View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Leiden University Scholary Publications

1 # Organs-on-chips: into the next decade

2 Lucie A. Low PhD[1†]; Christine Mummery PhD[2,3]; Brian R. Berridge[4]; Christopher P. Austin MD[1];
3 Danilo A. Tagle PhD[1†]
4 [1]National Center for Advancing Translational Sciences, National Institutes of Health, USA
5 [2]Leiden University Medical Center, the Netherlands
6 [3]University of Twente, the Netherlands
7 [4]National Institute for Environmental Health Sciences, National Institutes of Health, USA
8 [†]Co-corresponding author emails: lucie.low@nih.gov and danilo.tagle@nih.gov
9

Abstract

Organs-on-chips (OoCs), also known as microphysiological systems or "tissue chips" (the terms are synonymous), have garnered substantial interest in recent years owing to their potential to be informative at multiple stages of the drug discovery and development process. These innovative devices could provide insights into normal human organ function and disease pathophysiology, as well as more accurately predict the safety and efficacy of investigational drugs in humans. Therefore, they are likely to become useful additions to traditional preclinical cell culture methods and *in vivo* animal studies in the near term, and in some cases, replacements for them in the longer term. In the last decade, the OoC field has seen dramatic advances in the sophistication of biology and engineering, in the demonstration of physiological relevance, and in the range of applications. These advances have also revealed new challenges and opportunities, and expertise from multiple biomedical and engineering fields will be needed to fully realize the promise of OoCs for fundamental and translational applications. This Review provides a snapshot of this fast-evolving technology, discusses current applications and caveats for their implementation, and offers suggestions for directions in the next decade.

26 # [H1] Introduction

27 Drug development is slow and costly, driven mainly by high attrition rates in clinical trials[1].
28 Although remarkable increases in our understanding of the molecular underpinnings of human
29 diseases and our ability to model *in vivo* cell, tissue and organ-level biology have been made
30 over the past three decades, the number of US Food and Drug Administration (FDA)-approved
31 drugs per billion US$ spent on research and development has actually decreased monotonically
32 since 1950[2]. Drug development needs new approaches, paradigms and tools to reverse these
33 trends and thus deliver on the promise of science for patients[2].
34
35 Although animal models have contributed enormously both to our understanding of physiology
36 and disease, and to the development of new medicines, researchers have long been aware of
37 the frequent discordance between animal and human studies and therefore the need for
38 modeling and testing platforms that would be more predictive of human responses[3,4]. Indeed,
39 drug candidates may be terminated for lack of efficacy in animals, or discovery of hazards or
40 toxicity in animals that might not be human-relevant. Despite significant developments in
41 computational and *in vitro* biology and toxicology in the last two decades, currently over 80% of
42 investigational drugs fail in clinical testing, with 60% of those failures due to lack of efficacy and
43 another 30% due to toxicity[5].
44
45 To address some of these issues and offer alternative tools for preclinical stages, early "cell
46 culture analogs"[6,7] were explicitly designed to culture mammalian cells in linked chambers
47 perfused with a recirculating tissue medium, or "blood surrogate". Following on from these
48 models came a "heart-lung micromachine", integrating a lung cell culture model with a cardiac
49 device to assess the effects of drugs and therapeutics delivered to the human lung by aerosol
50 on cardiac function and toxicity *in vitro*. This first "lung-on-a-chip" research was published in
51 2010[8] and set the stage for organs-on-chips (OoCs, synonymously known as "tissue chips" or
52 microphysiological systems (MPS)) — microdevices engineered to contain (human) cells and
53 tissues and to model or mimic organ structures, functions, and reactions to biological
54 conditions, stressors or compounds.

The dramatic expansion of the OoC field in the past decade has been made possible by the convergence of multiple previously disparate technologies, including induced pluripotent stem cells (iPSCs) and mixed cell culture capabilties, genome editing, 3D printing, sophisticated cell sensors, microfluidics, and microfabrication engineering, which led to the demonstration that dynamic culture conditions significantly influence the physiological maturation and function of *in vitro* systems. Tissue chips offer promise in, for example, modeling multiple organs and tissues from individual donors of both healthy and disease dispositions, and investigating the responses of these tissues to environmental perturbations and therapeutics with known or unknown mechanisms of action. Worldwide investment from scientific funding bodies (Box 1) has enabled the development of a multitude of 3D tissue models, from relatively simple single cell type organoids to complex multi-cell type, multi-organ microfluidically-integrated systems (**Table 1**). Consortia, committees and workshops have emerged in Europe, the US and Asia to discuss state-of-the-science aspects of OoCs (Box 1).

In this Review, we will cover how OoCs have evolved over the last decade into a potentially transformational translational science paradigm. OoCs could impact drug discovery and development by offering novel tools for disease modeling and understanding, as well as providing alternative – and potentially more predictive – methods for assessment of toxicity and efficacy of promising new compounds and therapeutics. There are clear opportunities for this technology to provide more rapid, cost-effective, and accurate information on human diseases and drugs being developed to treat them, providing insights for academic, biopharmaceutical, and regulatory scientists that were previously not possible. We will explain how OoCs can model healthy and diseased phenotypes and discuss the promise of linked platforms for the creation of “body on chip” systems. Importantly, we will cover the limitations of OoCs and discuss how defining the context of use of OoC platforms is critical for their continued development. Current considerations and challenges will be detailed, and our predictions for the ongoing era of tissue chip research presented.

84 # [H1] Key features of organs-on-chips

85 OoCs are bioengineered microdevices that recapitulate key functional aspects of organs and
86 tissues. While there is wide diversity in the specific designs of each platform, OoCs range from
87 devices the size of a USB thumb drive to larger systems that reflect multiple linked organs
88 within the footprint of a standard 96-well laboratory plate. All OoC platforms have three critical
89 and defining characteristics: the three-dimensional nature and arrangements of the tissues on
90 the platforms; the presence and integration of multiple cell types to reflect a more
91 physiological balance of cells (such as parenchymal, stromal, vascular and immune cells); and
92 the presence of biomechanical forces relevant to the tissue being modeled (such as stretch
93 forces for lung tissues or hemodynamic shear forces for vascular tissues). One way that
94 biomechanical forces can be introduced to model fluid flow across the tissues is to include
95 microfluidic channels in the systems to deliver and remove cell culture media, and remove
96 associated cell metabolites and detritus. Organoids – another type of multi-cellular 3D tissue
97 model replicating some aspects of *in vivo* organ structure and function – are not classified as
98 OoCs due to their production through stochastic self-organization (rather than specific cell
99 seeding and growth protocols) and lack of cytoarchitectural structure (rather than provision of
100 scaffolding or specially-shaped culture chambers)[9].

101

102 **Table 1** highlights some specifics of how OoCs differ from two-dimensional cell cultures. Each
103 platform design, from 2D plates to complex 3D engineered systems, has advantages and
104 disadvantages. Therefore, the selection of a particular platform will depend on the context of
105 its use, such as the characteristics of the assays and their readouts. One key advantage for OoC
106 platforms is the ability to control cellular and specific tissue architecture to emulate chemical
107 gradients and biomechanical forces. This allows precision control over the biochemical and
108 cellular milieu to model *in vivo*-like environments and responses. Other advantages include the
109 ability to vascularize or perfuse tissues, either with inclusion of self-assembling endothelial cells
110 that form perfusable lumens, or by use of microfluidic channels that act as engineered
111 vasculature, bringing nutrients and fluidic flow to cells within culture chambers. Also, the ability
112 to incorporate real-time tissue function sensors such as microelectrodes or optical microscopy

113 markers (for example fluorescent biomarkers) allows for monitoring cell health and activity.
114 Figure 2 illustrates some of the diversity of OoC systems and shows how they can provide a
115 wide range of data outcomes that can be employed during drug development.

116

117 [H3] Common considerations and challenges

118 Before OoC platforms are implemented, careful consideration of a large number of variables
119 and challenges is needed to create and validate systems that reflect the context of use and
120 desired outcomes. Although not mututally exclusive, these challenges can be categorised as
121 either biological and technical.

122

123 [H2] Biological considerations and challenges

124 [H3] Defining context of use: When creating OoC systems, bioengineers are essentially reverse-
125 engineering human cellular systems; that is, taking apart and analyzing the components of the
126 biological system, identifying the key aspects and components needed for function, and using
127 these findings to reconstitute the functional system[10]. Reverse-engineering human tissues and
128 physiological systems is complicated due to an often-incomplete understanding of the
129 composition and interplay of any given tissue and system. Therefore, rather than attempt to
130 comprehensively model a complex system, it may be more useful to engineer simple tissues
131 that can still give relevant and useful answers for the specific field of study. For example, it may
132 be more beneficial to use discrete vascularized brain organoids[11-13] when modeling
133 glioblastoma, psychiatric disorders or developmental neurotoxicity than to create a complex
134 multi-organ system with cardiovascular, lymphatic and glymphatic components. However, a
135 multi-organ system could provide novel pathological insights into disease mechanisms for
136 disorders or toxicities that require interactions of more than one organ.

