Phage research in 'organ-on-chip' devices

Wai Hoe Chin

School of Biological Sciences,
Monash University
17 Rainforest Walk
Clayton, Vic. 3800, Australia
Tel: +61 3 9905 5486
Email: wai.chin@monash.edu

Jeremy J Barr

School of Biological Sciences,
Monash University
17 Rainforest Walk
Clayton, Vic. 3800, Australia
Tel: +61 3 9905 5486
Email: jeremy.barr@monash.edu

The use of 'organ-on-chip' devices in microbiology research presents enormous opportunities for fundamental and translational research[1–4]. Yet these approaches have not been widely embraced by the microbiology field. This is particularly evident with bacteriophage (phage) research applications. Traditionally phage research has been an early adopter of experimental techniques and approaches[5], having catalysed research in biotechnology, environmental biology, sequencing, and synthetic biology. Here we discuss some of the opportunities that organ-on-chip devices present to both phage and microbiology research, and provide a 'how to' guide for researchers interested in utilising this approach.

'Organ-on-chips' are micro-engineered biomimetic devices that replicate key functions, activities and physiological responses of entire living organs[6]. The approach has been used to develop beating hearts[7], simulate breathing lungs[8], sustain a gut microbiome[3,9] and even develop interconnected neurons of the brain[10]. Devices are typically micro-fabricated to contain channels that are lined with cultured human cells, which mimic organ-specific architecture and functions *in vitro*[6]. The device structure varies depending on the organ of interest. For instance, the gut-on-chip can comprise of a single[4] or double channel structure[9], with channel dimensions varying between 500–1000 µm wide and 150–250 µm high. The single-channel gut-on-chip forms the simplest structure, being enclosed by a glass slide upon which a layer of gut epithelial cells is grown. In comparison, the double-channel gut-on-chip is constructed by joining two single-channel devices together with a thin porous membrane separating the two channels. The membrane supports the gut cell layer within the top channel while the bottom channel represents the vascular system of the gut.

The fabrication, operation and experimentation of organ-on-chip devices typically require the convergence of numerous fields including engineering, cell biology and microbiology; presenting a high technical barrier for research applications. Yet overcoming these challenges allows us to probe the interactions between phages, their bacterial hosts and 'life-like' organs to answer therapeutic, ecological, and fundamental questions. For example, a mucus-producing lung-on-chip model was used to describe phage adherence to mucus layer, thereby forming a non-host-derived barrier against bacterial infection[4]. Other studies have demonstrated the maintenance of a gut microbiome and Coxsackie virus infection using a gut-on-chip model[3,9]; approaches that can be modified to investigate gut phage-bacteria interactions. In essence, the organ-on-chip provides researchers the benefit of *in vitro* amenability while experimenting with phages under biologically relevant conditions.

The organ-on-chip in four steps

Step 1: designing the organ-on-chip mould

The first step to creating an organ-on-chip is to fabricate a mould. Two commonly used options are photolithography and 3D-printing. Photolithography (Figure 1) is commonly used in engineering fields, but is technically challenging; requiring specialist equipment and reagents. However, this technique is virtually limitless in creating complex designs at the nanoscale[11]. The technique starts with depositing a photosensitive polymer on a substrate. By controlling ultraviolet (UV) light exposure on the substrate, the polymer will polymerise to the desired feature pattern, which is subsequently developed by washing away soluble unpolymerised regions.

