SensOoChip: Increasing the reproducibility and predictive power of organ-on-chips through multiparametric real-time monitoring and data modelling

Overall aim

1. The aim of this Challenge is to improve the utility and reproducibility of connected organ-on-a-chip (OoC) devices by integrating real-time multiparametric monitoring.

Duration

2. This is a three phase mega-Challenge with funding for up to five years.

3. Phase 1: nine months, Phase 2: up to three years, Phase 3 (subject to successful completion of Phase 2): up to two years.

Budget

4. Phase 1: up to £100k, Phase 2: up to £1.6M, Phase 3: up to £1M.

Sponsors

5. Sponsors define the Challenges in collaboration with the NC3Rs to set out the business case and 3Rs benefits, with a view to using the product developed. Sponsors are required to provide in-kind contributions to help solve the Challenge.

6. The Sponsors for this Challenge are AstraZeneca, Bayer AG, GSK, Merck Healthcare KGaA and Novartis.

Background

Advances in organ-on-a-chip technologies

7. OoC technologies are microfluidic cell culture devices that recapitulate the structure, function and (patho)physiology of human tissues and organs *in vitro*. The technology is increasingly delivering key tools that can improve disease modelling, safety and efficacy testing and reduce the reliance on animals by providing faster, cheaper and more physiologically relevant human cell-based models. The OoC field has grown rapidly in the past decade, from chips recapitulating single organ physiology to

those modelling clinical responses and disease (1). Multiple OoCs can now be connected incorporating immune components and vascularisation (2) to study organ cross-talk, such as the effects of drug metabolism on organ toxicity. They have been deployed successfully in a range of applications including safety assessment of an immuno-modulating antibody on a lung-on- and gut-on-chip (3), determining species differences in small molecule induced liver toxicity (4) and study of the gut microbiota (1,5). Despite the progress made, there are still barriers to the wider adoption of OoCs that limit their potential. These include biological, engineering, technical and practical challenges that must be overcome for the full potential of OoCs to be realised.

Addressing the limitations

8. To fully characterise the local OoC microenvironment, continuous monitoring of dynamic physiological parameters is needed. Current sampling approaches are labour intensive and time consuming with data collection often restricted to single snapshot measurements, and some analyses carried out “off-chip”. The amount of data collected is further limited as the sampling often requires destruction of the chip. The ability to incorporate longitudinal, non-invasive monitoring capabilities through the application of advanced engineering would deliver key benefits including:

    - Generation of detailed multiparametric data sets providing the potential for better understanding of the baseline biology of OoCs and their stability over time.
    - Collection of temporal data from a single chip allowing OoC experiments to be more scalable and removing chip-to-chip variability.
    - Automation of the sampling to streamline workflows and reduce the experimental burden on the user.
    - Improved reliability and reproducibility of OoCs through reducing the potential for variability caused by the individual user.

9. Longer term, OoCs that incorporate longitudinal monitoring could be used to improve absorption, distribution, metabolism and excretion (ADME) and toxicity assessments, and enable more robust studies of disease modelling and efficacy. To achieve the benefits of longitudinal multiparametric monitoring, two fundamental technical hurdles need to be addressed – these are sensors for real-time monitoring and analysis, and interrogation and application of multiparametric OoC datasets.

    <u>Sensors for real-time monitoring</u>

10. Sensors that enable real-time, minimally invasive monitoring are already being integrated into OoC systems (6,7). Electrical sensors, such as transepithelial/transendothelial electrical resistance (TEER), can measure tight junction formation and barrier integrity in real-time for up to 60 days in a human lung airway chip and for up to 12 days in a human gut chip (8). Electrochemical sensors, which incorporate a biological molecule as a response element immobilised on the electrode surface (e.g.

an enzyme, antibody, aptamer) can be used to measure analytes and biomarkers. For example, magnetic microbeads coupled to an electrochemical sensor unit have been used to measure transferrin and albumin secretion from hepatic spheroids to monitor the effect of acetaminophen for up to five days (9). Existing consortia are working to incorporate sensors for real-time monitoring (10), but there is still work to be done to ensure multiparametric data sets can be collected and importantly, shown to improve the quality of data and translational relevance when applied to a defined context of use.