137

138 Currently, OoCs can model certain aspects of a tissue but no single system completely
139 recapitulates a fully functional and integrated human tissue, let alone an organ. Rather, systems
140 are designed to model key aspects of a tissue – or its most characteristic features – to mimic
141 the morphological and functional phenotype of interest; where the phenotype being evaluated

142 depends on the question being asked. Despite the emerging diversity of OoC platforms (see [14]
143 for a recent review), identifying the base platform choice that can provide answers to the
144 research problem(s) in question remains challenging for end-users.
145
146 [H3] Cell sourcing: Regardless of system complexity, one universal issue faced by OoC
147 developers and users is renewable cell sourcing (**Box 2**). Choosing the appropriate cells for a
148 system is partly based on the context-of-use of the platform but also often based on the
149 availability of a particular cell source from commercial entities or from primary donors, which
150 each have advantages and disadvantages. Increasingly, iPSCs or adult stem cells sourced from
151 mass production of tissue organoids are seen as the answer to the lack of available primary
152 cells[15], and iPSCs have some compelling advantages. For example, iPSCs offer an almost
153 unlimited source of cells, and generating isogenic cell lines from them means that all tissues in
154 multi-OoC platforms could be from the same donor[16,17], thereby addressing a key source of
155 variability. However, to date, the phenotype of many iPSC-derived differentiated cells such as
156 cardiomyocytes is immature, and protocols for differentiation and maturation are non-
157 standardized and can be difficult to reproduce (**Box 2**).
158
159 [H3] Cell scaffolds: In addition to understanding a tissue's composition, engineering a tissue
160 requires understanding the functional interplay of cell types and the effect of the scaffold or
161 extracellular matrix **[G]** (ECM) on the function of the cellular architecture[18]. OoCs may use
162 decellularized scaffolds or seed cells within natural or synthetic hydrogels **[G]** to create an
163 environment conducive to cell growth, but the ECM composition and three-dimensional
164 arrangement affect cell survival, morphology and polarity[19-21] and so must be carefully chosen
165 and engineered to promote the formation of appropriate tissue characteristics. The choice of
166 the ECM material must be considered – hydrogels (networks of polymers that swell with water
167 application) are a widely used material due to their biocompatibility, support for cell adhesion,
168 and similarities to many soft tissues and *in vivo* ECM, but may be difficult to engineer and lack
169 standardized protocols for creation. The complexities of modeling even relatively simple tissues
170 with few cell types can be exponentially magnified when including vascularization, innate or

171 adaptive immune responses, and the frequent and often large variability in tissue sources
172 between donors/suppliers/batches. Recent advances in bioengineering allow new possibilities
173 for incorporation of biosensors into systems via the ECM. For example, incorporation of
174 fluorescent microgels containing peptides that are cleaved in the presence of specific
175 enzymes[22] offers the opportunity to use ECM for real-time readouts of OoC assays.
176
177 [H3] Linking multiple platforms: Linking multiple OoCs into multi-organ systems is not trivial and
178 requires consideration of aspects such as biological (allometric) scaling, maintenance of sterility
179 when building or connecting tissue modules, use of a common medium, incorporation of
180 bubble traps, and control of varying flow rates[23,24]. Additionally, a number of organs and tissues
181 are necessarily missing from even the most complex series of linked OoCs, necessitating the
182 need to account for missing organs. For example, how can a linked platform model important
183 diurnal or endocrine fluctuations – which affect cell and drug metabolism[25,26] – if tissues
184 producing or responding to those cues are absent? One solution has been the creation of
185 complex engineered 'microformulators' to formulate, deliver and remove culture medium at
186 defined time intervals, simulating the function of missing organ(s)[27]. However, this remains an
187 ongoing challenge.
188
189 [H3] Universal medium: Each tissue requires an adequate supply of specific nutrients and
190 growth factors relevant for that tissue, so for linked OoC tissue systems, a key challenge is
191 providing this kind of universal cell culture medium or "blood mimetic". So far, approaches to
192 address this issue have included scaling mixtures of culture media and engineering endothelial
193 barriers. For example, circulating a 50:50 mix of liver-specific and kidney-specific media in a
194 linked liver-kidney system recently enabled the nephrotoxic metabolites of aristolochic acid to
195 be determined[28]. However, as the number of linked systems increases, the success of the
196 scaling solution decreases, as every tissue ends up with a suboptimal culture medium, which
197 will impact the function and therefore physiological relevance of the system. Approaches for
198 linking systems may involve: creating single-pass or recirculating systems of culture medium
199 that can be replenished or modified over time[29,30]; or engineering platforms that allow culture

200 of tissues in individual modules but provide access to a circulating ‘blood surrogate’ medium by
201 inclusion of synthetic or endothelial barriers between tissue modules and the circulating
202 medium[31-33]. Some researchers have approached the universal medium problem by providing
203 tissues with appropriate individual support through variation of the surface chemistry of the
204 platform or scaffold on which cells are cultured (e.g. by silanes), while circulating a general
205 serum-free medium to introduce fluidic flow to the system[34,35].
206

207 ## [H2] Technical considerations and challenges

208 ### [H3] Platform design:
The characteristics of the assays that are intended to be run on an OoC
209 must be considered early in the design phase or when choosing a particular platform. Many
210 chips incorporate microfluidics, which can supply tissues with the nutrients and factors needed
211 for function and introduce important biomechanical forces such as the shear forces
212 experienced by cells adjacent to vasculature. However, microfluidic designs must carefully
213 model the resulting forces on the tissues because channel diameters, corners, and input/output
214 ports can influence flow rate and therefore tissue performance[36]. Ports for inflow and outflow
215 must be designed to maintain the sterility needed for cell culture while still allowing for culture
216 changes. Also, ‘bubble traps’ may need to be incorporated, as a bubble in a microfluidic channel
217 can completely block all flow[37].
218

219 Modeling biomechanical forces is appropriate in certain tissues; for example, stretch forces for
220 lung alveolar tissues[38]. An elegant solution from an early lung-on-a-chip introduced vacuum
221 channels running alongside a porous membrane onto which lung alveolar cells were seeded on
222 one side and lung endothelial cells on the other. Rhythmic application of the vacuum caused
223 stretching and relaxation of the cell-lined membrane and mimicked the biomechanical forces
224 associated with breathing[8]. This design has been adapted for many other tissues including
225 gut[39], heart[40], blood-brain barrier[41] and kidney glomerulus[42], highlighting how a simple design
226 concept can be useful for multiple applications.
227

228 The assays of interest for each platform will ultimately dictate platform design. For example,
229 chips replicating cardiac function likely need to allow access by a microscope and be fabricated

230 of optically clear materials to allow imaging of cardiac twitching [43,44]. Liver chips modeling
231 oxygen zonation may make use of microfluidic flow rates to create differing zones of oxygen
232 saturation[45]. Neural or muscular (cardiac or skeletal) platforms should incorporate multi-
233 electrode arrays **[G]** , or more microscale assays such as patch clamping or voltage clamping to
234 provide readouts of cell activity[40]. Inclusion of biosensors such as fluorophores can allow real-
235 time readouts of cell function; for example, metabolism, activity, or activation of certain
236 molecular pathways[46]. A recent automated multi-tissue organ system integrated an impressive
237 array of on-chip sensors including electrochemically activated immunobiosensors attached to
238 physical microelectrodes, mini-microscopes, in addition to optical pH, oxygen and temperature
239 monitors[47]. This technical feat highlights the ongoing engineering advances that are enabling
240 real-time non-invasive monitoring of OoC microenvironments.
241
242 [H3] Platform fabrication: Although hydrogels and other scaffolds can help structure the
243 internal cellular architecture of an OoC, the fabrication materials for the chip itself must be
244 carefully considered. Every material for platform fabrication has a surface chemistry that affects
245 how cells, fluids and compounds bind or absorb into the material. For example,
246 polydimethylsiloxane (PDMS) is a silicon-based organic polymer that is widely used for platform
247 fabrication because it is affordable and easy to work with via soft lithography methods,
248 allowing for fast prototyping and easy iterative design change, and it creates flexible,
249 biocompatible, optically clear platforms that allow modeling of biomechanical forces and real-
250 time tissue imaging. However, PDMS is gas permeable (which can be an advantage or
251 otherwise) and has a high absorbance for small hydrophobic molecules[48]. Therefore, PDMS
252 becomes problematic for drug studies as the PDMS-based platform itself can absorb a large
253 amount of the drug, or the resulting factors released from the cells may be leached from the
254 effluent. There is also a risk of cross-contamination for chambers or channels adjacent to each
255 other. So, mitigatory approaches for PDMS OoCs include treatment or coating of the polymer-
256 based surfaces of the device to prevent cell adhesion or drug loss[49-52]. Alternative materials for
257 chip fabrication include glass, silicon, and thermoplastics such as cyclic olefin coplastic (COC)
258 and poly(methyl) methacrolate (PMMA), with the material choice often being a trade-off

259 between the needs of the platform versus the availability, affordability or fabrication feasibility
260 of the materials.
261
262 Regardless of fabrication material choice, all OoC platforms require careful characterization of
263 adsorption/absorption profiles. Additionally, the biocompatibility of the materials to be used
264 must be considered and profiled, as unexpected toxicities could appear when repurposing
265 materials for platform fabrication[53].
266

267 [H1] Organs-on-chips for toxicity assessment

268 Toxicity and unknown safety of exposure to human tissues are large sources of failures of
269 potential drug candidates, and accounted for 40% of losses based on failure data from four
270 large pharmaceutical companies[5]. Traditionally, key individual tissues that are targeted for
271 toxicity assessments include liver, heart, kidney, vasculature, and brain. Methods of assessing
272 toxicity in these organs often use high-throughput but simple cell culture assays, which cannot
273 replicate a complex systemic response to a compound, or animals, which can model complex
274 responses but may not provide an accurate prediction of effects in humans.
275 Pharmacokinetic/pharmacodynamic (PK/PD) modelling **[G]** and physiologically-based
276 pharmacokinetic (PBPK) modeling **[G]** can be used to predict the absorption, distribution,
277 metabolism and excretion (ADME) of chemical substances in the body. However, these
278 modeling methods rely on data from other model systems and detailed anatomical and
279 physiological information where it is available. Animal studies are crucial for studying systemic
280 and longer-term effects in full biological systems, but the similarities and differences in
281 comparative physiology to humans can be anywhere on the spectrum between directly
282 translational to confounding or even completely unknown. Indeed, extreme and sometimes
283 tragic examples of the difficulty in translating from animals to humans can be seen in high
284 profile phase I clinical trial failures, although these events are thankfully rare [54,55]. These failures
285 were seen either during the 'first-in-human' phase[54] or during the dose escalation phase. The
286 drawbacks of current toxicity profiling highlight the intricacies of the translational process from
287 cell culture, to animals, and ultimately to humans, which can place clinical trial volunteers at

288 high-risk however carefully planned and executed a trial is. Additionally, there is a growing need
289 to predict the toxicity of novel modalities such as biologics, oligonucleotides and large
290 molecules (MW > ~900 Da) that are challenging or impossible to assess in standard animal
291 models. OoCs may have advantages for these modality-specific assessments by allowing
292 modeling of complex human responses in tightly-controlled *in vitro* systems that may be linked
293 to model organ crosstalk [56] and can be designed for specific contexts of use [57].
294
295 Single-tissue OoCs offer an alternative way to approach toxicity assessments of potential
296 compounds in various complex human 3D tissues[58]. In 2D liver cultures, hepatic cell line
297 cultures poorly represent primary human hepatocytes[59], and the latter cells rapidly de-
298 differentiate over 24 hours[60], limiting their usefulness in evaluating either subacute or chronic
299 exposure effects and systemic toxicities. An example of how OoCs could address such issues is a
300 recently developed 3D liver OoC system that can maintain healthy cell cultures for over 28 days
301 (**Table 2**) and mimic the *in vivo* environment of the liver (to include hemodynamic flow, oxygen
302 zonation and inclusion of immune components)[61,62], which opens new pathways for
303 ADME/toxicity studies. Oxygen zonation in this liver platform was achieved by controlling the
304 flow rate of medium through the platform to create zones of differing oxygen tension, and
305 coupling computational modeling of this tension to direct temporal and spatial monitoring of
306 oxygen-sensitive dyes in the system[45]. This highlights how use of biomechanical forces and
307 direct experimental assays from real-time biosensor readouts can be combined to provide
308 powerful tools for accurate replication of clinically-relevant toxicity profiles. Separation of the
309 sinusoid (vascular channel) and hepatic compartment by a porous membrane allows
310 physiologically-relevant addition of drugs, immune cells and other factors to the model [62].
311 Another recent study comparing a liver on a chip from rat, dog and human cell sources
312 elegantly showed species-specific differences in hepatotoxicity, highlighting the importance of
313 using human-specific cells for certain assays, while confirming the validity of the use of non-
314 human models for others[63] (**Table 2**).
315

