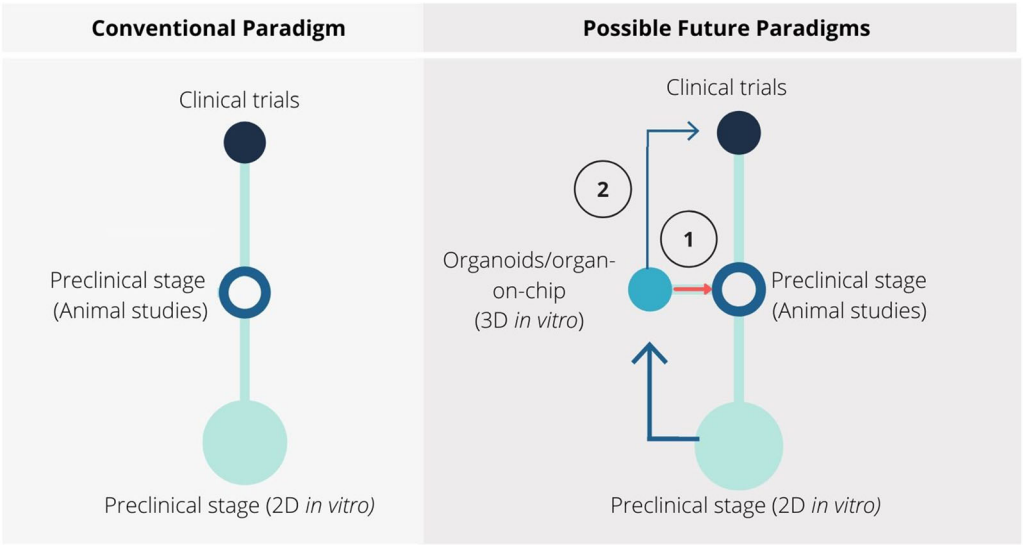

Figure 5. Possible hierarchy of inclusion of organoids/organ-on-chip in drug research.

to bring about regulatory acceptance of organoids and oversee the research required to establish them as a viable alternative to animal models in various stages of research and drug development.

9.4 *Fostering collaborations to promote innovation in this field*

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 organizing meetings, conferences, and symposiums to understand the cross-sector requirements.

9.5 *Regulatory engagement*

Engaging regulators during the early stages of development and use of non-animal methodologies can help in easing the path for regulatory acceptance and can also create more confidence in the use of non-animal methodologies.

10. Way forward

The shift to human relevant model systems will lead to paradigm shift in the fields of drug discovery and development and understanding human disease pathophysiology. While there have been significant strides to develop these technologies in the developed countries, India is beginning to take small steps in this direction.

In figure 5, we discuss two possible models on how organoids/organ-on-chip can be included in the current paradigm for drug research. The current hierarchy includes testing on cell lines, followed by animal studies, and the targets are subsequently tested in human clinical trials. The organoids/organ-on-chip could presently be used to supplement the post-preclinical stage to investigate the effect of candidate molecules on tissues and organs, with animal models only used to investigate systemic effects (PKPD studies) (Strategy 1). Hopefully, with significant strides in research and development, human-on-a-chip and multi-organ organoids could replace animal models post preclinical research and provide more human-relevant targets for clinical trials (Strategy 2).

The ICMR perspective paper on the need for alternatives to animals in the Indian context emphasizes on the need to promote top-down funding decisions towards human-based methods instead of newer animal models (Swaminathan *et al.* 2019). It further encourages forging national and international collaborations to generate open access and high-quality data to create a knowledge base of these alternative methods. Collaboration between academic, industry, and government is key if India needs to emerge as a key player in the innovation of these methods. The paper also encouraged the creation of 'Centres of Excellence' which could conduct alternatives to animal research in India. To replace animal models, we still require research to show the predictive value of these methods compared with animal models, and that research is still lacking.

Corroborating the mandates of the paper, ICMR has decided to establish its first 'Centre of Excellence in Human-Pathway-Based Biomedicine and Risk Assessment' facility in the premises of NARF-BR in Hyderabad. A collaboration between Atal Incubation Centre-CCMB and Humane Society International-India has also led to the creation of 'Centre for Predictive Human Model Systems' to promote non-animal and human-relevant methodologies in India.

These initiatives should help in the innovation and application of human-relevant methodologies to cater to drug development and bio-medical research relevant for our country, and also in development of India as a key player in the use of organoid technologies across the globe.

References

Bernhard H 2000 How closely related are humans to apes and other animals? how do scientists measure that? are humans related to plants at all? *Sci. Am.* https://www.scientificamerican.com/article/how-closely-related-are-h/

Biotechnology and Biological Sciences Research Council 2017. Non-Animal Technologies: New Vision, Strategy and Roadmap for UK - GOV.UK. https://www.gov.uk/government/news/non-animal-technologies-new-vision-strategy-and-roadmap-for-uk.

