

Towards More Predictive, Physiological and Animal-free *In Vitro* Models: Advances in Cell and Tissue Culture 2020 Conference Proceedings

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Abstract

Experimental systems that faithfully replicate human physiology at cellular, tissue and organ level are crucial to the development of efficacious and safe therapies with high success rates and low cost. The development of such systems is challenging and requires skills, expertise and inputs from a diverse range of experts, such as biologists, physicists, engineers, clinicians and regulatory bodies. Kirkstall Limited, a biotechnology company based in York, UK, organised the annual conference, *Advances in Cell and Tissue Culture* (ACTC), which brought together people having a variety of expertise and interests, to present and discuss the latest developments in the field of cell and tissue culture and *in vitro* modelling. The conference has also been influential in engaging animal welfare organisations in the promotion of research, collaborative projects and funding opportunities. This report describes the proceedings of the latest ACTC conference, which was held virtually on 30th September and 1st October 2020, and included sessions on *in vitro* models in the following areas: advanced skin and respiratory models, neurological disease, cancer research, advanced models including 3-D, fluid flow and co-cultures, diabetes and other age-related disorders, and animal-free research. The roundtable session on the second day was very interactive and drew huge interest, with intriguing discussion taking place among all participants on the theme of replacement of animal models of disease.

Keywords

3-D, 3Rs, animal-free, animal replacement, *in vitro*, microphysiological system, new approach methodologies, organoid, organ-on-a-chip, Three Rs, tissue microenvironment

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Introduction

The collaborative efforts by individuals having diverse skill sets and expertise have led to the accomplishment of many of the groundbreaking achievements in the field of *in vitro* biology and tissue engineering over the last few decades. These achievements have established that many of the most predictive, human-relevant *in vitro* models have the potential to replace animal models, and that these non-animal models can form an indispensable contribution to the drug discovery process.¹ The ACTC conference is an annual conference organised by Kirkstall Ltd, a UK-based biotechnology company. The aim of this conference is to bring together a multitude of individuals and institutions from science and technology backgrounds to present and discuss the latest developments in the fields of cell, tissue culture and *in vitro* modelling. This also helps foster networking and collaborative opportunities, utilising a rich and diverse 'expertise ecosystem' and 'community of practice'. In addition to the keynote lectures and session presentations, in the 2020 conference, a special focus session took place. This focus session was organised in the format of a roundtable discussion, where scientists from academia and industry, along with representatives from animal welfare organisations, participated in a discussion on existing challenges and information gaps, as well as potential approaches to

accelerate the implementation of human-relevant predictive science. This paper reports the proceedings of the conference, which took place on a virtual platform (www.kesonline.info) on 30th September and 1st October 2020. A total of 78 delegates, from 16 countries across four continents, participated in this conference. The geographical distribution of the delegates is illustrated in Figure 1.

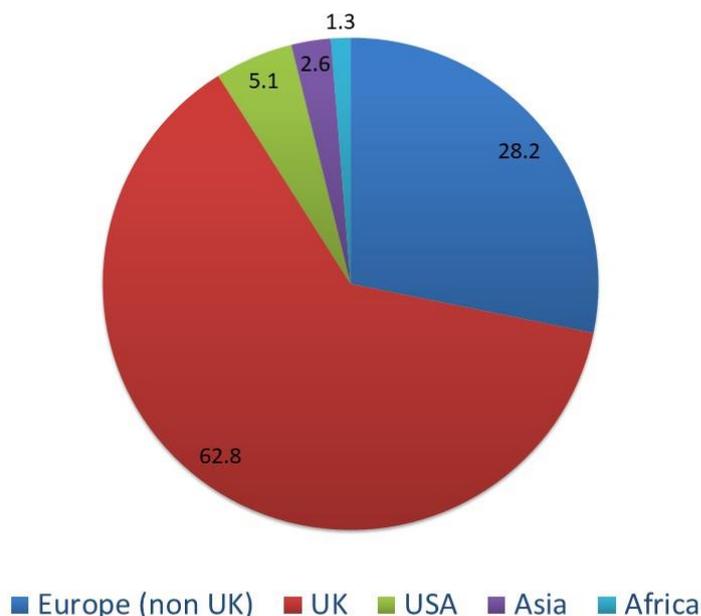


Figure 1. The proportion of the total number of delegates, as percentage values, for each geographical region.

Conference sessions

The conference included keynote lectures and presentations in the following seven sessions:

- Advanced Skin & Respiratory Models
- Novel Models for Studying Neurological Diseases
- *In Vitro* Cancer Research
- Advanced Cell Culture; 3-dimensional (3-D), Fluid Flow & Co-culture
- Diabetes and Other Age-related Disorders
- Animal-free Research
- *In Vitro* Models for Animal Replacement

Below, we summarise all the papers presented in the respective sessions (see also Table 1). The video recordings of the live presentations in this conference are available on request from Kirkstall Ltd (York, UK).

Table 1. A summary of the Conference proceedings.

Technique/model	Applications described	Presentation by:
Advanced Skin & Respiratory Models		
3-D human epithelial models (MucilAir™ and SmallAir™).	The study of SARS-CoV-2 pathogenesis (including viral infection kinetics, tissue-level tropism and induced transcriptional immune signatures) and evaluation of novel therapies.	Samuel Constant (K)
Models of the alveolar epithelial barrier of the human lung.	Testing for potential adverse biological impact of xenobiotics (e.g. chemicals, particles and nanomaterials), as well as for initial drug efficacy studies.	Martin Clift
Cell culture models of the alveolar mucosa, with the use of microfluidics and bacterial biofilms.	Reproduction of the air–blood barrier in healthy and inflammatory states; modelling of chronic infections by biofilm-forming bacteria.	Claus-Michael Lehr
Novel Models for Studying Neurological Diseases		
Advanced neurological models of peripheral nerve injury and regeneration, with the use of biomaterial scaffold and imaging technologies.	The improvement of nerve regeneration guidance channel implants.	John Haycock (K)
3-D hyaluronic acid–gelatine-based hydrogel model (HyStem™-HP) with glioblastoma cells and astrocytes.	Modelling the glioblastoma microenvironment, to understand the impact of non-neoplastic cells and extracellular matrix components on glioblastoma multiforme progression and therapeutic resistance.	Prospero Civita
Human iPSC-derived microglia and GB cells.	Evaluating the role of the membrane protein Cav-1 in immune response regulation and invasion in glioblastoma multiforme.	Catia Neto
A systematic approach to the evaluation of <i>in vitro</i> brain tumour studies.	Increasing the quality of research design and reporting.	Geoff Pilkington
<i>In Vitro</i> Cancer Research		
Barriers to the adoption of organoid technology in the pharmaceutical industry.	Helping to facilitate new technologies.	Victoria Marsh Durban (K)
Air–liquid interface (ALI) multi-layered co-cultures as non-small cell lung cancer models.	Cancer drug efficacy screening.	Dania Movia

A HepG2 3-D spheroid model.	The preclinical assessment of nano-biomaterials for cancer applications.	Melissa Tutty
Advanced Cell Culture; 3-D, Fluid Flow & Co-culture		
Microfabricated wound healing models, based on 3-D bioprinting technology, with immune cells and vascularisation.	Optimising wound healing by increasing the understanding of keratinocyte migration and re-epithelialisation.	John Connelly (K)
Human mesenchymal stem cells in chemically defined, xeno-free and albumin-free long-term culture.	The production of advanced therapy medicinal products and cell-therapy applications.	Mohammed Essameldin Abdelgawad
The <i>in vitro</i> management of 3-D cell cultures and explanted tissue by using perfusion bioreactor systems and integrated biosensors.	Manage repetitive non-destructive sampling, as well as the monitoring of metabolism, secretome and functional maturation, in 3-D cell-based and tissue-based models.	Sebastian Kress
A 'bone-on-chip' system.	The study of bone geometry, cell morphology and differentiation, as well as bone assembly.	Elisa Budyn
A microfluidic 24-element cell culture array, and a miniaturised microbioreactor with integrated sensing.	Cell-based cytotoxicity assays, synthetic biology, cell and gene therapy, and in 'organ-on-chip' systems.	Zulfiqur Ali
A dynamic perfusion-based blood–brain barrier model.	Cytotoxicity testing and drug permeation.	Basma Elbakary
3-D cell-based assays in animal-free nanofibrillar cellulose hydrogels.	Screening 3-D cultures against chemotherapeutic drug libraries; culturing cells for extended periods with stable metabolic activity (drug hepatotoxicity repeated dose assays).	Jonathan Sheard
The current status of organ-on-a-chip systems (microphysiological systems).	Exploring their potential as animal replacements, as well as progress and possible hurdles.	John Malcolm Wilkinson
Diabetes and Other Age-related Disorders		
A humanised cell culture system for EndoC-βH1 beta cells.	Cell stress in diabetes progression.	Lorna Harries (K)
A multicellular scaffold-based pancreatic ductal adenocarcinoma model.	Long-term pancreatic cancer post-treatment observations <i>in vitro</i> .	Eirini Velliou
A fully humanised beta cell line model system.	Assessing effects of the diabetic microenvironment on miRNA expression.	Nicola Jeffery