316 For the heart, which is another important target organ of toxicity, a number of heart-on-a-chip
317 systems have been developed that model the complex matrices of cardiomyocytes, (cardiac)
318 fibroblasts, endothelial cells and vasculature that interact *in vivo* in a highly ordered manner,
319 which can be easily perturbed by drugs, drug-drug interactions, or off-target side effects. Since
320 *in vitro* screens are now an integral part of drug development to characterize cardiac safety
321 liabilities, the current heart-on-a-chip systems are useful as they model human responses to
322 injury (**Table 2**), and show appropriately aligned sarcomeres, rhythmically synchronized beating
323 patterns, and physiologically relevant resting membrane potentials[44,64-67]. Other structures in
324 the heart, such as cardiac valves, have been bioengineered to assess the off-target cardiac side
325 effects of dopamine/serotonin production/reuptake influencing-drugs, such as pergolide, which
326 are used in clinical treatment for psychiatric disorders such as Parkinson's disease[68]. However, a
327 large problem with all cardiac OoC systems currently using iPSC-derived tissues is the fetal
328 phenotype of most resulting cardiomyocytes[69,70]. Despite this, recent advances using electrical
329 and mechanical stimulation to 'train' the developing cells or cardiac "organoid" growth in fatty
330 acid-based culture medium and inclusion of other relevant cell types seems to encourage a
331 significantly more mature phenotype[71-74], further expanding the potential use for OoC in the
332 cardiotoxicity field.
333
334 Other important tissues for toxicity profiling include those from the kidney, gut, and lung.
335 Developmental toxicity assays, including neurotoxicity, are also relevant for many exposure
336 studies. OoC models of the kidney (nephron and proximal tubules) can be used to model
337 readouts relevant for nephrotoxicity profiling such as filtration, reabsorption, transport of
338 various molecules, and action of protein transporters[75-78]. Indeed, a kidney-on-a-chip system
339 was used to elucidate that polymyxin-B nephrotoxicity may be caused by the cholesterol
340 biosynthesis pathway, highlighting how OoCs could not only be used to test the safety of novel
341 chemical molecules but also shed light on toxicological pathways of FDA-approved molecules[78]
342 (**Table 2**). Gut-on-chip systems can model certain aspects of the bioavailability and activity of
343 drugs, by creating *in vitro* intestinal epithelia and exposing these tissues to relevant
344 biomechanical forces, such as flow and peristalsis[79,80]. Inclusion of immune and microbiome

345 factors become critical for true human relevance, both of which by themselves are huge areas
346 of research, although there is progress being made in inclusion of these in both organoid[81] and
347 microfluidic systems[82-85]. For example, the "HuMix" model to recreate human-microbial
348 crosstalk allows researchers to investigate the causal relationships between the gastrointestinal
349 microbiota and certain human diseases, but could also be used in toxicology and
350 pharmacokinetic studies[82]. Toxicity profiling of inhaled substances can benefit from lung-on-a-
351 chip models that can recapitulate the air-liquid interface of the lung alveoli[8,86] and model
352 effects such as exposure to bacteria, drug-induced pulmonary edema and cigarette smoke[87].
353 Developmental neurotoxicity can be modeled in platforms containing 3D neural tissues. For
354 example, in a study that used RNA-Seq readouts from neural constructs exposed to 60 drugs of
355 known toxicity, a predictive model based on linear support vector machines had over 90%
356 accuracy in predicting the toxicological impact of 'blinded unknown' compounds[13], highlighting
357 the potential power of these types of 3D models for predictive toxicology. Other developmental
358 toxicological vulnerabilities have been assessed using placenta-on-a-chip models that can
359 recapitulate the ability of compounds to cross or affect the maternal-fetal barrier [88,89].
360 Readouts of vascular-related toxicity may be critical for therapeutics, and vascular networks on
361 OoCs have been used to investigate vascular toxicity with chemotherapeutics[29,90], and risk
362 factors for complications such as thrombosis from monoclonal antibody treatments [91].
363
364 Finally, linked multi-organ systems could expand OoC applications into organ interactions and
365 systemic toxicity profiling, and these are discussed further in section 6.
366

367 [H1] Disease modeling on a chip

368 In addition to being useful as tools for understanding toxicity in human tissues, OoCs also offer
369 ways to model disease states *in vitro*, thereby allowing mechanistic investigation not only of
370 disease pathologies but also of the efficacy and potential off-target effects of therapeutic
371 interventions. The potential enhanced understanding of human disease physiology from
372 modeling diseases on OoCs could help address the high attrition rates of promising compounds
373 seen during both lead optimization and clinical development stages due to lack of efficacy [5,92].

[H2] Stem cells and tissue chips – powerful partners

While many OoCs have been developed to model disease phenotypes using primary or cell line sources, the increasing use of iPSCs, plus the novel option of using the mass production of organoid technology as a way to source adult stem cells in biomedical research, has also led to the increased development of an array of diseases-on-chips including: cardiac (atrial and ventricular) myopathies[72,93,94]; asthma[95]; vascular abnormalities[96]; polycystic kidney disorders[97]; as well as neural disorders – including ones mimicking aspects of neurodegenerative and psychiatric disorder phenotypes[98,99] – and rare pediatric diseases such as Hutchinson-Gilford Progeria Syndrome [100]. However, a limitation associated with using stem cell-derived cells in OoCs include difficulties in producing an adequate number of mature, differentiated cells with the necessary purity for many tissues (for more see **Box 2**).

Despite these current limitations, one early example of the power of iPSCs' use in OoCs, coupled with genome editing technologies, investigated the rare childhood pediatric cardiomyopathy Barth Syndrome. Stem cell derived-cardiac tissues from patient donors were created and modeled on 'muscular thin films', which replicated the disordered sarcomeric organization and weak contraction properties seen in the disease[101]. Using genome editing techniques to 'correct' the faulty TAZ gene in the iPSC-derived cardiomyocytes, mitochondrial abnormalities underlying the disease were identified. These results highlight the potential use of OoCs as models for the critical stages of target validation where the creation of multiple tissue types from the same patient, and the generation of isogenic control tissues by genetic editing methods for any number of genetically-based diseases, can enable detailed and specific mechanistic studies for these disorders[102].

[H2] "You-on-a-chip" for common and rare diseases

Disease modeling on OoCs could contribute to the development of precision medicine. OoCs modeling angiogenesis[103], tumor growth[104], and intra- and extravasation[105,106], have all contributed to the development of vascularized and metastatic breast cancer models[107-110]. The treatment of patient-derived tumors on chips with chemotherapeutics enabled treatment

404 comparison and optimization[108], which is a step towards using this technology for precision
405 medicine. Tumor-on-a-chip platforms have also helped parse out the mechanistic effects of
406 different chemotherapeutic agents on the resulting 'microtumors'[90]. Other tumor-on-a-chip
407 models include neural glioblastoma[111], renal cell carcinoma[112], as well as lung[113], pancreatic[114],
408 colorectal[115], ovarian[116], prostate[117], and cervical[118] cancer, among many other types.
409
410 While many of these models were created with cancer cell lines, an obvious and powerful
411 opportunity arises when patient-derived primary or iPSC-derivatives are seeded onto OoC
412 models, creating "patient-on-a-chip" models. This could inform the stratification of cancer
413 patient populations into subpopulations that respond optimally to different chemotherapeutic
414 regimens or cocktails, but could also lead to development of "you-on-a-chip" for rare cancer
415 patients or those with unusual etiologies. Communities with rare diseases could benefit
416 tremendously from the opportunity to recreate these pathologies on chips (see [119] for a
417 review). For example, patient-derived pancreatic ductal epithelial cells can be used to create a
418 pancreas-on-a-chip to potentially understand the cystic fibrosis transmembrane conductance
419 regulator protein and its role in insulin secretion[120]. If iPSC protocols become available for
420 pancreatic cell creation – a current challenge with promising progress in the field[121] – then
421 modeling of an individual with cystic fibrosis on a chip becomes possible, which could prove
422 useful to understand the high risk of diabetes and glucose imbalance in this population.
423

424 [H2] Synergistic engineering to combine 3D models

425 Both OoC and organoid 3D models have strengths and limitations (**Table 1**), but innovative ways
426 to combine the technologies and introduce related ones such as 3D bioprinting – so-called
427 'synergistic engineering'[122]– adopts strengths from multiple 3D bioengineering fields to create
428 reliable predictive tissue models with the opportunities for higher throughput screening (see [123]
429 for a comprehensive review). For example, both organoids (which self-organize into three
430 dimensions) and bioprinted tissues (where cells are deposited in a specific manner) can be
431 seeded or printed in multi-well plates with media flow and inclusion of other biomechanical
432 forces, creating platforms with multi-tissue components that are amenable to larger scale
433 commercial production. An example of these combined technologies includes vascularized

organ 'buds' that can be perfused by a common medium[124] and bioprinting of endothelialized myocardium in a microfluidic perfusion bioreactor[125]. In the case of the latter, multiple bioengineering techniques were combined to create an innovative tool for predicting cardiovascular toxicity. First, endothelial cells were encapsulated into bioprinted microlattices to allow formation of an endothelial vascular bed, after which cardiomyocytes were introduced forming a myocardial tissue with good alignment to the bioprinted vascular bed. Finally, inclusion of the tissue construct into a microfluidic bioreactor allowed continuous vascular perfusion and real-time monitoring of cardiac contraction phenotypes for up to 2 weeks.

As with all disease models, the demonstration that these 3D tissue models effectively mimic the behaviors of the disease, as well as the responses to therapeutic drugs, *in vivo* is critical for their validation.