Cakir B, Xiang Y, Tanaka Y, *et al.* 2019 Engineering of human brain organoids with a functional vascular-like system. *Nat. Methods* **16** 1169–1175

Casey W 2017 The U.S. Strategic Roadmap: New Approaches to Evaluate the Safety of Chemicals and Medical Products, Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

Chi KR 2015 Orchestrating organoids. *The Scientist Magazine* https://www.the-scientist.com/lab-tools/orchestrating-organoids-34896.

Clark M and Steger-Hartmann T 2018 A big data approach to the concordance of the toxicity of pharmaceuticals in animals and humans. *Regul. Toxicol. Pharmacol*. **96** 94–105

Dekkers JF, Caroline LW, Hugo RJ, Inez B, *et al.* 2013 A functional CFTR assay using primary cystic fibrosis intestinal organoids. *Nat. Med.* **19** 939–945

Differding E 2017 The drug discovery and development industry in India—two decades of proprietary small-molecule R&D. *Chemmedchem* **12** 786–818

Directive 2014/23/EU of the European Parliament and of the Council of European Union. 2014 on the award of concession contracts text with EEA relevance http://data.europa.eu/eli/dir/2014/23/oj

Eder A, Vollert I, Hansen A and Eschenhagen T 2016 Human engineered heart tissue as a model system for drug testing. *Adv. Drug Deliv. Rev.* **96** 214–224

Egan M 2009 How useful are cystic fibrosis mouse models? *Drug Discov. Today Dis. Models* **6** 35–41

Franzen N, Wim H van Harten, Valesca PR, Peter L, *et al.* 2019 Impact of organ-on-a-chip technology on pharmaceutical R&D costs. *Drug Discov. Today* **24** 1720–1724

Giacomotto J and Laurent S 2010 High-Throughput Screening and Small Animal Models, Where Are We? *Br. J. Pharmacol.* **160** 204–216

Grimm D 2019 U.S. EPA to Eliminate All Mammal Testing by 2035. *Science | AAAS*. September 10,2019 https://www.sciencemag.org/news/2019/09/us-epa-eliminate-all-mammal-testing-2035

Herper M 2019 The Truly Staggering cost of inventing new drugs. forbes https://www.forbes.com/sites/matthewherper/2012/02/10/the-truly-staggering-cost-of-inventing-new-drugs/

Ingber D E 2018 Developmentally inspired human organs on chips. *Development* **145** https://doi.org/10.1242/dev.156125

Jie M, Hai-Fang L, Luyao L, Jie Z, *et al.* 2016 Integrated microfluidic system for cell co-culture and simulation of drug metabolism. *RSC Adv.* **6** 54564–54572

Kondo J and Masahiro I 2019 Application of cancer organoid model for drug screening and personalized therapy. *Cells* **8** 470

Kostadinova R, Franziska B, Dawn A, Laura S *et al.* 2013 A long-term three-dimensional liver co-culture system for improved prediction of clinically relevant drug-induced hepatotoxicity. *Toxicol. Appl. Pharmacol.* **268** 1–16

Lancaster MA *et al.* 2017 Guided Self-Organization and Cortical Plate Formation in Human Brain Organoids. *Nat. Biotechnol.* **35** 659–666

Lancaster MA and Meritxell H 2019 Disease modelling in human organoids. *Dis. Models Mech.* **12** doi: 10.1242/dmm.03934

Lancaster MA, Magdalena R, Carol AM, Daniel W, *et al.* 2013 Cerebral organoids model human brain development and microcephaly. *Nature* **501** 373–379

Lee SH, Nakwon C, and Jong H S. 2019 Pharmacokinetic and pharmacodynamic insights from microfluidic intestine-on-a-chip models. *Expert Opin. Drug Metab. Toxicol.* **15** 1005–1019

Li H, Saucedo-Cuevas L, Sujan S and Joseph G 2016 The neurobiology of Zika virus. *Neuron* **92** 949–958

Luo C, Lancaster MA, Rosa C, Joseph R N, *et al.* 2016 Cerebral organoids recapitulate epigenomic signatures of the human fetal brain. *Cell Rep.* **17** 3369–3384

Mariani J, Gianfilippo C, Ping Z, Alexej A, *et al.* 2015 FOXG1-Dependent Dysregulation of GABA/Glutamate Neuron Differentiation in Autism Spectrum Disorders. *Cell* **162** 375–390

Martignoni M, Groothuis GM, de Kanter R, 2006 Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. *Expert Opin. Drug Metab. Toxicol*. **2** 875–894

Maschmeyer I, Lorenz AK, Schimek K, Hasenberg T, *et.al.* 2015 A four-organ-chip for interconnected long-term co-culture of human intestine, liver, skin and kidney equivalents. *Lab Chip* **15** 2688–2699

May S, Samantha E and Lee P 2017 Organoids, organs-on-chips and other systems, and microbiota. *Emerg. Top Life Sci.* **1** 385–400

McGreevy JW, C H Hakim, McIntosh MA and Duan D 2015 Animal models of Duchenne muscular dystrophy: from basic mechanisms to gene therapy. *Dis. Models Mech.* **8** 195–213

Meng Q 2010 Three-dimensional culture of hepatocytes for prediction of drug-induced hepatotoxicity. *Expert Opin. Drug Metab. Toxicol*. **6** 733–746