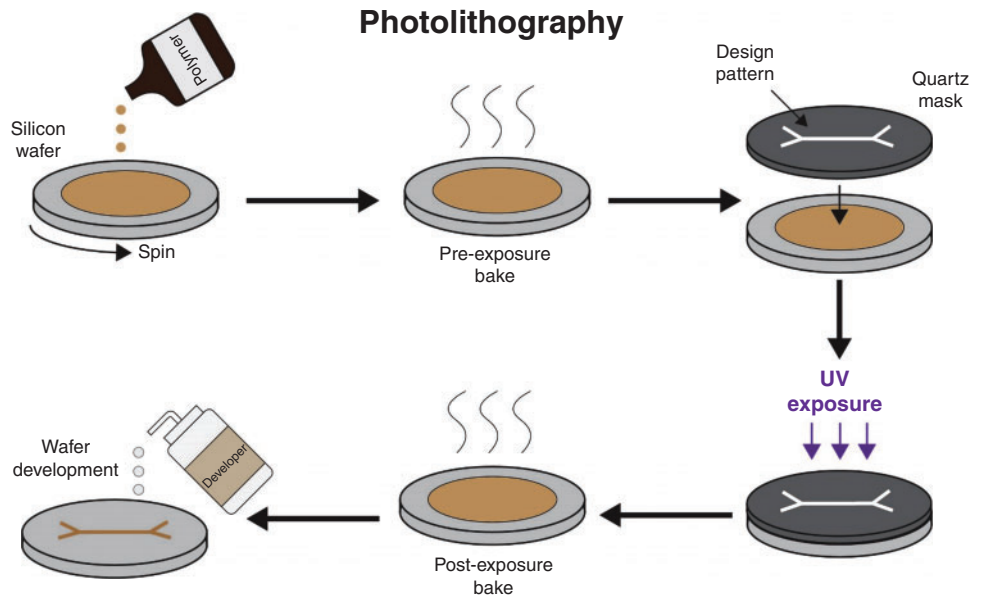

Figure 1. Organ-on-chip mould fabrication using photolithography. In this process, a photosensitive polymer (SU-8 is commonly used) is deposited onto a silicon wafer, baked, and overlayed with a quartz mask containing the desired features of the device. Exposure to UV light polymerises and solidifies the polymer to create the mould for subsequent use.

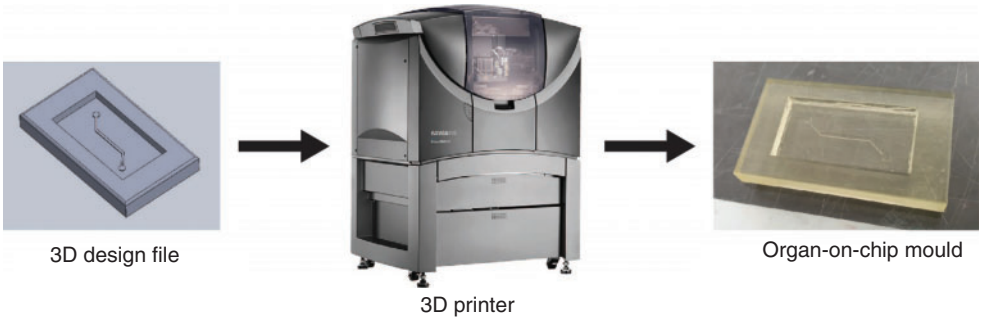

Figure 2. 3D printing the organ-on-chip mould. The mould is drawn using a modelling software, such as SolidWorks®, then converted to a 3D printer-readable file (.STL format) and sent to a 3D printer for device printing.

Alternatively, 3D-printing (Figure 2) offers a much quicker, easier, and cheaper route to fabricate organ-on-chip moulds. However, unlike photolithography, 3D printing has a much lower printing resolution, typically in the micrometre scale[12]. Nonetheless, the accessibility and speed that 3D-printing offers enable researchers to quickly create simple organ-on-chip moulds for subsequent manufacturing, set-up, and experimentation[11].

Step 2: making the organ-on-chip

Once a mould is obtained, a variety of materials can be used to manufacture organ-on-chip devices. However, none has matched polydimethylsiloxane (PDMS) for its advantages in biocompatibility, permeability to gases, optical transparency and material flexibility[13]. In addition to its advantages in biological applications, fabricating with PDMS is fairly straightforward (Figure 3) and does not require special expertise. The only specialist equipment required is a plasma cleaner to bond the PDMS device onto a substrate (typically a glass slide or another PDMS base). However, labs without access to this equipment can utilise a portable plasma 'torch' for bonding organ-on-chips (Corona SB, Elveflow Microfluidics). Alternatively, researchers can purchase ready-made devices that are immediately amenable to cell culture, such as the LiverChip® (CN Bio Innovations, United Kingdom) or Intestine Bio-Kit (Emulate Bio, USA). For further details on organ-on-chip fabrication methods, consult references[6,11,14].