[H1] 6. Creating a "Body on a Chip"

Linkage of multi-organ tissue systems is of clear benefit to model complex organ-organ interactions and inform PK/PD and PBPK modeling, ADME profiling, and quantitative systems pharmacology (QSP) and other computational modeling. Over the last decade, many efforts have been undertaken to integrate multiple systems and overcome the challenges associated with this (see [126] for a review). Indeed, US governmental funding from the Defense Advanced Research Project Agency (DARPA) was specifically allocated to create and link 10 organ systems (see Related links) that were viable for 28 days into a single 'body on a chip' as part of broader efforts by the US National Institutes of Health (NIH), FDA and DARPA to fund the development of tissue chips to advance regulatory sciences (see Related links). From this funding, two recent publications showed how a 10-organ "physiome on a chip" combined with QSP computational approaches could model distribution of *in vitro* pharmacokinetics and endogenously produced molecules[127]; and how a robotic 'interrogator' maintained the viability and organ-specific functions of eight vascularized, two-channel organ chips (intestine, liver, kidney, heart, lung, skin, blood–brain barrier and brain) for 3 weeks in culture [128].

The study of prodrugs[129], which are metabolized by the body from inactive to active compounds, could benefit, as could the development of novel compounds which that rely on (or cause) bioactivation[130]. Slow release mechanisms (e.g. slow-release painkillers and contraceptive injections or implants), or compounds produced by non-traditional methods such as synthetic biology or genetic engineering, could also be extensively assayed for unexpected side effects. Coupling these types of new molecular technologies with powerful computational modeling tools, including quantitative systems pharmacology (QSP)[131], machine learning[13], and artificial intelligence (AI)[132], could offer novel and helpful insights for current toxicological assessment. For example, capecitabine and tegafur (anticancer prodrugs) have been shown to be effective in a multi-organ pneumatic pressure-driven platform[133], and recently Boos et al[134] used a hanging-drop organoid system to test how products metabolized by human liver microtissues affect embryoid bodies. The prodrug cyclophosphamide (activated by cytochrome P450) was added to the system and a 50% drop of embryoid differentiation seen, demonstrating how powerful synergistically engineered microfluidic systems can be not only for prodrug investigation, but also embryotoxicity in this case.

Challenges with linking systems include how to: scale the organs of interest (e.g. allometrically, based on body size, or metabolically[24]); model fluid flow dynamically through the system and scale flow appropriately for each tissue[23]; supply all tissues with adequate growth factors and culture medium support (for example via a blood surrogate culture medium[7] or by separation of cultures by endothelial barriers[135]); and design and fabricate these complex systems. One approach to linking systems that avoids many challenges faced with physically linking organ cultures involves functional coupling such as running media through physically separate systems sequentially to model multi-organ ADME. In the case of Vernetti et al[136], this approach showed that organ-specific processing of the tested compounds was consistent with clinical data, and additionally uncovered that a liver-bioactivated microbiome metabolite crosses the blood-brain barrier using a neurovascular unit OoC[137,138].

491 A number of physically linked systems via microfluidics and pneumatic or peristaltic pump
492 mechanisms have been published (Figure 3) and include systems that have revealed, for
493 example, novel mechanisms of aristolochic acid nephrotoxicity[28], the metabolic coupling of
494 endothelial and neuronal cells in the neurovascular unit[139], and inflammatory crosstalk between
495 the gut and liver[140]. For example, Chen et al[140] examined an integrated gut-liver transwell OoC
496 and showed that modulation of bile acid metabolism was seen in the linked system.
497 Meanwhile, in an inflammatory state (modeling endotoxemia by increasing circulating
498 lipopolysaccharide levels), hepatic biotransformation and detoxification pathways showed
499 changes, highlighting that even relatively simple OoC models can give valuable information on
500 organ interactions.
501
502 Additionally, a number of multi-organ systems demonstrating utility in toxicology and disease
503 modeling applications are appearing in the literature, including systems modeling homeostatic
504 mechanisms[32,141], hepatic metabolism and off-target cardiotoxicity[34,142], and the female
505 reproductive tract and menstrual cycle[143] that reproduced a 28 day hormonal cycle in a
506 platform including ovarian tissue, fallopian tube, uterus and cervix, but also included a liver
507 module for reproductive toxicology utility (Figure 3A). Synergistically engineered multi-tissue
508 organoid-based platforms linked by microfluidics are also joining the expanding cadre of multi-
509 organ OoC tools[47,133,144,145]. Importantly, many of these systems incorporate a variety of real-
510 time assays and biosensors for ongoing cell health and function readouts and can support
511 extended cell culture (<28 days), allowing chronic and repeated testing of compounds for
512 systemic toxicity evaluation[35,146]. Some of these linked systems are becoming more broadly
513 available to researchers either through contract research organization (CRO)-based services or
514 purchase of off-the-shelf systems, although the latter are generally simpler organoid-based
515 higher throughput multi-well plate systems. Manufacturing the more complex OoC systems
516 designed by engineering labs is still an obstacle to widespread implementation in biomedical
517 labs.
518

519
[H1] Replication, validation and commercialization

520 As OoCs become increasingly commercially available, reproducibility of the technology at
521 multiple sites is becoming critically important. Negotiating legal frameworks to facilitate sharing
522 of proprietary information and technologies between organizations can can be lengthy.
523 Meanwhile, sometimes critical exchange of reagents and trained personnel can become costly,
524 and unexpected obstacles can emerge from simple processes such as shipping cells and
525 resources. Some questions that arise from these obstacles include: should cells be shipped in
526 differentiated or undifferentiated forms? Should platforms be seeded with cells, or should the
527 recipient fabricate the systems from shared molds instead? Can cells be shipped in OoC plates
528 in a frozen state and simply thawed prior to use by end-users? Thorough consideration of the
529 most straightforward processes can become complex and expensive.
530

531
[H2] Robust, reproducible, reliable platforms

532 The US government has provided almost a decade of support for OoC development, and
533 although the DARPA 'body-on-a-chip' program has now ended other federal agencies continue
534 to support US-based OoC development, and agencies in Europe and elsewhere are also
535 supporting OoCs (**Box 1**). In particular, the National Center for Advancing Translational Sciences
536 (NCATS) has created two new programs since 2016 that focus on creation of reproducible,
537 reliable, and automated systems that are accessible to the wider community. The Tissue Chip
538 Testing Centers (see Related links) initiative began in 2016 to support two independent centres
539 charged with onboarding developers' tissue chips, monitoring reproducibility of assays and
540 outcomes, and investigating additional parameters that are of use to the community. The first
541 publication addressing independent validation of a kidney proximal tubule model was recently
542 published[147] and a number more are forthcoming. To encourage the development of robust
543 automated systems with smaller laboratory benchtop footprints, the NCATS Tissue Chips in
544 Space program also promises advances for the technical development in the field (**Box 1**). These
545 programs, plus commercial pressures, are pushing the move towards more 'turn-key' OoCs to
546 help reduce or remove the need for the specialized infrastructure and highly-skilled personnel,
547 which is currently often required for OoC implementation.
548

549 ## [H2] Commercial considerations and hurdles

550 [H3] Increasing throughput: Most complex non-organoid tissue chips are currently very low
551 throughput, where only dozens of replicates (at most) can be performed at any one time.
552 Consequently, during the early stages of drug discovery, at which many thousands of potential
553 hits can be identified in a short time-frame through standard high-throughput screening assays,
554 the use of such chips is likely to be considered cost- and time-prohibitive for pharmaceutical
555 companies at present. Technological advances to create more automated, miniaturized OoC
556 systems that can become 'turn-key' technologies for facile use will be crucial to increasing
557 throughput and the number of replicates per platform.
558
559 [H3] Scaling up of reliable manufacturing processes: One difficulty with many OoCs is how to
560 scale-up system manufacturing to an industrial pace. Most early OoC designs are bespoke and
561 fabricated in-house at the developers' institutions, where fabrication is limited by cost and
562 availability of both manufacturing equipment and personnel. Therefore, academic laboratories
563 should focus on early quality control of the chips produced in-house, to ensure reliability and
564 reproducibility before scale-up can occur. This means careful compilation of standard operating
565 procedures for chip design and creation, and designing clear quality control procedures that can
566 be easily followed at other laboratories or manufacturers. Since most academic laboratories are
567 not equipped for scale-up of production, the creation of spin-off or start-up companies, or
568 formation of partnerships with manufacturing firms to mass-produce chips, becomes
569 necessary. At this stage, it would be extremely useful for all manufacturers to conform to Good
570 Manufacturing Practice guidelines (see Related links) such as those set forth by the US FDA,
571 which cover issues including equipment verification, process validation, sanitation and
572 cleanliness of manufacturing facilities, and appropriate training of personnel. While this
573 guidance is to ensure the safety and reliability of manufacturing processes for foods, drugs, and
574 devices for medical use, and is therefore not necessary for OoC manufacturing, it would still
575 provide excellent standards for reliability of chip production across all fields and help to broadly
576 increase confidence in the systems. In order to increase end-user confidence in the reliability
577 and fidelity of mass-produced platforms, additional considerations should be taken that all
578 biological assays are created on chips under Good Laboratory Practices, as this is critical for

579 preclinical toxicology testing and has been identified as a major reason for drug development
580 attrition rates[148]. In addition, there is a need for independent "qualification" labs to test OoCs
581 and their usage with available cell types, much like the NCATS Tissue Chip Testing Centers (see
582 Creating a "Body on a Chip") or the European Union Reference Laboratory for Alternatives to
583 Animal Testing European Centre for the Validation of Alternative - EURL ECVAM (see Related
584 links).
585
586 [H3] Onboarding versus outsourcing: Due to the expense and complication of technology
587 transfer for some OoCs, developers may face the decision between supplying a commercial
588 product for purchase to be used independently in a customer's laboratory, or offering services
589 through a CRO to OoC consumers. If researchers decide to commercialize their OoC platforms,
590 technology transfer and onboarding processes should become seamless, reliable and
591 standardized for every customer. Meanwhile, retaining the personnel, infrastructure and
592 resources necessary for OoC use within a CRO-based service means customers should expect
593 high standards of the research produced. However, the flexibility and adaptation of the chips
594 for specific contexts of use may be limited because CROs may not offer particular assays or
595 services. As this burgeoning field is still young, many developers and companies are choosing to
596 adopt aspects of both business models. Some offer OoC devices that can be onboarded
597 relatively easily but may need specialized equipment and/or extensive technical support. Other
598 CROs perform experiments in-house in collaboration with academic or industry researchers to
599 help advance continuing R&D on the system.
600
601 [H3] Managing expectations: While the potential of OoCs is exciting, the technology is at an
602 early stage, so providing realistic caveats and limitations to potential consumers is critical to
603 avoid overselling its current capabilities. Some challenges faced within the field may be
604 resolved over the next decade or so – issues with cell sourcing will continue to be addressed as
605 the stem cell field matures, for example. Other limitations may take longer to resolve – for
606 example, reduction and refinement of animal use are laudable and achievable aims and are

within the realm of possibility already, but full replacement of animals in drug development is generally seen as unlikely in the near future.