Analysis, interrogation, and application of multiparametric OoC datasets

11. An improved workflow that delivers large datasets for improved understanding and characterisation of control and treated OoC systems could also enable more accurate and robust comparison with historical compound data and facilitate the identification of novel drug effects previously undetected due to the limited sampling capabilities. In the longer term, advanced computational modelling approaches such as integrated pharmacokinetic (PK) and pharmacodynamic (PD) modelling, quantitative systems pharmacology and toxicology (11), and machine learning (12) could be applied to these datasets to enable robust decisions around compound progression and improve their clinical translation (13).

The Challenge

12. This Challenge aims to capitalise on the advances already made in the field of OoCs to develop engineering capabilities focused on the:

    - Integration of multiparametric, inline monitoring of an established liver OoC system to increase the quantity and quality of data collected.
    - Connection and monitoring of a second organ – the heart.
    - Demonstration that the multiparametric datasets generated can be interrogated and modelled to improve understanding of the local physiological environment, reproducibility of OoCs and the effect of drug administration.

13. The Challenge will focus initially on integrating a set of sensors for inline monitoring of key parameters for the liver. Liver-on-chip is arguably the most characterised and biologically understood of current OoC models and has been shown to accurately model parameters such as ADME as well as direct effects such as drug-induced liver injury (14-16).

14. It is important that sensors for common physiological parameters such as oxygen demand, secretion of proteins, release of biochemical signalling molecules and enzymatic activity can be integrated, with relative ease, into other OoCs, not least as there is an increasing drive to connect multiple OoCs to model more holistic systems and the effects of drug metabolism. The second organ for this Challenge should be a heart-on-chip model as cardiotoxicity is another principal cause of drug failures and

recalls and many liver-derived metabolites can cause cardiac safety issues. A connected heart-liver OoC will enable the study of metabolism of drugs by the liver and their effects on the heart (17).

15. The liver and heart, connected in a single OoC experiment, will serve as exemplars to demonstrate that useability, robustness, and reproducibility are improved through continuous inline monitoring, and that the data generated can deliver a step-change in the understanding of the physiology of OoCs and the safety assessment of drug candidates.

3Rs benefits

16. The increasing global shift to use new approach methodologies (NAMs) has accelerated the momentum to move to human-based models to improve safety assessments to protect human health. OoCs are key enabling NAM technologies and increasing their robustness and reliability as set out in this Challenge will reduce barriers to use, permitting more detailed and human relevant studies of physiology and disease. This Challenge has the potential to deliver 3Rs benefits in the pharmaceutical industry by:

    - Improving the early identification of drugs with target and/or chemistry-related toxicity and preventing these from progressing into animal studies.
    - Ensuring the drugs that do progress to *in vivo* studies are safer with less potential for toxicity to be identified in the animals.
    - Improving mechanistic toxicity and pharmacology (PK/PD) studies *in vitro,* replacing the need to use animals.

Industry benefits

17. The Challenge will also deliver additional industry benefits through improved assessment of biological therapeutics where animals are not suitable models, enabling more human-relevant preclinical data to be generated. In the longer term, the approach developed through this Challenge could form part of a suite of *in vitro* and *in silico* approaches to provide an absolute replacement of animal studies for a large part of the drug discovery and development process. The approaches developed will also impact across multiple sectors including cosmetics, fast moving consumer goods, food and chemicals.

Key deliverables

18. The aim of this Challenge is to develop multiparametric, continuous monitoring for connected liver-heart OoC devices and apply computational approaches to the datasets generated to demonstrate improved utility and reproducibility.

19. Delivery of this Challenge must:

- Develop a connected liver-heart OoC system that can be monitored for up to two to three weeks to permit interrogation of multiple readouts and biomarkers.
- Integrate sensors to enable repeated measurements that are minimally invasive to the OoC systems and reduce the interventions needed by the user.
- Provide a platform that is more user friendly that current models, and increases confidence in robustness, reproducibility and predictivity through standardisation of the devices and harmonisation of assays performed.
- Deliver a system that addresses the current limitations of single use, snapshot measurements from OoCs.
- Have throughput amenable to drug administration studies – in the range of 24 parallel chips for repeat dosing.
- Incorporate required cells from readily available sources – including induced-pluripotent stem cells and primary cells and cell lines.
- Demonstrate that the datasets generated can advance the understanding of the baseline physiology of the OoCs.
- Evidence the ability to identify and characterise drug-induced effects on:
    - Liver toxicity.
    - Compound metabolism and clearance.
    - Cardiac toxicity.
- Deliver a dynamic model of the liver-heart system with reference compounds agreed with the Sponsors.
- Include strong project management processes to ensure timely delivery of milestones.