Step 3: recreating the 'organ' in the organ-on-chip

Any given organ is functionally and architecturally complex. Therefore, we must be mindful that organ-on-chips serve to approximate these complexities by 'building the organ' using tissues or cells in culture. Nonetheless, with a fair amount of creativity and innovation, these approximations can recapitulate key functions and fundamental architecture of an organ unit. Recreating the functioning organ-on-chip relies on tissue culture work that is no different to traditional cell culture in flasks (Figure 4). Researchers

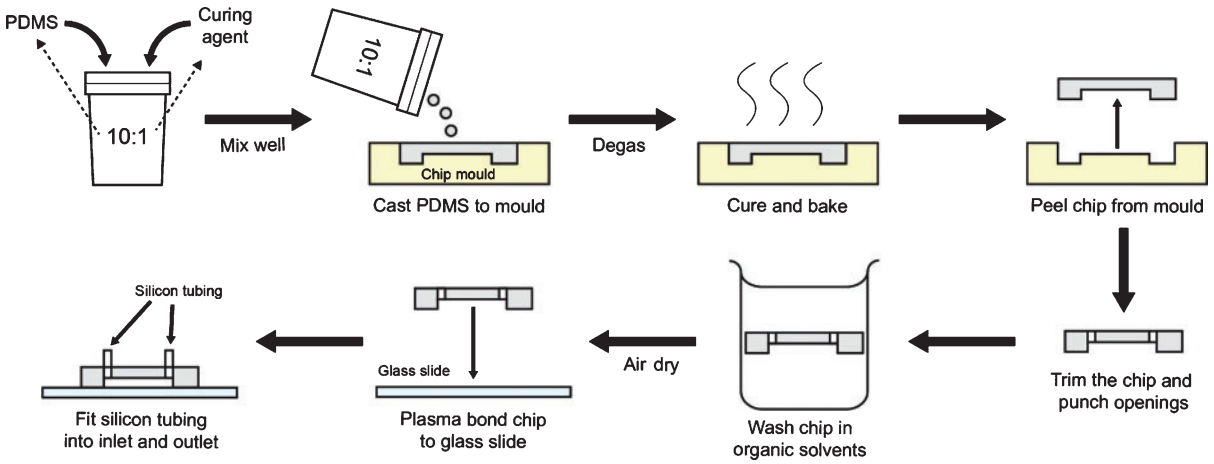

Figure 3. Workflow for organ-on-chip manufacture using PDMS. PDMS is a viscous fluid that solidifies when mixed at a recommended ratio of 10:1 with its curing agent. The mixture is then cast into the mould and baked at 95°C for curing. The PDMS chip is then peeled from the mould, trimmed and washed with organic solvent to remove residual uncured PDMS. The PDMS is then plasma bonded onto a glass slide, although other substrates can be used. Plasma activates the PDMS surface chemistry so that it forms irreversible chemical bonds when in contact with glass. Openings are punched into the PDMS and flexible silicon tubing fitted to create the device.