Meigs L, Smirnova L, Rovida C, Leist M, Hartung T. 2018 Animal testing and its alternatives - the most important omics is economics. *ALTEX* **35** 275–305

Mohs RC and Greig NH 2017 Drug discovery and development: Role of basic biological research. *Alzheimers Dement. (N Y)* **3** 651–657

Onozato D, Misaki Y, Anna N, Takumi A, *et al.* 2018 Generation of intestinal organoids suitable for pharmacokinetic studies from human induced pluripotent stem cells. *Drug Metab. Dispos*. **46** 1572–1580

Passini E, Britton OJ, Lu HR, *et al.* 2017 Human *in silico* drug trials demonstrate higher accuracy than animal models in predicting clinical pro-arrhythmic cardiotoxicity. *Front Physiol*. **8** 668

Perrin S. 2014 Preclinical research: Make mouse studies work. *Nature* **507** 423–425

Plenge RM, Scolnick EM and Altshuler D 2013 Validating therapeutic targets through human genetics. *Nat. Rev. Drug. Discov.* **12** 581–594

Prasad V and Mailankody S 2017 Research and development spending to bring a single cancer drug to market and revenues after approval. *JAMA Intern. Med*. **177** 1569–1575

Qirui W, Liu J, Wang X, Feng L, *et al.* 2020 Organ-on-a-Chip: Recent Breakthroughs and Future Prospects. *BioMed. Eng. OnLine* **19** 9

Regulation (EC) No 1223/2009 of the European parliament and of the council of 30th November 2009 on cosmetic products n.d. *Official Journal of the European Union,* L342/59-209. *https://ec.europa.eu/health/sites/health/files/endocrine_disruptors/docs/cosmetic_1223_2009_regulation_en.pdf*

Research Nestester, 2019. Organoids: Market Insights, Size, Trends & Forecast Till 2027. *https://www.researchnester.com/reports/organoids-market/2154*.

Rittirsch D, Hoesel LM and Ward PA 2007 The disconnect between animal models of sepsis and human sepsis. *J. Leukoc. Biol.* **81** 137–143

Saito Y, Muramatsu T, Kanai Y, Ojima H, Sukeda A, Hiraoka N, *et.al.* 2019 Establishment of patient-derived organoids and drug screening for biliary tract carcinoma. *Cell Rep.* **27** 1265–1276

Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV *et al.* 2013 Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc. Natl. Acad. Sci. USA* **110** 3507–3512

Sosa-Hernández JE, Villalba-Rodríguez AM, Romero-Castillo KD, Aguilar-Aguila-Isaías MA, García-Reyes IE 2018 Organs-on-a-chip module: a review from the development and applications perspective. *Micromachines (Basel)* **9** 536

Spence JR, Mayhew CN, Rankin SA, Kuhar MF, Vallance JE, *et al.* 2011 Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. *Nature* **470** 105–109

Sriram D, Chintala R, Parthasaradhi BVV, Nayak SC, Mariappan I and Radha V 2020 Expression of a novel brain specifc isoform of C3G is regulated during development. *Sci. Rep.* **10** 18838

Stokes JM, Yang K, Swanson K, Jin W, Cubillos-Ruiz A, *et al.* 2020 A deep learning approach to antibiotic discovery. *Cell* **180** 688–702

Swaminathan S, Kumar V and Kaul R 2019 Need for alternatives to animals in experimentation: An Indian perspective. *Indian J. Med. Res.* **149** 584–592

Takasato M, Er PX, Chiu HS, Maier B, Baillie GJ *et al. 2015* Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. *Nature* **526** 564–568

Tiriac H, Belleau P, Engle DD, Plenker D, Deschênes A, *et al.* 2018 Organoid profiling identifies common responders to chemotherapy in pancreatic cancer. *Cancer Discov.* **8** 1112–1129

United States Government Accountability Office. 2019 Federal Agencies Should Assess and Report on Their Efforts to Develop and Promote Alternatives. GAO-19-629 Animal Use in Research *https://www.gao.gov/assets/710/701635.pdf*

Vaira V, Fedele G, Pyne S, Fasoli E, Zadra G, *et al.* 2010 Preclinical model of organotypic culture for pharmacodynamic profiling of human tumors. *Proc. Natl. Acad. Sci. USA* **107** 8352–8356

van de Wetering M, Francies HE, Francis JM, Bounova G, Iorio F, *et al.* 2015 Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell* **161** 933–945

Walsh A, Cook R, Sanders M, *et al.* 2016 Drug response in organoids generated from frozen primary tumor tissues. *Sci. Rep.* **6** 18889

Worp H, van der B, Howells DW, Sena ES, Porritt MJ, Rewell S, *et al.* 2010 Can animal models of disease reliably inform human studies? *PLoS Med.* **7** https://doi.org/10.1371/journal.pmed.1000245

Zhang B, Lai BFL, Xie R, Huyer LD, Montgomery M, Radisic M. 2018 Microfabrication of AngioChip, a biodegradable polymer scaffold with microfluidic vasculature. *Nat Protoc.* **13** 1793–1813

Corresponding editor: Subhash C Lakhotia