One approach to managing expectations has been employed by government funding agencies in the US where creating partnerships between research and regulatory agencies, such as the NIH and FDA, over the last decade has allowed regulators access to OoC developers and their unpublished data to help inform system development. Conversely, it has enabled researchers to design useful platforms to provide data for regulatory assessment. This has led to familiarity of the technology among the regulatory community in the US, which ultimately can help pave the way for OoC data inclusion in IND (Investigational New Drug) **[G]** and NDA (New Drug Application) **[G]** packages in the future.

[H2] Validating organs-on-chips

As OoCs continue along a path towards widespread commercialization, validation must be considered. Importantly, the term 'validation' means different things to various stakeholders, but could be considered as involving three stages or principles[149]. First, physiological validation could be defined in the context of 'analytical performance', including addressing features such as sensitivity, specificity and precision (essentially reproducibility). This validation step is necessary to create a tissue chip that appropriately and reliably mimics the tissue of interest and responds in relevant ways to compounds of known action or toxicity, and it should be performed by OoC developers. Second, qualification or validation to show biological *in vivo* relevance should come next, although there is debate in the field as to whether animal or human responses should be used for this stage. Animal responses are broadly used in current drug development, which supports the argument that they should be the 'gold standard' for OoC responses to be compared against. Conversely, predicting human responses is the aim for the field, which supports the focus on generation of human responses on OoCs. Reproducibility and setting the standards for qualification currently fall under the remit of, for example, the NCATS Tissue Chip Testing Centers. The third stage, industrial validation, or OoC adoption by industry and regulatory agencies, will involve the generation of data from proprietary compounds and submission of that data to regulatory agencies. All of these stages of validation

637 are currently underway. In the US, the FDA has also partnered with a number of OoC
638 companies to get hands-on experience with OoC data, as they expect this type of data to be
639 submitted to them in the near future.
640
641 Taken together, the three stages/principles of validation/qualification described above will help
642 address international guidelines for novel methods, for example the Organisation for Economic
643 Co-operation and Development (OECD) Guidance Document on the Validation and International
644 Acceptance of New or Updated Test Methods for Hazard Assessment (see Related links) These
645 guidelines describe necessary assay details for validation such as the rationale, the endpoints
646 and limitations, protocols, variability, performance with reference and known chemicals, and
647 comparisons to existing assays. Importantly, the OECD guidelines also state that data
648 supporting the validity of the method must be available for review. To address this need for all
649 stakeholders, the NIH's NCATS also funds an MPS Database, which is tasked with integrating all
650 the data from the Testing Centers, as well as data from a number of other NIH-funded
651 developers, FDA users, and commercial OoC suppliers. This centralized database acts as a public
652 repository for a broad range of OoC data and will prove useful for developers, industry and
653 regulatory bodies over the coming years, with a recent report highlighting functionality for data
654 visualization, inter- and intra-study reproducibilities and power analyses calculations [150].
655
656 Additionally, underpinning the needs of the above validatory steps, the accurate
657 standardization of methodologies used for generating empirical data should be considered. The
658 term 'standardization' brings on new challenges with respect to what 'standardization' means
659 for either technical, analytical or biological aspects of OoCs. So, 'performance standards' should
660 be established for the analytical validation and biological qualification of OoCs. To this end, the
661 deposition of technical, analytical and biological data into the MPS-Database will help set some
662 of the standards, reducing the need for each user to develop their own methodologies, assays
663 and analytical methods. At the same time, many US government-funded researchers are
664 working with regulatory and industrial end-users to evaluate what should be considered
665 accepted metrics that are translatable to other laboratories and applications.
666

667 # [H1] Emerging opportunities and prospects

668 There are multiple stages at which OoC platforms could be implemented in drug discovery and
669 development, and the platform type may differ depending on the stage (see Figure 1). High-
670 throughput plate-based OoCs with relatively simplistic (but cheap and fast to produce) tissue
671 constructs could prove useful for target identification, lead selection and lead optimization.
672 Low- to medium-throughput OoC platforms that model more complex tissue-tissue or organ-
673 organ interactions could be more useful for preclinical single or double organ toxicity and
674 efficacy studies. Multi-organ systems – while perhaps the most complex and expensive to
675 develop – offer promise for reducing the need for animal studies and for use in parallel with
676 phase I and II clinical trials. Finally, OoC platforms from patient stem-cell-derived sources could
677 be used during later clinical trial phases (III and IV) as well, for *in vitro* therapeutic testing
678 before *in vivo* administration, or for concurrent monitoring of approved therapeutics.
679 Ultimately, the potential safety and efficacy of a drug or drug candidate could be evaluated
680 using OoCs in generic, or even individualized, human platforms, giving “first-in-human” testing
681 a new connotation.
682
683 Coupling OoC technology with techniques such as gene editing[151] (particularly when a series of
684 disease-relevant mutations are introduced onto a single genetic background) offers powerful
685 ways to increase the predictive power of these tools further in disease modeling and toxicology.
686 We also see opportunities to discover and validate clinically-translatable biomarkers by creating
687 datasets to correlate *in vitro* OoC readouts with clinical outcome measures. For example, using
688 OoCs to produce ‘omics’-based (and even real-time) readouts could promote the identification
689 and evaluation of appropriate endpoints surrogate to those in the clinic, which could provide
690 valid and reliable measures of change in human subjects. These endpoints and readouts could
691 be quantified and assessed for clinical benefit and compared to traditional enzymatic,
692 biochemical or histopathological assays, as well as offer ways to assess both short- and long-
693 term clinical changes. Ultimately, the use of OoC readouts detailing changes in molecular
694 signatures that have been validated against traditional methods and demonstrated clinical
695 relevance could become a common practice in drug development.

696

697 In order to help smooth the adoption and implementation of OoCs in the drug development
698 process, continued engagement and discussions with OoC developers and end-users is critical,
699 as is engaging with regulatory bodies. A 2017 report predicted that the global OoC market could
700 grow by 38% per year to become a US$117M/year industry in 2022 (based on market analysis
701 by Yole Développement) – with the potential to become a multi-billion dollar industry. In
702 support of this predicted growth and the utility of OoCs at various stages of drug development,
703 a recent anaylsis predicted up to a 26% reduction in R&D costs in the pharmaceutical industry
704 by adopting OoC technology[152], and it is anticipated that OoC data will be included in IND and
705 NDA submissions to the US FDA in the near future.
706
707 There is optimism that OoC systems may one day outperform traditional models, making the
708 understanding of human diseases and development of drugs to treat them more rapid,
709 efficient, and cost-effective, and in so doing replace, reduce and refine (the "3Rs") the use of
710 laboratory animals. Nevertheless, much work remains to address the challenges discussed in
711 this article, and thereby determine and realize the potential of this technology. According to
712 the 2018 Gartner report (see Related links) on the hype cycle of emerging technologies, OoCs
713 (referred to as 'biochips' in this report) are now in the 'Peak of Inflated Expectations' phase.
714 Disillusionment and a stall in progress often occurs after this phase because the technology fails
715 to live up to the preliminary, and often inflated, expectations, before the field recovers and
716 productivity resumes, with more modest expectations. Therefore, the aim for emerging
717 technologies is to reach this productive plateau as quickly as possible, when 20-30% of the
718 potential audience has adopted the innovation. Right now, this is estimated to be 5-10 years for
719 OoCs. It will take the coordinated global efforts of the OoC community to help this technology
720 reach that potential global audience and ultimately, help transform science, medicine, and
721 patients' lives.

722 **[bH1] Box 1: Collaborative tissue chip development efforts**

723 In 2010, the US Food and Drug Administration (FDA) and the US National Institutes of Health
724 (NIH) created a Joint Leadership Council to help speed the translation of biomedical discoveries
725 at the laboratory bench to commercial availability of new therapeutics. Under this mandate,
726 the Advancing Regulatory Science program was initiated, with awards issued to address
727 distinct, high priority areas of regulatory science. Based on the promise from these funded
728 projects, from which the seminal lung-on-a-chip work was published[8], the NIH and FDA
729 partnered with the Defense Advanced Research Projects Agency (DARPA) to fund two 5-year
730 programs for the development of OoCs. The NIH program, called "Tissue Chips for Drug
731 Screening" (see Related links), awarded funding to develop 3D microsystems to represent
732 multiple tissue types and also concurrently funded a program to explore the use of stem cells
733 and progenitor cells to differentiate into the multiple cell types that would be needed to
734 populate the microsystems. DARPA's MPS program (see Related links) focused on developing a
735 reconfigurable platform of at least 10 human organs or tissues in an integrated system that
736 could mimic and replicate biological crosstalk between tissues. While both initial programs
737 ended in 2017, the NIH continues to offer funding for further development of OoCs in an
738 expanding array of programs, including for disease modeling, inclusion of immune factors,
739 modeling of Alzheimer's Disease, use in the context of clinical trials, and as part of the NIH Help
740 End Addiction Long-term (HEAL) initiative (see Related links) to address the US opioid epidemic.
741

742 The FDA has offered advice and guidance from a regulatory standpoint for the past decade, and
743 recently signed Memorandums of Understanding with a number of commercial tissue chip
744 companies to on-board the technology to FDA laboratories. Additionally, the IQ Consortium
745 (see Related links), a non-profit organization consisting of pharmaceutical and biotechnology
746 company representatives, partnered with US government funding agencies in 2016 to add end-
747 user stakeholder perspectives to the field. The IQ Consortium recently published a series of
748 manuscripts on the characterization and use of OoC sytems in safety and toxicity profiling
749 applications [56,153] and for modeling skin[154], lung[155], the GI tract[156], kidney[157] and liver [158].
750