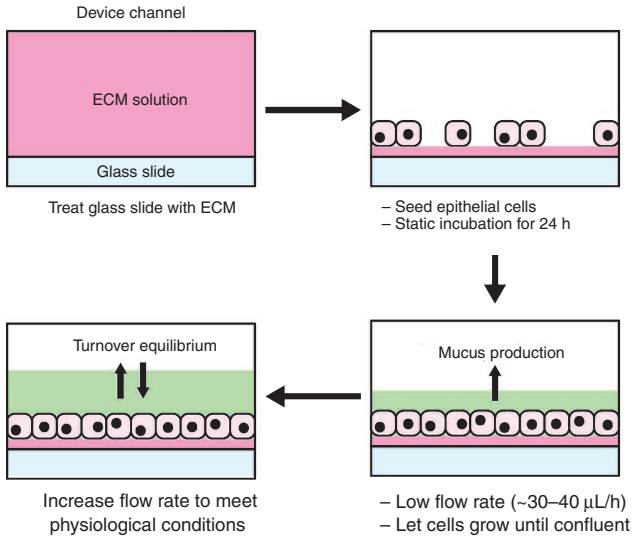

Figure 4. Workflow for reconstructing a mucus-secreting organ-on-chip. First, the device is first treated with biopolymers to provide cells with an extracellular matrix (ECM) to attach and grow within the channel[15]. The desired cell lines are propagated, harvested using standard trypsinisation and carefully seeded into the device using a micropipette. Cells are then incubated under static cell culture conditions to allow cell attachment to the ECM-treated substrate. Subsequently, the attached cells are perfused with tissue culture media to drive cell growth and differentiation within the organ-on-chip. In this example, a mucus-secreting gut epithelial cell line is used to recreate a gut-on-chip device.

need only to scale their techniques to efficiently handle tissue culture at the microfluid-level – a simple act of replacing serological pipettes with micropipettes.

Difficulties in transitioning culture cells from flasks into the organ-on-chip are often encountered, but can be overcome with a few simple solutions. Toxicity from uncured PDMS in the devices can potentially cause cell death, but is easily eliminated through organic solvent washes[16]. Determining the optimal cell seeding density will vary depending on the device and cell line used and often requires troubleshooting. Cell layer maintenance within the device requires a continual flow rate that does not impose excessive shear stress to the cells. Again, this will depend on the cell line used as some cell lines, such as endothelial lines, are more robust in withstanding high shear stress[17]. Consulting publications that have used similar cell lines and devices will provide a ballpark figure to start troubleshooting.

Step 4: operating the organ-on-chip

As outlined in step 3, cell growth and maintenance within the organ-on-chip is dependent on constant perfusion with culture media. Syringe pumps and pressure-driven systems are two widely adopted approaches to perfuse organ-on-chip devices, each with their advantages and limitations. Setting up syringe pumps is simpler and requires less tubing, but has limited flow control and sample inoculation options. Conversely, pressure-driven systems are computerised setups made up of multiple components to regulate air pressure that will drive fluid flow from a media reservoir into the device. Connecting these components requires various

adaptors and considerable tubing length, but offer increased flexibility for device control and inoculation. Furthermore, the computer interface in these systems offers fast response times and can incorporate flow sensor feedback loops that provide superior fluid flow stability compared to syringe pumps[18].

Moving forward: phage research in organ-on-chips

Traditionally, investigations of phage-bacteria interactions have been confined to *in vitro* broth culture. While these studies have proven instrumental for our understanding of phage biology, they neglect the complex environment and interactions seen *in vivo*. Recently, animal models have demonstrated the surprising diversity and stability of the phageome[19], and tissue culture-based *in vitro* studies have shown surprising interactions between phage and eukaryotic cells and tissues[4,20–22]. Organ-on-chip systems offer a unique way to study phage interactions within life-like systems that are cheap, accessible, and experimentally amenable.

Phage therapy approaches utilising organ-on-chip

Phages are known for their antimicrobial properties and are currently being pursued as an alternative to antibiotics in treating bacterial infections. Today, animal models are still the 'bread-and-butter' for preclinical testing of therapeutics, including the therapeutic validation of phages. However, animal models are costly, labour-intensive, and ethically questionable[9]. There are further concerns regarding the suitability of animal infection models to recapitulate human pathological conditions. Organ-on-chip models provide a middle ground between traditional static cell cultures and animal models for preclinical testing. A recent example was the use of a gut-on-chip to reproduce Coxsackie virus infection of a highly differentiated human villus intestinal epithelium, which reproduced cytopathic effects[3]. The use of organ-on-chip devices for phage therapy approaches offers large potentials, including the validation of antimicrobial capacity within an organ of interest, pharmacokinetic and pharmacodynamics studies, and tracking the emergence of phage resistance.