Animal-free Research		
A systemic approach to transforming biomedical research and regulation.	Promoting the development and uptake of new technologies.	Carla Owen (K)
A bioinformatic analysis to identify differentially expressed genes.	The identification of bisphenol A-regulated genes in ER ⁺ breast cancer.	Kerri Palmer
A non-animal-derived affinity reagent (ssDNA aptamer) against a peptide hormone.	For research and diagnostic purposes in Alzheimer's disease and ovarian cancer.	Sheree Smith
An <i>in vitro</i> model of human nociceptors derived from human dental pulp stem cells.	Investigating the role of MrgprX1 in pain signalling via its interaction with the pain receptor, Transient Receptor Potential Ankyrin 1 (TRPA1).	Hayley McMillan
In Vitro Models for Animal Replacement		
Current status of medical device biocompatibility testing <i>in vitro</i> .	Promoting the development and uptake of new technologies.	Helena Kandarova (K)
An organ-on-chip model of non-alcoholic steatohepatitis.	Drug development.	Tomasz Kostrzewski
Current regulatory guidelines and technological development of 3-D models.	Promoting the development and uptake of new technologies.	Janette E Turner
Inflamed human alveolar epithelial lentivirus immortalised (hAELVi) cells.	Efficacy testing of orally inhaled anti-inflammatory drugs.	Julia Katharina Metz
Roundtable Discussion (all delegates)		
<i>Changing the world: Good science is not enough to persuade researchers to replace animal testing.</i>	Topic discussed	Introduction by:
	<i>The need for economic justification.</i>	John Malcolm Wilkinson
	<i>The role of scientific skills in bringing about the change.</i>	Bhumika Singh
	<i>The end game: A strategy to end animal modelling for the benefit of patients.</i>	Alex Irving, Andre Menache
	<i>Need for regulatory change.</i>	Janette E. Turner
<i>Catalysing the world's transition to human-specific medical research and testing.</i>	Jarrold Bailey	

(K) = Keynote Presentation.

Advanced Skin & Respiratory Models

Prof. Rosalind Hannen (Queen Mary University of London, UK) chaired the session, which started with the keynote lecture by Dr Samuel Constant, Epithelix, Switzerland. The lecture described the 3-D human epithelial models, MucilAir™ and SmallAir™, for studying severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pathogenesis and evaluating novel therapies against this virus.²⁻⁴ These models have been successfully used for the development of antivirals against influenza, rhinoviruses, respiratory syncytial virus, among others. The current research strategies to stop SARS-CoV-2 infection were outlined by Dr Constant, who also highlighted how these reconstituted human airway epithelial models can be used to characterise viral infection kinetics, tissue-level tropism and transcriptional immune signatures induced by the SARS-CoV-2. The session talks are outlined below:

Advancing in vitro models of the alveolar epithelial barrier for their anatomical and physiological relevance for toxicology testing

Dr Martin Clift (Swansea University Medical School, Swansea, UK) highlighted the increased attention given to the development of advanced *in vitro* technologies, specifically models of the alveolar epithelial barrier of the human lung.⁵ Most focus has been given to constructing systems based upon multiple cell types, since these provide the ability to formulate models that exhibit anatomically correct structures.⁶ These systems have been used with great effect to study the potential adverse biological impact of numerous xenobiotics (for example, chemicals, particles and nanomaterials), as well as for initial drug efficacy studies.⁷

Modelling respiratory infections for developing novel anti-infectives

Prof. Claus-Michael Lehr (Helmholtz Institute for Pharmaceutical Research, University of Saarland, Germany) described the cell culture models of the alveolar mucosa, an approach to implementing microfluidics, and growing bacterial biofilms on pulmonary cell cultures. Regardless of the recent pandemic caused by coronavirus, the problem of antimicrobial resistance continues to increase, while the number of new antibiotics and even the number of companies engaging in their development is decreasing. Besides the need for new targets and molecules for anti-infective compounds (for example, pathoblockers), there is a need to deliver them across any biological barriers that might prevent access to the target site. Relevant barriers in this context are the body's outer epithelia — including the gut, skin and the lung, the bacterial cell envelope, and non-cellular barriers — such as mucus and bacterial biofilms. To study the cellular interactions of drugs and nanoparticle deposition in the deep lung, Prof. Lehr's group has pioneered human alveolar epithelial cell models.^{8,9} More recently, their research has been inclined toward microfluidic devices and more complex co-cultures, allowing the reproduction of the air–blood barrier in healthy and inflammatory states, and the modelling of chronic infections by biofilm-forming bacteria.¹⁰⁻¹² *In vitro* studies on such systems suggest that novel self-assembling nanocarriers,¹³ capable of co-delivering tobramycin and modern quorum sensing inhibitors, may permit a significant reduction in the dose of the antibiotic to eradicate the infection and reduce the risk of inducing antimicrobial resistance.

Novel Models for Studying Neurological Diseases

The session was chaired by Prof. Geoffrey Pilkington (University of Portsmouth, Portsmouth, UK) and Prof. John Haycock (University of Sheffield, Sheffield, UK), who also gave the keynote lecture, 'Combining biomaterial scaffold and imaging technologies for advanced neurological models *in vitro*'. Prof. Haycock described the use of biodegradable scaffolds to produce 2-D and 3-D *in vitro/ex vivo* models of peripheral nerve injury and regeneration, to provide improved nerve regeneration guidance channel implants. The work focused largely on chick embryo dorsal root ganglia cultures, assessed under sophisticated microscopical imaging conditions — but, ultimately, human model systems would be developed, informed by the chick embryo studies. Human tissue from early *post-mortem* cadavers or human peripheral nerve induced pluripotent stem cell (iPSC)-derived cultures might benefit these complex systems, but each brings with it technical, practical and ethical obstacles.

In this session, Dr Prospero Civita (University of Portsmouth and Cardiff University, UK) and Dr Catia Neto (Cardiff University, UK) presented studies on the tumour microenvironment (TME) in glioblastoma (GB), a pivotal factor in supporting tumour progression and therapeutic resistance. A method for evaluating *in vitro* brain tumour studies was also described.

Modelling the glioblastoma microenvironment by using hiPSC-derived cerebral organoid, incorporating iPSC-derived microglia and patient-derived Glioblastoma Multiforme (GB) cells: A sophisticated tool for disease modelling and preclinical drug screening

Dr Prospero Civita (University of Portsmouth and Cardiff University, UK) presented a 3-D hyaluronic acid–gelatine-based hydrogel model (HyStem™-HP) with glioblastoma (GB) cells and astrocytes, to understand the impact of non-neoplastic cells and extracellular matrix (ECM) components on GB progression and therapeutic resistance. The study showed that non-neoplastic astrocytes, in both 2-D and 3-D *in vitro* co-culture models, form tunnelling nanotubule (TNT) connections with GB cells, and transfer non-neoplastic mitochondria via a TNT connection; this mechanism may be related to GB drug response, as well as proliferation and migration.^{14,15} To understand the impact of each cellular component within the brain upon GB progression, Dr Civita and his group used iPSC technology to generate human cerebral organoids,¹⁶ i.e. 'mini-brains', which include patient-derived glioma cells and iPSC-microglia cells to generate an *in vitro* microenvironment similar to the human brain, in order to study GB as a human disease and to provide a valuable *in vitro* model for high-throughput therapeutic screening. To enhance the utility of the model for therapeutic testing and drug delivery, the tumour microenvironment would, optimally, include the cellular components of the blood–brain barrier and potentially blood flow, in addition to being carried out under appropriate cerebral oxygen conditions.