751 In Europe, the Institute for human Organ and Disease Model Technologies (hDMT, see Related
752 links), headquartered in the Netherlands, leads the way on integrating state-of-the-art human
753 stem cell technologies with biotechnical fields to support the development and validation of
754 human organs and disease models-on-chip. The hDMT consortium helped co-ordinate one of
755 the European Union's Horizon 2020 research and innovation programs termed Organ-on-Chip
756 Development (ORCHID, see Related links), and in late 2018 launched the new European Organ-
757 on-Chip Society (EUROoCS, see Related links) that will encourage development and
758 coordination of tissue chip research in Europe. Other countries are following the hDMT
759 example and are establishing similar organ-on-chip networks in Israel, UK, the Scandinavian
760 countries and Switzerland.
761
762 One key tenet of collaborative partnerships for tissue chip development has been the
763 involvement of different stakeholders to help advance each of their missions. For example,
764 partnership of tissue chip developers with the Comprehensive *in vitro* Proarrhythmia Assay
765 (CiPA, see Related links) initiative helps provide tools to fulfill CiPA's mission of engineering
766 assays for assessment of the proarrhythmic potential of new drugs with improved specificity
767 compared with current assays, while demonstrating the utility of tissue chips for toxicity
768 screening.
769
770 Another collaboration between the NIH and the Center for Advancement of Science in Space
771 (CASIS, see Related links) allows researchers to use the microgravity environment on the
772 International Space Station (ISS) to conduct biomedical research. The program, which partners
773 with the International Space Station National Laboratory (ISS-NL), is using microgravity as a tool
774 to investigate Earth-based disease pathologies such as formation of kidney stones that would
775 otherwise be difficult or take too long to model on Earth. Moreover, researchers and space
776 payload developers work collaboratively to adapt OoC platforms and make them robust enough
777 for rocket launch, spaceflight, integration into ISS facilities, and splash-down. This is leading to
778 advances in the technical engineering of robust platforms capable of higher throughput (>24
779 replicates running concurrently) with a much smaller footprint. The systems are turn-key

780 enough to be “astronaut-proof”, meaning that non-scientist workers (in this case astronauts,
781 most of whom are not trained in laboratory techniques) can perform the necessary
782 interventions – both in space and in the future on Earth in a variety of applications[159].
783

784 **[bH1] Box 2: Cell sourcing for 3D tissue engineering**

785 The common aphorism of "all models are wrong but some are useful" is apt when considering
786 cell sourcing for microphysiological systems (or any bioengineered tissue models). No cell
787 source is perfect; many have serious caveats; but even the most problematic cell source can
788 provide useful information if used appropriately based on the question being asked. Cells
789 seeded in tissue chips come from three main sources: commercially available cell lines; primary
790 cells from human donors; and induced pluripotent stem cell (iPSC)-derived sources.
791

792 ***[bH2] Commercially available cell lines:*** Cell lines should have extensive validation of purity and
793 viability when received from reliable sources (such as the American Type Culture Collection)
794 and are often proliferative as well as easy to culture and transfect. These cells have clear and
795 reliable culture protocols, generally respond in stable and predictable ways and will likely
796 contribute to high reproducibility. Commercially available cells can be excellent sources of hard-
797 to-find cell types, or when primary and iPSC sources are unavailable. However, these cell lines
798 are approximations for the primary cell types found *in vivo* and should be periodically evaluated
799 to see how far from the primary cell phenotype the new generations are straying.
800

801 ***[bH2] Primary cells:*** The clear advantage of using cells from human donors is that the cells
802 capture the phenotype (presumably genetically and functionally) of the mature adult state.
803 Primary cells can model disease pathologies when sourced from donors with certain diseases
804 and can accurately reflect clinical population variance in their phenotypes. However, because
805 genetic and epigenetic differences arise during a donor's lifetime, variability between donors or
806 batches can be hard to identify and track. For some primary tissues (for example: neural cells),
807 access from donors may not even be possible. In many cases, primary cells are available
808 because the tissue has been removed or biopsied for diagnostic purposes and can be displaying
809 pathological phenotypes. Primary cells also require specialized culture and media to retain their
810 phenotypes, which can be problematic in linked tissue chip systems, as a common media could
811 prove suboptimal for the different tissues. Finally, adult stem cells grown as organoids (for later

812 seeding in OoCs) only represent the epithelial component of the tissue, not the stroma or
813 vasculature, limiting their application.
814
815 ***[bH2] iPSCs:*** Stem cell-derived sources are a potential solution to cell sourcing difficulties for
816 tissue chips because they are potentially infinitely renewable and can be from either healthy or
817 diseased populations. These iPSCs provide huge potential for populating tissue chips because
818 individuals could have platforms created that model their tissues and disease phenotypes. This
819 also allows creation of isogenic cell lines for genetic disorders, in which the resulting iPSCs can
820 be genetically engineered to either harbor the disease-specific mutation or not, allowing
821 opportunities to study the genetic impact of a disorder with unparalleled specificity.
822
823 Drawbacks of iPSC-derived tissues include the immature or fetal phenotypes (for example:
824 cardiomyocytes; kidney; and liver) of the cells, which can limit their utility. The time and
825 resources needed for creation and passaging of cell lines, and later differentiation, is long (nine
826 months or more for some neural tissues) and expensive compared to the ease of buying
827 commercially available cells. Also, cells may retain an 'epigenetic memory' of their donor
828 tissues[160] depending on the number of passages, which can limit directed differentiation for
829 specific tissues.
830

831 **Figure legends**

832 Figure 1 |**Utility of OoCs in a variety of stages of drug development**

833 Drug development is a dynamic environment for data feedforward and feedback between
834 multiple stages and processes, being described as a 'dynamic map' [161]. These dynamic maps
835 provide a framework for understanding modern drug development and include activities and
836 processes such as Lead Identification, Clinical Research and Development and Regulatory
837 Review. OoCs can be informative in a number of these neighborhoods. On this schematic of an
838 OoC surrounded by the multiple stages and processes of drug development, green components
839 represent the known current or shortly predicted use of OoCs and blue components represent
840 the possible and predicted utility. Many OoCs are currently at the 'Basic science research stage'.
841 Use of OoCs in the 'Medical landscape' stage includes use for precision medicine and patient-
842 specific treatments. 'Clinical research and development' use would include patient subgroup
843 stratification and projects under the NIH "Clinical Trials on a Chip" program, as an example.
844 'Regulatory review' refers to IND and NDA data. 'Post marketing' refers to adverse drug
845 reaction reporting and drug repurposing efforts. References are included for examples of OoC
846 use in these areas. [PR: permissions for need to be included].

847

848 Figure 2 | **Examples of features and platform designs for organs on chips**

849 Diverse platform design and key design features for organs on chips allow a broad range of data
850 readouts which can be used for computational modeling as part of the drug discovery process.
851 A broad diversity of tissue platforms highlights key common features – the 3-dimensions for
852 tissue culture, inclusion of multiple cell types, and modeling of biomechanical forces that
853 recreate the in vivo environment.

854 a) Transwell systems allow barrier modeling and fluid flow across a permeable membrane for
855 media exchange and cell-cell interaction. In this example, Caco2 and mucus-
856 secreting HT29-MTX intestinal cells create the gut apical side, with immature dendritic cells
857 seeded on the basal side and left to mature, creating a barrier model of the gut. On the
858 right, barrier function of transwells can be measured by trans-epithelial electrical
859 resistance (TEER) or secretion of e.g. mucin from cells in both single and linked OoCs.
860 Adapted with permission from [140].

861 b) Platforms with diamond-shaped cell chambers (2mm wide by 1mm high) allow for seeding
862 with human endothelial colony-forming cell-derived endothelial cells (ECFC-EC, in green)
863 which self-organize into perfusable microvasculature, with cell media supplied via
864 microfluidic channels flowing from bottom to top. Seeding with colorectal cancer cells
865 (HCT116 cells, in blue) forms vascularized microtumors which can be used to screen
866 chemotherapeutics for safety and efficacy. Histology allows clear localization and
867 visualization of cell interactions, such as the vascularization of microtumors and the
868 perfusion of media through the system (rhodamine B dextran, in red). Adapted with
869 permission from [29].

870 c) A vascularized liver acinus model (vLAMPS – left) consisting of cells in collagen sandwiched
871 between three glass layers allows 3D layering of multiple liver cell types representing the
872 liver acinus. (Right) Oxygen zonation can be computationally modeled by calculating the
873 rate of media flow in the microfluidic channels, creating 3 distinct zones (oxygen rich;
874 intermediate; and oxygen-poor) on the platform which recreate the liver sinusoid and
875 establish a metabolic gradient similar to that seen *in vivo*. LECM; liver extracellular matrix.

876 PET; polyethylene terephthalate. LSECs; liver sinusoidal endothelial cells. Adapted with
877 permission from [62].
878 [PR: permissions for panels a, b, and c need to be included].
879

880 Figure 3 | **Examples of linked multi-organ systems, which can help understand systemic or off-**
881 **target drug effects and create “body-on-a-chip” systems.** The modules and media can be
882 linked by (A) pneumatic or electromagnetic pumps, (B and C) peristaltic flow, or (D) media
883 circulated by hydrostatic flow driven by gravity.

884 a) (Left) This female reproductive system MPS contains 5 tissue modules (ovary, cervix,
885 uterus, fallopian tube and liver) and models the hormonal profile of the female
886 menstrual cycle and pregnancy which can be useful for assessing female reproductive
887 toxicity. (Right) The modules are linked by a complex series of internal valves and pumps
888 under the tissue construct inserts and flow of tissue-specific media and hormones are
889 driven by pneumatic pumps powered by electromagnets. Adapted from [143].
890 b) (Left) A simplified schematic of a linked multi-organ system for investigating
891 doxorubicin-induced toxicity on liver, heart, bone, and various other tissues e.g. brain.
892 The platform consists of individual tissue constructs cultured in multiple modular
893 ‘inserts’, set into a platform with the same footprint as a standard 6-well laboratory
894 plate. In this example, 4 tissue types can be replicated in triplicate on a single plate.
895 (Right) Schematic of the side view of the platform. Underneath each tissue insert lies a
896 permeable membrane lined with endothelial cells, perfused by a recirculating vascular
897 medium driven by peristaltic pump. The system allows for optimal cell culture for each
898 tissue type as well as inclusion of common circulating factors such as immune cells,
899 hormones and exosomes. Adapted with permission from [162].
900 c) A robotic system with inbuilt microscope, peristaltic pump, and automatic fluid handling
901 named the ‘Interrogator’ can house up to 10 OoCs for PK/PD and PBMK modeling.
902 Reproduced with permission from [128].
903 d) This commercially available multi-organ system from Hesperos Inc. cultures liver, cardiac
904 and skeletal muscle and neurons on a microfluidic chip. Each tissue module is cultured
905 on a plate modified by proprietary surface chemistries to help cells adhere to the
906 surface and act as ECM, and media reservoirs contain a serum-free common medium
907 which is gravity-fed by placing the chip on a laboratory rocker. Cardiac, skeletal and
908 neuronal modules contain microelectrode arrays (MEA) to stimulate and record activity

909 in tissue subtypes. Adapted with permission from Schaffer C (November 30 2017) "3D-
910 Bioprinting Conference Showcases Versatility" Genetic Engineering and Biotechnology
911 News magazine Vol 37, No 21. Published by Mary Ann Liebert Inc. publishers.
912 https://www.genengnews.com/magazine/305/3d-bioprinting-conference-showcases-
913 versatility/
914 [PR: permissions for panels a, b, c, and d need to be included].
915 .