Phase 1 deliverables

20. It is expected that the biology of the liver OoC model is already well characterised and evidence for this should be provided as part of the Phase 1 application.
21. The deliverables for Phase 1 are:
    - Selection of a suite of readouts that can be collected longitudinally for up to two to three weeks. These should be evidenced as to how they will demonstrate:
        - Baseline liver function and metabolism.
        - The effect of drugs on known biomarkers of altered liver function.

- Detail on how the readouts selected will permit:
  - Monitoring of the viability of the OoC and its stability over time and establishment of baseline organ function.
  - Improved and earlier detection of altered function and toxicity.
  - Increased user confidence in the capability of OoCs.
- Plans for and, where possible early evidence of, integration of sensors into the functioning liver model to gather data on the specified readouts.
- Robust plans for the development of data interrogation approaches for Phase 2.
- Identification of a heart-on-chip model and plans for the sensor readouts that will be incorporated in Phase 2.

Phase 2 deliverables

22. Phase 2 includes essential and desirable deliverables. **Essential deliverables** are:

23. Inline sensor integration

- Extensive characterisation and qualification of sensors in the liver model to cover the proposed readouts (calibration, sensitivity, specificity/selectivity, durability and stability).
- Contractors must demonstrate:
  - There is no cross-talk between sensors.
  - That materials used in the sensors are not harmful to cells and are inert towards the compounds.
  - That the data produced is reproducible.
  - That sensors and equipment can make multiple readouts for up to two to three weeks.
  - Compatibility with existing lab equipment (e.g. microscopes for high content imaging, plate readers, lab automation devices).

24. Qualification

- The system developed should improve on the quality and quantity of readouts when compared to current established assays, by providing the following evidence:
  - Robust qualification of the final liver OoC model is required, using the integrated sensors with positive and negative controls.
  - Established concentration-effect relationships using positive and negative reference drugs.

- Consideration of donor-to-donor variability where appropriate.
- Final tissue analyses at the end of the experimental period (e.g. histology, imaging, omics) and comparison to the data generated from the sensors.

25. Data generation and handling
    - Demonstration of how the multiparametric datasets generated can be analysed and interpreted to improve the understanding of OoC physiology and the effects of compound administration.
26. Second organ integration
    - Integration of sensors into a heart OoC and the ability to connect and reliably measure multiple readouts simultaneously with sensors from the multi-organ models up to two to three weeks.
    - Demonstration that the viability and baseline functionality of the organs is preserved when the OoCs are connected.
    - Development of plans for how the model will be made widely available upon Challenge completion.
27. The Challenge also includes **desirable deliverables** that are intended to maximise the potential of the model in the longer term. The desirable deliverables are:
    - Demonstration of liver-driven compound effects in the heart model (measured on-chip or off-chip).
    - Delivery of a dynamic PK/PD model of the liver and heart.
    - The addition of circulating immune cells and demonstration of their effect.
    - The ability to control compound dosing and exposure.
28. The application must include a plan to commercialise the platform and disseminate to the wider bioscience community.

Phase 3 deliverables

29. Phase 3 awards are subject to assessment on the delivery of Phase 2. The Phase 3 deliverables are:
    - A multi-lab qualification study to assess inter- and intra- lab reproducibility.
    - Testing of Sponsor compounds and comparison of data to that previously generated.
    - Engage new end-users for testing.
    - Development of standard operating procedures.
30. Further qualification and modelling with larger datasets. Such approaches may include, but are not limited to, methods to integrate mutiparametric data such that predictions and decisions can be made

from an integrated data set rather than individual readouts in isolation, quantitative systems pharmacology modelling to translate data into clinically relevant predictions, and/or machine learning applications. These approaches should be tested and qualified using control and treated data from a minimum of three compounds with different mechanisms of action.

31. Include the development of a software tool to collect and analyse the data generated, if appropriate.

32. Dissemination to the wider bioscience community.

In-kind contributions

33. The Challenge will be supported through the provision of in-kind support from the Sponsors. The in-kind support offered includes:

    - Expertise on use of OoC models within the pharmaceutical industry, including safety pharmacology.
    - Input on end-user requirements.
    - Compounds to test and associated preclinical and clinical data.
    - In-house testing to assess transferability and reproducibility of the model.

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