Understanding the glioblastoma immuno-environment: The impact of Caveolin-1 on microglia phenotype

Dr Catia Neto (Cardiff University, UK) presented work on understanding the microenvironment of glioblastoma multiforme (GB), which is a highly lethal brain tumour. Microglia, the brain immune cells, are highly abundant in GB, and are responsible for creating an inflammatory microenvironment that promotes tumour progression.¹⁷ Caveolin-1 (Cav-1) is involved in multiple signal transduction events and cytoskeletal dynamics,

interacting with multiple pathways. In GB, Cav-1 was identified as an independent prognostic marker,¹⁷ and in immune cells it seems to be involved in pro-inflammation.¹⁸ Dr Neto concluded that iPSC-derived microglia can respond to pro-inflammatory and anti-inflammatory stimuli. In microglia, Cav-1 might be involved in inflammation. Suppression of Cav-1 in iPSC-derived microglia drives tumour invasion. The use of human iPSC-derived microglia along with GB cells provides a sophisticated model for evaluating the role of the membrane protein Cav-1 in immune response regulation and invasion in GB. The use of high-passage, homogeneous GB cells, however, restricts the utility of the system. Thus, freshly isolated GB cells from human brain tumours or human GB organoids, used together with iPSC-derived microglia sub-populations, may better reflect the *in situ* tumour microenvironment.

Systematic approach to review in vitro methods in brain tumour research (SATORI-BTR): Developing preliminary guidance for evaluation of in vitro studies in brain tumour research

Geoff Pilkington, on behalf of Karen Pilkington (University of Portsmouth, UK), presented an interesting project on the evaluation of *in vitro* brain tumour studies. The project used a systematic process to identify, assess and agree criteria for assessing *in vitro* brain tumour research. Forty-eight draft criteria were reviewed by using a Delphi (expert consensus) process across two rounds by a panel of senior researchers from nine countries. This process has resulted in preliminary guidance for appraisal of *in vitro* brain tumour studies.¹⁹ Further development and dissemination of the guidance is planned. Some of the gaps in this field are a lack of widely accepted criteria for assessing the quality and human relevance of *in vitro* studies, making implicit knowledge and expertise used by those in the field when assessing *in vitro* research explicit, and gaining consensus from senior researchers located across different institutions and countries.²⁰ This project attempted to address these gaps by agreeing a set of provisional criteria for assessing the quality and human relevance of *in vitro* brain tumour research with the experts. These provisional criteria could be used by those designing or reporting such research. The criteria are generic and could be adopted or adapted by other fields of *in vitro* cancer research.²¹

***In Vitro* Cancer Research**

This session, chaired by Prof. Geoff Pilkington, included a keynote lecture by Dr Victoria Marsh Durban (Cellesce, UK). In her talk, 'Making 3-D possible: Large scale organoid production using bioprocess design', Dr Durban described the barriers to the adoption of organoid technology in the pharmaceutical industry — namely, the high skill and labour requirements for organoid culture, and the inability to produce large batches of organoids with the homogeneity required for screening applications. She illustrated the organoid expansion process at Cellesce, and the comparison of bioprocess-expanded organoids to those expanded by manual process. The lecture also covered proof-of-principle studies for colorectal cancer organoids in drug screening. Other presentations in this session were:

Air–liquid interface (ALI) multi-layered co-cultures mimic biochemical mechanisms of the cancer cell–fibroblast crosstalk involved in non-small cell lung cancer (NSCLC) multi-drug resistance (MDR) in patients

Dr Dania Movia (Trinity College Dublin, Ireland) described the use of a cell culture system as an *in vitro* tool for cancer drug efficacy screening. Lung cancer is the leading cause of

cancer-related deaths worldwide. Patient prognosis is extremely poor for advanced NSCLC, its most common form. Inhaled therapeutics are under development. However, at present, reliable preclinical models to support the development of such drugs do not exist.²² Dr Movia described a 3-D multi-layered cell culture of human NSCLC cells grown at the ALI, established by her research group as the first *in vitro* tool for screening the efficacy of orally inhaled drug products for NSCLC treatment.^{23,24} The study demonstrated the establishment of a cancer cell–fibroblast crosstalk, capable of modulating multi-drug resistance and feedback activation signalling processes *in vitro* as per *in vivo* conditions.

The development of a HepG2 3-D spheroid model for the preclinical assessment of nanobiomaterials for cancer applications

Ms Melissa Tutty (Trinity Centre for Health Sciences, Ireland) presented work on the development of a HepG2 3-D spheroid model system. There are high attrition rates and slow progress to the clinic associated with nanobiomaterials (NBMs). Current safety profiling methods fall short when assessing the risk of these materials, with only 60–70% of hepatotoxins being detected by conventional screening methods.²⁵ This is thought to be in part due to inappropriate and reductionist 2-D *in vitro* preclinical assessment methods and inter-species variation in animal models.²⁶ This talk presented an alternative method for assessing NBMs accurately, by using more *in vivo*-like 3-D cell culture in the form of spheroids.²⁷ The work involved screening NBMs specifically, utilising a 3-D model of the liver and incorporating relevant cell types.

Advanced Cell Culture, 3-Dimensional (3-D), Fluid Flow and Co-culture

Professors Elisa Budyn (Université Paris-Saclay, France) and John Haycock (University of Sheffield, UK) co-chaired this session. The keynote lecture by Dr John Connelly (Queen Mary University of London, UK) was titled ‘Engineering vascularised and immune-responsive human skin equivalents via indirect bioprinting’. Dr Connelly noted that the diversity of chronic skin wounds, which can be very painful and debilitating, presents several challenges in determining optimal individual treatment regimes. Dr Connelly’s research group utilised microfabricated wound healing models, in combination with high content imaging and systems biology, to develop systems-level understanding of keratinocyte migration and re-epithelialisation.²⁸ He also presented unpublished data on the development of bioinks for 3-D human skin equivalents, methods for 3-D bioprinting vascularised tissue models by using sacrificial biomaterials, and the incorporation of immune cells into human skin equivalents. The session talks are outlined below:

Harnessing an innovative completely chemically defined, xeno-free and albumin-free controllable cellular microenvironment for long-term culture of human mesenchymal stem cells (hMSCs) for the production of advanced therapy medicinal products (ATMPs) and cell-therapy applications

Prof. Mohammed Essameldin Abdelgawad (Helwan University, Cairo, Egypt) presented an innovative, chemically defined and controllable cellular microenvironment, optimised for either hMSCs or hMSCs-extracellular vesicles (EVs), allowing long-term survival, expansion and maintenance of the phenotypic, proliferative, differentiation and paracrine-signalling/secretome features of hMSCs. This approach is valuable, as it paves the way for

production, scaling up and diversification of pure hMSCs and/or their EVs for various precision and targeted therapies of urgent diseases, like Covid-19 infection.

Advanced in vitro management of 3-D cell cultures and explanted tissue

Dr Sebastian Kress (University of Natural Resources and Life Sciences, Vienna, Austria) highlighted a platform for facilitating the management of *in vitro* cell and tissue cultures. The use of 3-D cell cultures has gained importance in medicine and pharmaceutical research. However, commercially available systems are operated under conditions not yet optimised for 3-D applications — for example, lack of availability of tailor-made, minimally invasive monitoring systems with integrated sensors to monitor, optimise and standardise culture conditions. In this talk, Dr Kress presented a platform technology combining perfusion bioreactor systems with integrated biosensors to facilitate *in vitro* management of 3-D cell cultures and explanted tissue. This provides a potential solution for repetitive time-resolved non-destructive sampling, and monitoring of metabolism, secretome, and functional maturation of 3-D cell-based and tissue-based models (*manuscript in preparation*). This might enable the reduction of sample size and variation, bridging the gap between *in vitro* and *in vivo* studies.