Glossary

extracellular matrix (ECM) – supporting network of macromolecules providing structural and biochemical support to surrounding cells. Promotes cell adhesion and cell-cell communication and produces biochemical cues for tissue growth and maintenance. The ECM is tissue-specific and in animal tissues consists of fibrous elements (collagen, elastin), and links proteins (laminin, fibronectin) and other molecules.

hydrogels - highly absorbent and hydrophilic biocompatible 3D polymer networks used to contain cells or drugs for tissue engineering applications. Can consist of natural (collagen, gelatin, agarose) or synthetic components and respond to environmental conditions such as pH. May have both liquid and solid properties. Other uses include wound dressings, contact lenses.

multi-electrode arrays (MEAs) – arrays of 10-1000s of tightly spaced microelectrical sensors designed to record from single cells to networks of cells at sub-millisecond timescales. Can also be used to stimulate cells with precise spatial and temporal characteristics. Used in electrically-excitable tissues such as cardiac, muscular, neural.

pharmacokinetic/pharmacodynamic (PK/PD) modeling – integration of pharmacokinetics (PK – movement of drugs through the body) and pharmacodynamics (PD – body's biological response to drugs) into a mathematical model describing dose-concentration-response relationships. Can be used to predict effect and efficacy of drug dosing over time.

physiologically-based pharmacokinetic (PBPK) modeling – mathematical modeling of body compartments (predefined organs or tissues) combined with known parameters of concentrations, quantities and transport between compartments used to predict absorption, distribution, metabolism and excretion (ADME) of synthetic or natural chemical substances within the body.

IND (Investigational New Drug) – An application submitted to the US Food and Drug Administration (FDA) to administer novel drug to humans. The first step in the drug review process, which includes information on animal studies, manufacturing protocols, and clinical and personnel protocols. Data gathered becomes part of the New Drug Application (NDA).

NDA (New Drug Application) – An application submitted to the US FDA requesting permission to sell and market a drug in the US. Information submitted includes data from the IND and is reviewed for safety and efficacy, benefit versus risks, appropriate labelling information, and manufacturing and processing methods.

Related Links

Defense Advanced Research Project Agency (DARPA) funded linked 10 organ system:
https://www.darpa.mil/program/microphysiological-systems

US National Institutes of Health (NIH), FDA and DARPA funded development of tissue chips to advance regulatory sciences: https://www.nih.gov/news-events/news-releases/nih-fda-announce-collaborative-initiative-fast-track-innovations-public

Tissue Chip Testing Centers: https://ncats.nih.gov/tissuechip/projects/centers
National Center for Advancing Translational Sciences (NCATS) Tissue Chips in Space:
https://ncats.nih.gov/tissuechip/projects/space

Good Manufacturing Practice guidelines: https://www.ecfr.gov/cgi-bin/text-idx?SID=cb7c830642b365274d824a432e118e77&mc=true&node=pt21.8.820&rgn=div5

European Union Reference Laboratory for Alternatives to Animal Testing European Centre for the Validation of Alternative (EURL ECVAM): https://ec.europa.eu/jrc/en/eurl/ecvam

Organisation for Economic Co-operation and Development (OECD) Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment: https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd-gd34.pdf

Organs on chips - 2017 market overview analysis by Yole Développement:
http://www.yole.fr/OrgansOnChips_Market.aspx#.XIP6dVNKiV4

The Gartner Hype Cycle for Emerging Technologies 2018:
https://www.gartner.com/smarterwithgartner/5-trends-emerge-in-gartner-hype-cycle-for-emerging-technologies-2018/

984 IQ Consortium: https://iqconsortium.org/

985

986 human Organ and Disease Model Technologies (hDMT): https://www.hdmt.technology/

987

988 ORCHID: https://h2020-orchid.eu/

989

990 Comprehensive *in vitro* Proarrhythmia Assay (CiPA): http://cipaproject.org/

991

992 The Center for Advancement of Science in Space (CASIS): https://www.iss-casis.org/

993

994 **Table 1. Key features of two-dimensional and three-dimensional engineered tissues.**

	Conventional 2D systems	3D systems	
		Organoid	Organ-on-chip
Production characteristics	Grown on rigid flat surfaces, often as a cellularly homogeneous monolayer	Embedded in hydrogels/suspended in 'hanging drops', and left to self-organize into multiple cell types	Multiple relevant cell types seeded into engineered chambers with perfusion and/or biomechanical forces included
Production complexity and speed	Generally straightforward and fast (minutes-days)	Generally straightforward, but slower (days-weeks) depending on cell sources	Variable complexity (depends on platform design), slower (days to weeks) depending on cell sources and required tissue maturation metrics
Level of control over cell architecture	High	Very low	High
Maturation of iPSC-derived cells allowed by platform*	Immature	Improved but still highly immature	Platform designs can improve and encourage cell maturity[164]
Resulting cell morphology	Unnatural, with limited ECM composition and contact with cells	Similar size and shape to *in vivo*, allows relevant ECM interaction during cell proliferation	Similar size and shape to *in vivo*, allows relevant ECM interaction throughout cell lifetime
Diffusion of signal factors and nutrients	Short distances possible	Ineffective transport to interior can cause cell death or immaturity	Allows precisely controlled temporal and spatial gradients
Vascularization or perfusion?	Not possible, generally perfusion via media change	Depends on cell types but likely creates non-functional vessels; externally perfused; can include fluid flow across tissue surfaces	Yes - by microfluidic channels or design which can include/create endothelialized vessels
High throughput feasibility?	Yes	Possibly, depending on tissue[165,166]	Depends on platform design; generally low to medium throughput
On-platform assay and analysis difficulty	Low difficulty, easy access to cells and readouts	Tissue function analyses possible; cell separation not possible	Real-time tissue/organ function analyses possible
Variability and *in vivo* relevance of resulting tissues in manufactured platform	Low variability and relevance - simple, homogeneous cultures	Can be high variability and low relevance as there is little control over resulting cell subtypes and location	Can be low variability and high relevance - allows high levels of control over cell type and placement

995 *immaturity of iPSC-derived cells still a general issue

996 **Table 2 – Examples of single tissue OoCs for toxicological assessment**

Tissue/Organ	Platform Characteristics	Challenge	Response	Reference
Liver "SQL-SAL"	Human hepatocytes, stellate, immune and endothelial cells are layered in glass and PDMS microfluidic chip. Fluorescent biosensors included. Survival to 28 days.	1. Troglitazone and nimesulide (hepatotoxic) 2. Trovafloxacin + LPS and levofloxacin + LPS (immune-mediated hepatotoxicity) 3. Methotrexate (fibrotic injury) 4. Caffeine (negative control)	1. Time and dose-dependent LDH release, apoptosis, plus decreased albumin and urea secretion 2. Increased LDH release and apoptosis with trovafloxacin + LPS but not levofloxacin + LPS 3. Increased fibrotic markers 4. No effect	Vernetti et al 2016[61]
Liver	Primary hepatocytes places across porous membrane from LSECs, +/- Kupffer and stellate cells. Rat, dog, human species comparisons possible.	1. Bosentan (cholestatic) 2. Acetaminophen (hepatotoxic) 3. Methotrexate (fibrotic injury)	1. Species-specific albumin decrease; correlated to clinical response in humans; bile salt transport inhibition 2. Glutathione and ATP depletion; formation of ROS; decreased albumin secretion 3. Lipid accumulation (steatosis) and fibrosis	Jang et al 2019[63]
Cardiac	Self-organized iPSC-derived cardiomyocytes in 3D microfluidic device	1. Isoproterenol (β-adrenergic agonist) 2. E-4031 (hERG blocker) 3. Verapamil (multi-ion channel blocker) 4. Metoprolol (β-adrenergic antagonist)	Cardiac beat frequencies in line with clinical data including dose-dependent changes and arrhythmias concordant with human cardiotoxicology data	Mathur et al 2015[64]
Kidney	Primary human kidney proximal tubule epithelial cells seeded to form a lumen in microfluidic platform	Polymyxin B	Increased KIM-1 and injury-associated miRNAs	Weber et al 2018[78]

997 ATP, adenosine triphosphate; hERG, Human ether-a-go-go-related potassium channel; KIM-1, kidney injury molecule 1; LSECs, liver sinusoidal
998 endothelial cells; LPS, lipopolysaccharide; LDH, lactate dehydrogenase; PDMS, polydimethylsiloxane; ROS, reactive oxygen species.
999

1000 ## References

1001

1002 1 Paul, S. M. *et al.* How to improve R&D productivity: the pharmaceutical industry's grand
1003 challenge. *Nature Reviews Drug Discovery* **9**, 203, doi:10.1038/nrd3078
1004 https://www.nature.com/articles/nrd3078#supplementary-information (2010).
1005 2 Scannell, J. W., Blanckley, A., Boldon, H. & Warrington, B. Diagnosing the decline in
1006 pharmaceutical R&D efficiency. *Nature Reviews Drug Discovery* **11**, 191,
1007 doi:10.1038/nrd3681
1008 https://www.nature.com/articles/nrd3681#supplementary-information (2012).
1009 3 Seok, J. *et al.* Genomic responses in mouse models poorly mimic human inflammatory
1010 diseases. *Proceedings of the National Academy of Sciences* **110**, 3507-3512,
1011 doi:10.1073/pnas.1222878110 (2013).
1012 4 Hay, M., Thomas, D. W., Craighead, J. L., Economides, C. & Rosenthal, J. Clinical
1013 development success rates for investigational drugs. *Nature Biotechnology* **32**, 40,
1014 doi:10.1038/nbt.2786
1015 https://www.nature.com/articles/nbt.2786#supplementary-information (2014).
1016 5 Waring, M. J. *et al.* An analysis of the attrition of drug candidates from four major
1017 pharmaceutical companies. *Nature Reviews Drug Discovery* **14**, 475,
1018 doi:10.1038/nrd4609
1019 https://www.nature.com/articles/nrd4609#supplementary-information (2015).
1020 6 Sweeney, L. M., Shuler, M. L., Babish, J. G. & Ghanem, A. A cell culture analogue of
1021 rodent physiology: Application to naphthalene toxicology. *Toxicology in Vitro* **9**, 307-
1022 316, doi:http://dx.doi.org/10.1016/0887-23s33(95)00007-U (1995).
1023 7 Sin, A. *et al.* The Design and Fabrication of Three-Chamber Microscale Cell Culture
1024 Analog Devices with Integrated Dissolved Oxygen Sensors. *Biotechnology Progress* **20**,
1025 338-345, doi:10.1021/bp034077d (2004).
1026 8 Huh, D. *et al.* Reconstituting Organ-Level Lung Functions on a Chip. *Science* **328**,
1027 1662-1668, doi:10.1126/science.1188302 (2010).