Gut-on-chip: moving gut phageome and microbiome studies from faeces to mucus

The human gut is home to a diverse repertoire of microbial species. This gut microbiome is comprised of trillions of microbial cells that influence our health, well-being and even psychological behaviour[23]. Numerically, the gut viruses, of which phages account for ~90%, are as abundant, if not more, than their microbial counterparts[24]. However, very little is known regarding the nature of phage-bacteria interactions within the gut. This is primarily due to the difficulty in studying and sampling the gut environment directly. Faecal samples are often used as a proxy to direct sampling, yet the faecal microbial communities differ significantly from intestinal mucosa[25]. Gut-on-chip devices address these limitations by providing a life-like environment for phage-bacteria experimental studies (Figure 4). This relatively simple set-up mimics essential aspects of the *in vivo* gut, namely the mucus layer, luminal flow, and spatial elements of the cell layer. Using gut-on-chip devices, it was demonstrated that phages were able to adhere to gut-produced mucus layer and as a result, exhibit enhanced antimicrobial activity within the mucus layer, providing a layer of non-host-derived immunity[4,20]. A microbiome gut-on-chip approach demonstrated the recapitulation of pathogenic microbially induced inflammation and the correction of these effects through probiotic and antibiotic therapies[26]. Finally, recent cell culture studies demonstrated that phages targeting the gut pathogen *Clostridium difficile* had increased antimicrobial affects when in co-culture with human gut cell lines[22]. These studies illustrate the potential of phage and microbiology studies within organ-on-chip devices.

Phage-bacteria ecology and evolution using organ-on-chip

To date, most evolutionary and ecological hypotheses attempting to explain phage-bacteria diversity in nature are confined to test-tube experiments and mathematical models. However, these are limited by the complexity of the experimental environment and assumptions of the models tested. Comparatively, the organ-on-chip approach allows for experimental investigations of these hypotheses under life-like conditions, adding increased complexity and biological relevance. Building off recent organ-on-chip microbiome devices[4,26], researchers are now able to study emergent microbial properties, such as co-evolutionary phage-host dynamics, experimental evolution of microbial communities, and investigations of gut phage-bacteria ecology. These devices are further amenable to the introduction of genetically modified phages and bacteria, including the insertion of fluorescence markers for real-time visualisation[27] or antibiotic or CRISPR locus for quantification of target populations[28,29]. The collective evolutionary and ecological results obtained may validate models and further explain gut microbiome diversity.

Conclusion

Phages have been at the forefront of many biological advances. Today, not only are they impacting the medical field through therapeutic applications, but also continually fueling fundamental

research, such as evolutionary biology and ecology. However, experimental phage research has been mostly confined *in vitro* and *in silico*. To that, we propose organ-on-chips as an experimental approach to further propel phage and microbiology research. The amenability of organ-on-chips allows researchers to conduct various phage and microbiological studies within life-like conditions; without the cost associated with animal models. Despite requiring high interdisciplinary knowledge, the organ-on-chip remains accessible to non-engineers through collaborations or simpler alternatives in setting up the platform.

Conflicts of interest

The authors declare no conflicts of interest.

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Biographies

Wai Hoe Chin, BSc (Hons), graduated with a Bachelors in Biomedical Sciences with first class honours from The University of Edinburgh. He is currently a first-year PhD student in Monash University under supervision by Dr Jeremy Barr. His research adopts the gut-on-chip platform to investigate phage-bacteria co-evolution and ecology within the gut.

Jeremy J Barr, BBiot (Hons), PhD, is a Lecturer and ARC DECRA Fellow at the School of Biological Sciences, Monash University. He leads a research group investigating the tripartite interactions between bacteriophages and their bacterial and human hosts (https://thebarrlab.org).