Studying human bone formation in a bone-on-chip

Prof. Elisa Budyn (Université Paris-Saclay, France) presented a model based on decellularised human bone with re-cellularisation with human bone cells, to study bone geometry, cell morphology and differentiation, and bone assembly. A ‘bone-on-chip’ system, composed of one or more decellularised bone pieces, re-cellularised with either human primary adult MSCs or fetal osteoblast progenitors, provided the relevant 3-D environment for the cells to differentiate into stem cell-derived osteocytes (SCDOs). These systems were cultured for up to 26 months, and mechanically stimulated in either three-point bending or compression to mimic exercise. Synchrotron ATR FTIR and mechanical tests showed fetal cells produced highly mineralised and fatty tissue compared to adult cells. These systems displayed multi-level spatial reorganisation of the cells, and the collagen fibres.²⁹ Hence, this bone-on-chip system makes it possible to simultaneously study the effects of mechanical stimulations and age on bone cell differentiation, their calcium mechanobiology and the quality of the bone they form over very long periods of time (at least over two years).

Miniaturised microbioreactor for controlled and lower cost cell culture

Prof. Zulfiqur Ali (Teesside University, UK) presented a microfluidic 24-element cell culture array for cell-based cytotoxicity assays, and a miniaturised microbioreactor with integrated sensing that is amenable to use with downstream analytics. Figure 2a shows that each culture element (2 mm diameter) has two pairs of horizontal and vertical pneumatically actuated valves (which are normally closed) for fluidic control. The culture array is fabricated as a three-layer (fluidic, flexible polydimethylsiloxane membrane and actuation) structure by casting on an SU-8 mould.³⁰ A separate microbioreactor system, driven by pressurised fluid and having integrated pH, dissolved oxygen and optical density measurement potential, was also described. This microbioreactor element comprises three layers (sensor, fluidic and headspace), joined by adhesive layers (Figure 2b). It can be operated in batch, continuous and perfusion modes, as well as allowing the collection of

supernatants for further downstream analytics.³¹ The developed system can be used flexibly for a variety of applications, including synthetic biology, cell and gene therapy and organ-on-a-chip.

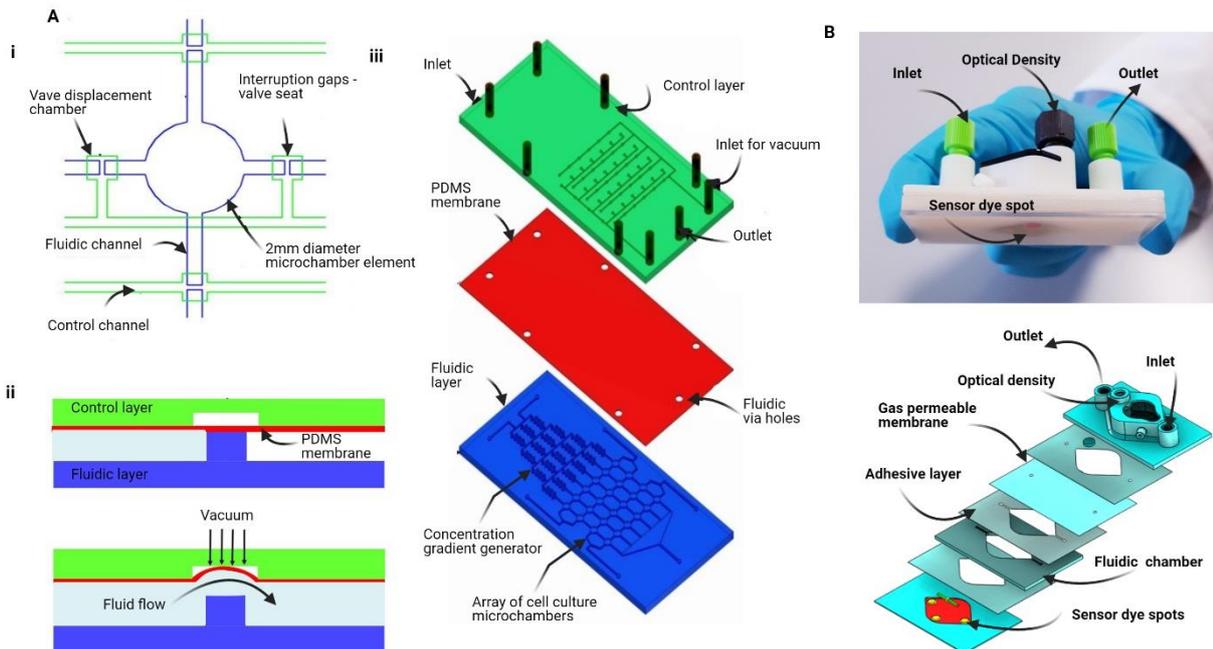


Figure 2. Miniaturised microbioreactor for controlled and lower cost cell culture (Zulfiqur Ali, Teesside University, UK). (a) Control by fluid flow by (i) two pairs of row and columnar valves (ii) with opening and closing of normally closed valves by applying or releasing vacuum for (iii) 24-element cell culture array with layer structure (b) microbioreactor element comprising sensor layer with dye spots, fluidic chamber layer and headspace layer that are joined by successive adhesive layers.

A dynamic perfusion-based blood–brain barrier model for cytotoxicity testing and drug permeation

Dr Basma Elbakary (Aston University, UK) presented work on the development of a more physiologically relevant blood–brain barrier (BBB) model. The BBB serves to protect and regulate the central nervous system (CNS) microenvironment. The development of an *in vitro* mimic of the BBB requires, particularly for drug permeation studies, the recapitulation of the correct phenotype of the *in vivo* BBB. However, the majority of widely used BBB models demonstrate low transendothelial electrical resistance (TEER) and poor BBB phenotype. The application of shear stress is known to enhance tight junction formation and hence improve the barrier function.^{32,33} A high TEER primary porcine brain microvascular endothelial cell (PBMEC) culture was utilised to assess the impact of shear stress on barrier formation by using the Kirkstall Quasi Vivo® 600 (QV600, Kirkstall Ltd, UK) multi-chamber perfusion system. The application of shear stress resulted in a reorientation and enhancement of tight junction formation on both coverslip and semi-permeable inserts, in addition to enhancing and maintaining an appropriate TEER for longer, when compared to static conditions. The functional consequences of the shear stress were demonstrated by a reduction in the flux of mitoxantrone across the PBMEC monolayers. The QV600 perfusion system may service as a viable tool to enhance and maintain the high TEER PBMEC system for use in *in vitro* BBB models.³⁴

Automated 3-D cell-based assays in animal-free nanofibrillar cellulose hydrogels

Dr Jonathan Sheard (UPM, Helsinki, Finland) outlined the potential uses of plant-derived hydrogels (GrowDex[®]) in cell culture applications. The animal-derived hydrogels that are often used for 3-D assays have known challenges — for example, batch variation and the presence of undefined components. GrowDex hydrogels (UPM, Finland) are made from plant-derived nanofibrillar cellulose (NFC), are fully defined and reproducible, and ready to use. They are biocompatible with cell culture, resembling the ECM whilst allowing the free diffusion of small molecules (such as nutrients, drugs and oxygen). Shear thinning properties, temperature stability and customisable stiffness, all facilitate their use in a multitude of applications, including automated 3-D cell-based assays. GrowDex has been shown to be an effective support matrix for many cell types including primary tumour cells,³⁵ stem cells,³⁶ liver cells,³⁷ pancreatic islets³⁸ and cancer cell lines,³⁹ allowing them to form spheroids or organoids. The screening of these 3-D cultures against chemotherapeutic drug libraries revealed differences in drug responses between 2-D and 3-D culture conditions. Primary hepatocytes and liver cell lines were shown to form 3-D spheroids with polarised structures, and could be cultured for extended periods with stable metabolic activity, thus enabling drug hepatotoxicity tests in repeated dose assays. Multicellular structures can be recovered for downstream analysis without impact to cell viability, phenotype, or function.

Status of organ-on-a-chip technology and progress towards replacement of animals

Dr John Malcolm Wilkinson (Technology for Industry Ltd, UK) gave an overview of the current status of organ-on-a-chip systems and their potential to replace animals. Organ-on-a-chip technology (now also known as microphysiological systems; MPSs) has made great progress in recent years, but has failed to make significant inroads into the replacement of animal testing. Dr Wilkinson reviewed the major commercial and academic efforts to develop robust, physiologically relevant and, more importantly, cost-effective assays and tests that can be used in the study of disease or for screening new drug candidates. Microfluidic MPSs have been under development for over 15 years and have been very much a technology-driven field until now, but there is now a shift as more attention is being paid to the market requirements and needs of customers. The companies involved are mostly start-ups and they are still refining their business models. Some are selling products, but most are following grants or research contracts. There have been many academic research papers, but they have not been significantly adopted by the pharmaceutical industry. Recent workshops have identified several of the challenges and hurdles that are being faced. These include technical and economic hurdles and slow regulatory acceptance, as well as communication gaps between potential users and developers. The 2016 'transatlantic think tank for toxicology' (t⁴) workshop report⁴⁰ set out clear functional and performance standards, as well as economic criteria that will need to be met, both at the general level and for some specific applications.

Pharma companies have a requirement for high-throughput screening (HTS) methods for lead discovery. This includes the screening of large compound libraries for activity against biological targets, often through automation, miniaturisation of assays, and large data analyses. Although the cost and timescale per test is minimal in this type of screening, the physiological relevance, and hence the clinical predictivity, is low. At the other end of the scale, complex *in vitro* models — such as multi-organ models with dynamic fluid flow that represent the biological complexity of an integrated system — are tools being used in the latest research. However, the cost of such research and utilisation of these models remains high. The interplay of the range of characteristics of non-animal

models and methods regarding these factors, is illustrated in Figure 3. The challenge is to develop and implement models that yield robust, reproducible data that are predictive for human biology. Small and medium biotechnology companies, Pharma and academic institutions, are all investing resources to address these challenges.

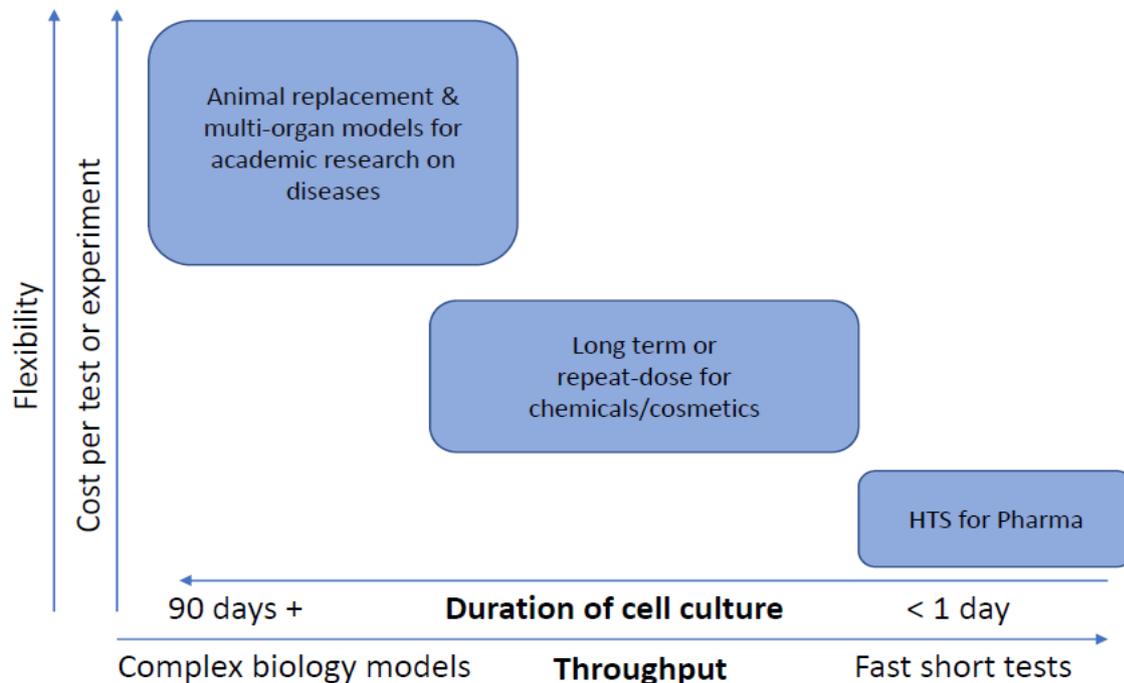


Figure 3. The competing pressures affecting the use of non-animal models. HTS: high-throughput screening.

Diabetes and other age-related disorders

Dr Victoria Kearns (University of Liverpool, UK) chaired this session. Prof. Lorna Harries (University of Exeter, UK), in her keynote lecture, ‘Cellular stress and beta cell identity changes’, presented a humanised cell culture system for EndoC- β H1 beta cells.⁴¹ The session talks were as follows:

A multicellular scaffold-based pancreatic ductal adenocarcinoma model — Towards animal-free research

Dr Eirini Velliou (University of Surrey, UK; now at University College London, London, UK) presented a novel 3-D scaffold-based hybrid, multicellular pancreatic tumour model consisting of pancreatic cancer cells, stellate and endothelial cells.^{42–44} This scaffold-assisted pancreatic cancer model recapitulates the tumour niche due to adjustable mechanical and biochemical properties and structural integrity, along with enabling cell–cell and cell–extracellular matrix (ECM) interactions. The model remains viable with physiological features for up to two months, enabling the conduct of fractionated treatment and long-term post-treatment observations *in vitro*.

MicroRNAs (miRNAs) responsive to the diabetic microenvironment in the human beta cell line EndoC- β H1 may target genes involved in cell function and survival

Dr Nicola Jeffery (University of Exeter, UK) presented a cell model that mimics the diabetic microenvironment and could be used to study microRNAs. MicroRNAs are small non-coding RNA species that comprise a key component of cellular stress responses. Altered expression of miRNAs has been reported in the islets of human donors with type-2 diabetes, but the effects of different aspects of the diabetic microenvironment, and the responses of beta cell genes at the level of gene ontology pathways, have largely gone unexplored. Dr Jeffery's group set out to identify the miRNAs dysregulated by different aspects of the diabetic microenvironment and to classify their targets into functional pathways. Dr Jeffery detailed a fully humanised beta cell line model system that showed an improved glucose sensitivity, exhibited by changes in the expression of protein, genes and miRNA. Furthermore, global miRNA screen and validation showed altered expression of miRNAs associated with glucose function, cell function and survival.

Animal-free research

Dr Alpesh Patel (Animal Free Research, UK) chaired this session. Dr Carla Owen (Animal Free Research, UK) gave the keynote lecture, 'A systemic approach to transforming biomedical research and regulation'. The session talks were as follows:

Identification of bisphenol A-regulated genes in ER⁺ breast cancer using animal-free approaches

Miss Kerri Palmer (University of Aberdeen, UK) presented her work on non-animal approaches to study bisphenol A-regulated genes in ER⁺ breast cancer. The exact mechanisms of bisphenol A (BPA) in the development of breast cancer are not fully understood. The bioinformatic analysis presented in this talk identified BPA-regulated differentially expressed genes between non-malignant breast tissue and ER⁺ breast cancer. This analysis also identified specific pathways regulated by these genes, and identified adipose tissue as potentially having a more prominent role in breast cancer development. The endocrine disrupting chemical BPA is found in the environment at low levels and can leach from products, such as plastic bottles, leading to unintentional exposures.⁴⁵⁻⁴⁷ These exposures may influence breast cancer development, but the exact mechanisms are incompletely understood.⁴⁸⁻⁵⁰ The bioinformatic analysis presented in this talk identified BPA-regulated differentially expressed genes between non-malignant breast tissue and ER⁺ breast cancer. This analysis also identified specific pathways, regulated by these genes, that may present future targets for breast cancer prevention. Furthermore, adipose tissue was identified as potentially having a more prominent role in breast cancer development and progression; a view that is also being increasingly shared by others.^{51,52}

Raising an aptamer against the peptide hormone, kisspeptin, for potential diagnostic use in Alzheimer's disease and ovarian cancer

Ms Sheree Smith (Leeds Beckett University, UK) described the synthesis of a non-animal-derived affinity reagent (ssDNA aptamer) against the peptide hormone, kisspeptin. This aptamer could be used for research and diagnostic purposes in Alzheimer's disease and ovarian cancer. This paper also highlighted the ease of aptamer generation and its

applicability to many other fields of research. Whilst aptamers have limitations, such as susceptibility to degradation and cross-reactivity,⁵³ increasing the number of positive selection rounds can counteract the issues with cross-reactivity. Post-selection chemical modifications also exist to strengthen binding affinities and resist degradation.⁵⁴ Overall, aptamers have huge potential, and some are already commercially available.

Endogenous Mas-related G-protein-coupled receptor X1 (MrgprX1) activates and sensitises TRPA1 in a human model of peripheral nerves

Dr Hayley McMillan (Queens University, Belfast, UK) described a study that aimed to investigate the role of MrgprX1 in pain signalling via its interaction with the pain receptor, Transient Receptor Potential Ankyrin 1 (TRPA1) by using an *in vitro* model of human nociceptors derived from human dental pulp stem cells. Peripheral nerve equivalents (PNEs) were generated by using a fibronectin differential adhesion protocol, established previously.⁵⁵ MrgprX1 and TRPA1 protein expression was investigated by using immunocytochemistry. MrgprX1 receptor signalling, and the mechanisms through which it couples to TRPA1, were studied by Fura-2-based Ca²⁺ imaging. Immunocytochemistry confirmed endogenous protein expression of both MrgprX1 and TRPA1 in PNEs. Ca²⁺ imaging results showed that MrgprX1 couples to the G_{αq/11} pathway and activates TRPA1. In addition, MrgprX1 sensitises TRPA1 to agonist stimulation via protein kinase C (PKC). Hence, the study concluded that MrgprX1 both activates and sensitises TRPA1 in a model of human peripheral nerves, suggesting an important role for this receptor in the modulation of pain.

***In vitro* models for animal replacement**

This session was chaired by Dr Virginia Pensabene (University of Leeds, UK). In the keynote lecture, 'Medical device biocompatibility testing *in vitro* — Are we there yet?', Dr Helena Kandarova (Slovak Academy of Sciences, Slovakia) addressed the status of the use of alternative methods by the medical device industry and the implementation of validated *in vitro* assays into the ISO standard 10993 for biocompatibility testing. She described the development, validation and implementation of an *in vitro* test based on *in vitro* 3-D reconstructed human skin models for intra-cutaneous testing of extracts from medical devices. The new ISO standard 10993-23 describing this method was published in January 2021. Dr Kandarova noted that it took more than 10 years to develop, validate and implement this method into the ISO standards. She also highlighted that in order to accelerate the regulatory acceptance of these novel approaches, it is necessary to include regulators from the beginning of the validation process. The team participating in this international multicentric validation project has been awarded two Society of Toxicology (SOT) prizes. It was also recognised by Lush (UK) as a 'Commended Project' in the 2020 Lush Prize 'Lobbying for Alternatives' category. The session talks are outlined below:

Comparing an in vitro organ-on-chip model of non-alcoholic steatohepatitis (NASH) to murine models and liver tissue from patients

Dr Tomasz Kostrzewski (CN Bio Innovations Ltd, Cambridge, UK) described a liver microphysiological system (MPS) for the culture of primary human liver cells over a number of weeks, and the procedure for disease state induction in the model through the use of physiologically relevant stimuli. The phenotype of the model was analysed in detail

and shown to correlate with patient samples at the transcriptional and proteomic level.^{56–58} To demonstrate its utility in drug development, the MPS was treated with the bile acid analogue, obeticholic acid, which caused dose-responsive reductions in a wide range of disease endpoints. Non-alcoholic steatohepatitis (NASH) is a disease that involves multiple tissues and peripheral immune cells. Therefore, the model could be further enhanced by the addition of other cell types, or by being interlinked with other tissue models — for example, adipose, gut or pancreas models (Figure 4).

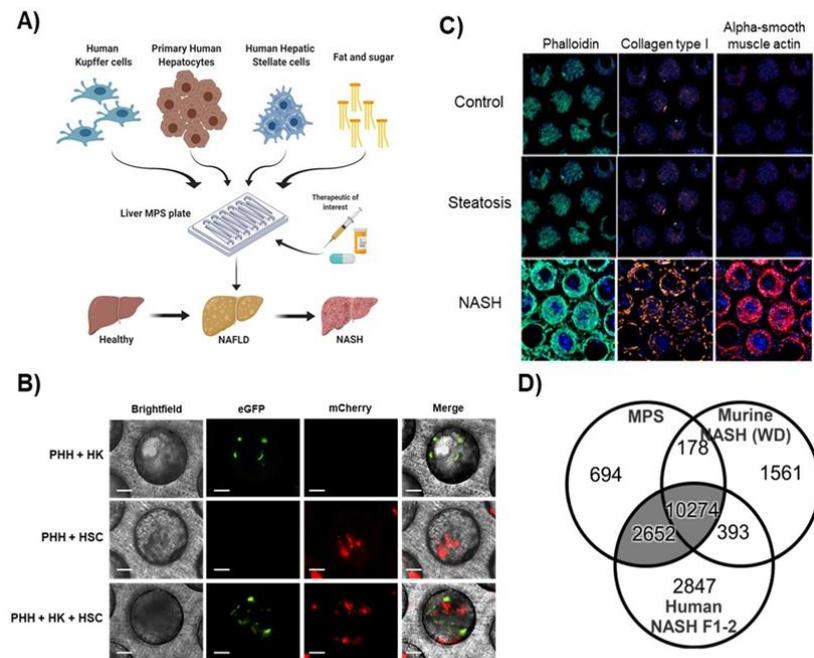


Figure 4. A liver MPS model of the non-alcoholic fatty liver, representing the human disease. (a) Primary human hepatocytes (PHH), Kupffer cells (HK) and hepatic stellate cells (HSC) were co-cultured in a microphysiological system (MPS; PhysioMimix™) for up to 1 month, in the presence of fat and sugar. The three cell types form 3-D liver microtissues in the MPS. (b) To allow cell localisation within microtissues, HK were transduced with an adenovirus expressing GFP, and HSC with an adenovirus expressing mCherry (TxRed). (c) The liver microtissues in the non-alcoholic steatohepatitis (NASH) condition (Fat) were stained with Oil Red O to observe intracellular fat accumulation and compared to healthy (Lean) controls (scale bar ¼ 350 mm). (d) The transcriptomic profile of the NASH MPS microtissues was compared to a western diet (WD) murine model and NASH patients with F1-F2 fibrosis; the presence of abundant transcripts was compared between samples.

Current regulatory guidelines and technological development of 3-D models: Closing the gap

Dr Janette E. Turner (Safer Medicines Trust, Kingsbridge, UK) provided an overview of the challenges and opportunities involving new approach methodologies (NAMs). The scientific progress of 3-D models over the last 10–15 years has resulted in estimates that a reduction in total research and development costs for drug development of up to 26% is expected through organ-on-chip (OOC) or microphysiological system (MPS) technologies.

Dr Turner examined how these New Approach Methodologies (NAMs), with specific reference to 3-D models, are increasingly being recognised as viable models in some aspects of human drug discovery — however, they are not being fully adopted across all countries or regulatory agencies. The need for harmonisation and standardisation in this field was discussed. The development and adoption of these models in non-regulatory settings was also highlighted, to show how their use may help provide a better understanding of disease and help tailor precision medicine treatments.

The potential of inflamed human alveolar epithelial lentivirus immortalised (hAELVi) cells as an in vitro test system

Dr Julia Katharina Metz (PharmBioTec Research and Development GmbH, Germany; Department of Pharmacy, Saarland University, Saarbrücken, Germany) described a stepwise *in vitro* approach to investigate lung inflammation. In the development of an *in vitro* test strategy for orally inhaled drug products, the adverse outcome represented by the reduction of the epithelial barrier during inflammation was addressed. The hAELVi cells⁹ were stimulated with the proinflammatory cytokines tumour necrosis factor- α (TNF- α , 25 ng/ml) and interferon- γ (INF- γ , 30 ng/ml) for 48 hours. The apparent permeability coefficient (P_{app}) of the tracer molecule sodium fluorescein (NaFlu) rapidly increased when the TEER fell below 245 $\Omega\cdot\text{cm}^2$. This value was calculated by a piece-wise linear fitting of the transepithelial electrical conductivity (TEEC), the reciprocal of the TEER (Figure 5).⁵⁹ The approach included the combination of a human cell line (hAELVi) and a standardised protocol to induce inflammation, thus potentially enabling a better comparison with human data and improving reproducibility. By calculating a specific threshold value (critical transepithelial electrical resistance, TEER value), a categorisation of the inflammation level was possible.

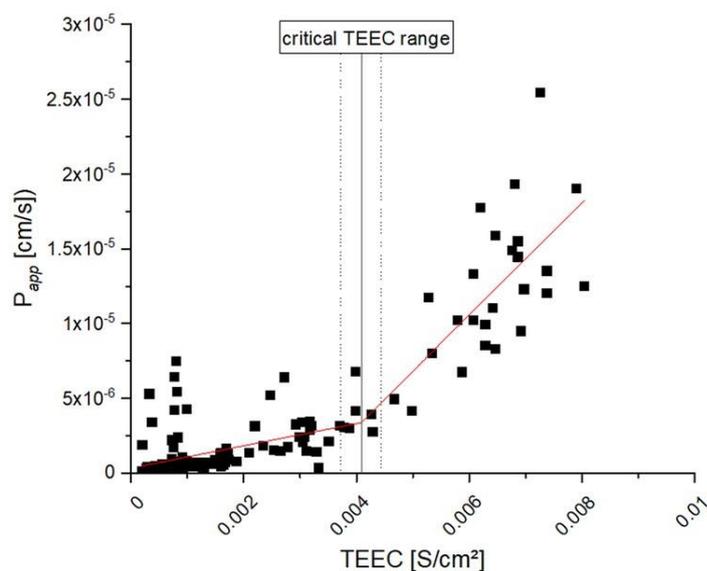


Figure 5. Critical transepithelial electrical conductivity (TEEC) of hAELVi cells to perform a piecewise linear fitting for the calculation of the 'critical TEER' at $245 + 20 \Omega\cdot\text{cm}^2$. At this value, the inflammation of the hAELVi cells opens the tight junctions and an increased permeability of NaFlu is measurable (figure taken with permission from Metz et al.⁵⁹).

Changing the world: Good science is not enough to persuade researchers to replace animal testing — A roundtable discussion

At the end of the conference, a roundtable discussion was organised, with a special focus on animal-free research. This highly interactive session, moderated by Dr Wilkinson (Technology for Industry Ltd, UK), started off with five short talks (five minutes each) delivered by pre-selected panel members (see Table 1). These talks aimed to make introductory position statements to steer the discussion toward thought-provoking open-ended questions, followed by 35 minutes of dialogue among the participants of the session. All the delegates of the conference were invited to participate in the roundtable discussion. The five panel talks were:

- *The need for economic justification* (John Malcolm Wilkinson, Technology for Industry Ltd, UK);
- *The role of scientific skills in bringing about the change* (Bhumika Singh, Kirkstall Ltd, UK);
- *The end game: A strategy to end animal modelling for the benefit of patients* (Alex Irving and Andre Menache, Patients Campaigning for Cures, UK & EBVS® European Veterinary Specialist in Animal Welfare Science, Ethics and Law);
- *Need for regulatory change* (Janette E. Turner, Safer Medicines Trust, Kingsbridge, UK);
- *Catalysing the world's transition to human-specific medical research and testing* (Jarrod Bailey, Center for Contemporary Sciences, USA)

Summary of the roundtable discussion

Many core *in vitro* assays are still reliant on animal (non-human) cell types. Substituting the existing animal tests with more such *in vitro* tests may be risky, as relevance to humans and predictivity may be lost. New approach methodologies (NAMs), with specific reference to 3-D models, are increasingly being recognised as viable models in some aspects of human drug discovery. Microphysiological systems (MPSs) may be employed in many areas of drug discovery, and perhaps hold the greatest potential for delivering personalised treatments and patient screening of therapies. But, due to legacy regulations which mandate animal use in preclinical testing and an undeveloped process of validating these models, these systems are not being fully adopted across all countries or regulatory agencies. Regulatory acceptance may be accelerated by generating data based on comparisons of animal *in vitro* and *in vivo* outcomes with the corresponding human *in vitro* and *in vivo* outcomes, and by following harmonisation and standardisation approaches. It was noted that, although advances have been made in the scientific research to adhere to the principles of the Three Rs, the extent of engagement required at many fronts in the current society to implement the outcome is inadequate. Much more collaboration during the development of MPS (or any other novel platform) is needed, so that all stakeholder perspectives and requirements are captured early on.

There was consensus among delegates that the way forward in 'changing' the world toward adopting human-relevant methodologies is by establishing collaborations with all of the relevant stakeholders, educating them, training current and new students, and setting up new organisations dedicated to promoting the scientific advantages of such approaches. The following are the key comments and ideas that emerged from the discussion:

— Imparting confidence in all parties is key. We need all stakeholders together. These stakeholders include companies requiring registration of drug or chemicals, contract research organisations (CROs), regulatory bodies, non-governmental organisations, *in vivo* scientists and technicians, consultants and innovators.

— There has been a communication gap between regulators, end users and developers, except in the fields of genetic toxicology and safety pharmacology. This gap has been recognised, and activities toward bringing together all stakeholders are taking place (c.f. the US EPA Accelerating the Pace of Chemical Risk Assessment (ACPRA) initiative). Regulators should clarify: i) their needs; ii) establish what data are required to address that need; iii) accept performance criteria; and iv) provide a clear pathway for the evaluation of new methods if adoption is to succeed.

— The sharing of success stories from end users/developers would help educate regulators, entrepreneurs and investors: “The replacement of mice for Botox testing is a success story.”

— Establishment of relevant organisations is important. One recent example of this is set-up of the Center for Contemporary Sciences, USA (CCS; contemporarysciences.org), launched in 2020 to help catalyse a paradigm shift in research to human-specific investigation. Dedicated solely to this vision, the organisation is developing educational, academic, collaborative and policy programmes to achieve it (Figure 6).

Educating	Educating grant-making, corporate, governmental and academic institutions
Supporting	Supporting innovative biotech companies
Connecting	Connecting entrepreneurs and investors
Improving	Improving adoption of more effective, human-based technologies
Increasing	Increasing the pipeline of PhD, MBA and JD students to new testing technologies

Figure 6. Center for Contemporary Sciences’s (contemporarysciences.org), USA, vision of “saving and improving lives by catalysing the world’s transition to human-specific medical research.” The organisation hopes to help to achieve this through educational, academic and policy programmes, as well as facilitating collaborations and bridge-building between stakeholders, including funders and investors.

Other organisations, including the UK-based NC3Rs (<https://www.nc3rs.org.uk/>), launched in 2004, and the North American 3Rs Collaborative (NA3RsC; <https://na3rsc.org/>), have been propagating the importance of the Three Rs by promoting training, funding and creating collaborative opportunities.

— Educating and training of stakeholders, students and innovators is imperative. Showcasing scientific breakthroughs, publishing reviews, and conducting proactive workshops to solve research issues by using human-specific methods, would all help to increase the pipeline of knowledgeable early career scientists.

— Working with educational establishments is crucial, to ensure that students are informed about new approach methodologies (NAMs), preferably via course content: “I only had a brief introduction to the Three Rs during pharmacology undergrad, but was fortunate to be funded by the NC3Rs for my PhD. They have some really interesting educational resources online which would definitely benefit pre-PhD level students to consider.”

— There is a need for smart data interpretation and stratification of assays, depending on the complexity and the context of the research question being investigated.

Discussion

The ACTC conference consisted of seven theme-based sessions, including keynote lectures, session talks and a roundtable panel discussion, as part of the two-day programme. The overall aim of this event was to bring the scientific community up to date with the latest research and technological advances in the cell and tissue culture field, primarily around the concept of *in vitro* modelling for drug discovery and to further the understanding of disease mechanisms. Due to the imperative need to find more predictive human-relevant systems and valid replacements or screens for animal experiments, increased attention has been given to the development of advanced *in vitro* technologies. For example, models of the alveolar epithelial barrier of the human lung,^{5,22,60} pancreatic tumours,^{42–44} the blood–brain barrier,³⁴ and others, are described in this report and summarised in Table 1. It is now increasingly believed that the extension of the models beyond single cells to multiple cell types would allow them to exhibit anatomically more similar structures to the tissues they represent, thus increasing the accuracy and predictive power of such models.⁶

These systems have been used to great effect in the study of the potential adverse biological impact of numerous xenobiotics (e.g. chemicals, particles and nanomaterials), and for initial drug efficacy studies.⁷ Despite the clear advantages of multi-cellular *in vitro* models compared to monoculture systems, due to the facilitation of important cell–cell interplay,²² the static nature of multi-cellular *in vitro* systems is unable to provide the physiological, dynamic environment of the *in vivo* human scenario. Therefore, focusing on re-creating the dynamic physiological environment, together with the use of the multi-cellular models, is imperative to progress in this area of replacement technologies. This advancement of multi-cellular models — in essence, making them dynamic in nature, and so more similar to human health or disease *in vivo* — will result in increased sensitivity and make in-roads toward their wider use as *in vitro* animal replacement strategies. Theoretically, this is all possible, but it has not yet been achieved.

The ability to make versatile models and understand the intrinsic role of the microenvironment requires the creation of clinically relevant systems that embrace the complexity of the tissue under investigation — for example, the human brain. Despite considerable research, modelling brain tumours is still challenging due to the difficulties in

reproducibility and the inherent complexity of both the neoplasm and the organ in which it grows. Current preclinical glioblastoma (GB) models, although progressive, fail to address the critical issue of the interaction between tumour cells and normal host tissue. Recently, Civita's group developed all human GB models where both non-neoplastic microglial and astrocytic cells were shown to grow with GB cells within a 3-D extracellular matrix, which confers resistance to several drugs with different modes of action. New and highly sophisticated *in vitro* 3-D approaches, stemming from either patient-derived tumour cells or pluripotent stem cells, could provide *in vitro* models to study diffuse gliomas or metastatic brain cancer. However, these models still have limitations due to the absence of the stromal component or functionalised vasculature (the blood–brain barrier). This type of model also often lacks reproducibility. The incorporation into the system of stromal cell types, along with human serum supplementation and appropriate oxygen concentrations, could significantly improve the model in this respect. An optimised model could potentially be used to test a wide range of nano-delivery vehicles and agents.

In another example, the *in vitro* model of the lung presented by Dr Movia offers a refinement of early-phase clinical testing that may facilitate better selection of drugs with disease-specific activity and, subsequently, drugs that are more likely to succeed in Phase 3 trials.^{22,61} This model could be further improved for cancer studies by inclusion of the complexity of the lung tumour microenvironment (TME). The TME is a complex system comprising cancer cells, ECM and stromal cells. Physical interactions take place between the ECM and non-small cell lung cancer (NSCLC) cells, regulating tumour growth/invasion, and abrogating the effects of drug intervention. The optimisation of a controlled cellular microenvironment is very tedious, expensive and time-consuming. The robust biomechanical and biophysical control of the TME that is incorporated in the pancreatic duct adenocarcinoma model presented by Dr Velliou (University College London, UK), would improve accuracy in disease modelling and treatment screening.

With regard to age-related and chronic disorders, the ability of human cell models to maintain their quality to reproduce relevant features of human diseases over long time periods will be key to furthering our understanding of chronic diseases such as diabetes. The ability of these models to reproduce and predict the responses to treatments will allow them to contribute to new therapies.

In addition to the novel experimental approaches described above, computational systems biology tools represent additional avenues for exploration at multiple biological scales. These tools will enable more accurate comparisons between *in vitro* and *in vivo* models, and more detailed integration of multi-parameter datasets and further improvement of experimental design. Large scale studies, which employ these tools along with detailed pathological analysis — for example, a combination of histomorphology, molecular, genetic and metabolic characterisation — will be required, in order to develop a full, scalable, sensitive and reproducible *in vitro* model that can be robustly employed in the evaluation of compound efficacy and safety.

As discussed earlier, *in vitro* experiments could inform optimal clinical drug use and improve understanding of tumour or disease pathobiology. The inclusion of general accepted criteria and guidance for *in vitro* studies could further improve the quality and reproducibility of the experiments for preclinical drug testing and delivery. The systematic approach presented by Prof. Karen Pilkington, employing strategies mainly used for clinical trials, has provided a sound base for discussion among *in vitro* experts, despite the low response rate in the initial survey carried out as part of the project.²¹ The results of the study provided a list of criteria for best practice in *in vitro* research and testing, which are now being used by a panel of experts to produce documentation with the criteria and guidelines that will help to standardise practice in a range of *in vitro* techniques, thus enhancing the quality and reproducibility of such work.

So, what more do we need to do to achieve full, scalable, reproducible, sensitive, predictive and, most importantly, human-relevant *in vitro* models?

— Focus on characterising the models and comparing them to the human organ being modelled with respect to structure and function. Large scale studies should be designed to achieve this, with detailed all-round pathological analyses (for example, a combination of histomorphology, molecular, genetic and metabolic parameters).

— Understand as fully as possible the *in vivo* tests that we are attempting to replace, and the questions at the regulatory stage that they have been designed to answer.

With regard to increasing acceptance and uptake of the models, the following factors should also be considered:

— Accelerating the uptake and use of the models by major public sector and industrial stakeholders, as well as their acceptance by legislative and regulatory bodies, is crucial.

— There is an essential need for harmonisation and standardisation of alternative approaches, with all relevant stakeholders engaged throughout this process. This would help to expedite the point above.

Conclusions

As in previous years, the ACTC conference succeeded in providing a systematic platform for researchers to present and discuss their scientific approaches and findings with a scientifically diverse community. This report detailed the proceedings of the latest conference that was held virtually over two days (30th September and 1st October 2020). The virtual platform augmented the geographical reach of the event, and the benefit was evident by the enrichment of the scientific participation from the UK, Europe, and wider geographies including North America, Asia and Africa. The content of the sessions helped the participants to learn more about the state-of-the-art, understand the current challenges, and explore how the new methodologies could address these challenges, in the current landscape of the cell and tissue culture field.

To conclude, replication of the tissue microenvironment, multi-cellularity, utilisation of patient-derived cell types where appropriate, and integration of dynamic flow systems, are the future directions for advanced research in the *in vitro* modelling field. Microphysiological systems do hold the potential to significantly increase the success rate of candidate therapies in clinical development. A common — and arguably the most important — message that emerged from the discussions during the event was that the engagement of relevant stakeholders in the implementation of the new technologies at all levels needs to be strengthened. These stakeholders are not sufficiently aware of the existence and capabilities of human-specific methods. Funding of, and confidence in, human methods are not as sufficient as the evidence demands. All these factors need attention and appropriate action — greater funding, training and awareness, collaboration, investigation opportunities, and confidence in human-relevant approaches.

Conflict of Interest

The authors declare that the report was prepared in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Bhumika Singh is an employee of Kirkstall Ltd., UK, a manufacturer of advanced tissue culture fluidic devices.

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Author Contribution

BS drafted the manuscript. All authors contributed to the content of the manuscript.

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