1028 **Early organ-on-chip paper recreating the alveolar-capillary interface of the human**
1029 **lung incorporating cyclic biomechanical stretch forces and showing replication of *in***
1030 ***vivo* responses.**
1031 9 Rossi, G., Manfrin, A. & Lutolf, M. P. Progress and potential in organoid research. *Nature*
1032 *Reviews Genetics* **19**, 671-687, doi:10.1038/s41576-018-0051-9 (2018).
1033 10 Ingber, D. E. Reverse Engineering Human Pathophysiology with Organs-on-Chips. *Cell*
1034 **164**, 1105-1109, doi:https://doi.org/10.1016/j.cell.2016.02.049 (2016).
1035 11 Pamies, D. *et al.* A human brain microphysiological system derived from induced
1036 pluripotent stem cells to study neurological diseases and toxicity. *ALTEX*,
1037 doi:10.14573/altex.1609122 (2016).
1038 12 Plummer, S. *et al.* A Human iPSC-derived 3D platform using primary brain cancer cells to
1039 study drug development and personalized medicine. *Scientific Reports* **9**, 1407,
1040 doi:10.1038/s41598-018-38130-0 (2019).
1041 13 Schwartz, M. P. *et al.* Human pluripotent stem cell-derived neural constructs for
1042 predicting neural toxicity. *Proceedings of the National Academy of Sciences of the United*
1043 *States of America* **112**, 12516-12521, doi:10.1073/pnas.1516645112 (2015).
1044 14 Rothbauer, M., Rosser, J. M., Zirath, H. & Ertl, P. Tomorrow today: organ-on-a-chip
1045 advances towards clinically relevant pharmaceutical and medical in vitro models.
1046 *Current Opinion in Biotechnology* **55**, 81-86,
1047 doi:https://doi.org/10.1016/j.copbio.2018.08.009 (2019).
1048 15 Kasendra, M. *et al.* Development of a primary human Small Intestine-on-a-Chip using
1049 biopsy-derived organoids. *Scientific Reports* **8**, 2871, doi:10.1038/s41598-018-21201-7
1050 (2018).
1051 16 Ramme, A. P. *et al.* Towards an autologous iPSC-derived patient-on-a-chip. *bioRxiv*,
1052 376970, doi:10.1101/376970 (2018).
1053 17 Vatine, G. D. *et al.* Human iPSC-Derived Blood-Brain Barrier Chips Enable Disease
1054 Modeling and Personalized Medicine Applications. *Cell Stem Cell* **24**, 995-1005.e1006,
1055 doi:https://doi.org/10.1016/j.stem.2019.05.011 (2019).

1056 18 Caliari, S. R. & Burdick, J. A. A practical guide to hydrogels for cell culture. *Nature*
1057 *Methods* **13**, 405, doi:10.1038/nmeth.3839
1058 https://www.nature.com/articles/nmeth.3839#supplementary-information (2016).
1059 19 Crapo, P. M., Tottey, S., Slivka, P. F. & Badylak, S. F. Effects of Biologic Scaffolds on
1060 Human Stem Cells and Implications for CNS Tissue Engineering. *Tissue Engineering Part*
1061 *A* **20**, 313-323, doi:10.1089/ten.tea.2013.0186 (2013).
1062 20 Safaee, H. *et al.* Tethered Jagged-1 Synergizes with Culture Substrate Stiffness to
1063 Modulate Notch-Induced Myogenic Progenitor Differentiation. *Cellular and Molecular*
1064 *Bioengineering* **10**, 501-513, doi:10.1007/s12195-017-0506-7 (2017).
1065 21 Trappmann, B. *et al.* Matrix degradability controls multicellularity of 3D cell migration.
1066 *Nature Communications* **8**, 371, doi:10.1038/s41467-017-00418-6 (2017).
1067 22 Shin, D. S. *et al.* Synthesis of microgel sensors for spatial and temporal monitoring of
1068 protease activity. *ACS biomaterials science & engineering* **4**, 378-387,
1069 doi:10.1021/acsbiomaterials.7b00017 (2018).
1070 23 Wikswo, J. P. *et al.* Engineering Challenges for Instrumenting and Controlling Integrated
1071 Organ-on-Chip Systems. *IEEE Transactions on Biomedical Engineering* **60**, 682-690,
1072 doi:10.1109/TBME.2013.2244891 (2013).
1073 24 Wikswo, J. P. *et al.* Scaling and systems biology for integrating multiple organs-on-a-
1074 chip. *Lab on a Chip* **13**, 3496-3511, doi:10.1039/C3LC50243K (2013).
1075 25 Johnson, B. P. *et al.* Hepatocyte circadian clock controls acetaminophen bioactivation
1076 through NADPH-cytochrome P450 oxidoreductase. *Proceedings of the National*
1077 *Academy of Sciences* **111**, 18757, doi:10.1073/pnas.1421708111 (2014).
1078 26 Bass, J. & Takahashi, J. S. Circadian Integration of Metabolism and Energetics. *Science*
1079 **330**, 1349, doi:10.1126/science.1195027 (2010).
1080 27 Cyr, K. J., Avaldi, O. M. & Wikswo, J. P. Circadian hormone control in a human-on-a-chip:
1081 In vitro biology's ignored component? *Experimental Biology and Medicine* **242**, 1714-
1082 1731, doi:10.1177/1535370217732766 (2017).
1083 28 Chang, S.-Y. *et al.* Human liver-kidney model elucidates the mechanisms of aristolochic
1084 acid nephrotoxicity. *JCI Insight* **2**, doi:10.1172/jci.insight.95978 (2017).

1085 **Physically coupled liver and kidney OoCs used to uncover the mechanism of**
1086 **nephrotoxicity of aristolochic acid via bioactivation in the liver, showing utility of**
1087 **coupled OoCs for understanding toxicities.**
1088 29 Phan, D. T. T. *et al.* A vascularized and perfused organ-on-a-chip platform for large-scale
1089 drug screening applications. *Lab on a Chip* **17**, 511-520, doi:10.1039/C6LC01422D
1090 (2017).
1091 30 Zhang, C., Zhao, Z., Abdul Rahim, N. A., van Noort, D. & Yu, H. Towards a human-on-chip:
1092 Culturing multiple cell types on a chip with compartmentalized microenvironments. *Lab*
1093 *on a Chip* **9**, 3185-3192, doi:10.1039/B915147H (2009).
1094 31 Materne, E.-M. *et al.* The Multi-organ Chip - A Microfluidic Platform for Long-term
1095 Multi-tissue Coculture. *JoVE*, e52526, doi:doi:10.3791/52526 (2015).
1096 32 Maschmeyer, I. *et al.* A four-organ-chip for interconnected long-term co-culture of
1097 human intestine, liver, skin and kidney equivalents. *Lab on a Chip* **15**, 2688-2699,
1098 doi:10.1039/C5LC00392J (2015).
1099 **Development of a multi-organ integrated OoC with pulsatile flow which reliably**
1100 **supports homeostasis over 28 days and allows ADME profiling and repeated dose drug**
1101 **testing.**
1102 33 Tsamandouras, N. *et al.* Integrated Gut and Liver Microphysiological Systems for
1103 Quantitative In Vitro Pharmacokinetic Studies. *The AAPS Journal* **19**, 1499-1512,
1104 doi:10.1208/s12248-017-0122-4 (2017).
1105 34 Oleaga, C. *et al.* Multi-Organ toxicity demonstration in a functional human in vitro
1106 system composed of four organs. *Scientific Reports* **6**, 20030, doi:10.1038/srep20030
1107 https://www.nature.com/articles/srep20030#supplementary-information (2016).
1108 **Development of a multi-organ pumpless OoC with common medium under serum-free**
1109 **conditions, maintaining tissues to 14 days and profiling accurate acute responses to**
1110 **therapeutic compounds.**
1111 35 Oleaga, C. *et al.* Human-on-a-Chip Systems: Long-Term Electrical and Mechanical
1112 Function Monitoring of a Human-on-a-Chip System (Adv. Funct. Mater. 8/2019).
1113 *Advanced Functional Materials* **29**, 1970049, doi:10.1002/adfm.201970049 (2019).

1114 36 Stone, H. A., Stroock, A. D. & Ajdari, A. Engineering Flows in Small Devices: Microfluidics
1115 Toward a Lab-on-a-Chip. *Annual Review of Fluid Mechanics* **36**, 381-411,
1116 doi:10.1146/annurev.fluid.36.050802.122124 (2004).
1117 37 Lochovsky, C., Yasotharan, S. & Günther, A. Bubbles no more: in-plane trapping and
1118 removal of bubbles in microfluidic devices. *Lab on a Chip* **12**, 595-601,
1119 doi:10.1039/C1LC20817A (2012).
1120 38 Kaarj, K. & Yoon, J.-Y. Methods of Delivering Mechanical Stimuli to Organ-on-a-Chip.
1121 *Micromachines* **10**, doi:10.3390/mi10100700 (2019).
1122 39 Kim, H. J., Huh, D., Hamilton, G. & Ingber, D. E. Human gut-on-a-chip inhabited by
1123 microbial flora that experiences intestinal peristalsis-like motions and flow. *Lab on a*
1124 *Chip* **12**, 2165-2174, doi:10.1039/C2LC40074J (2012).
1125 40 Maoz, B. M. *et al.* Organs-on-Chips with combined multi-electrode array and
1126 transepithelial electrical resistance measurement capabilities. *Lab on a Chip* **17**, 2294-
1127 2302, doi:10.1039/C7LC00412E (2017).
1128 41 Herland, A. *et al.* Distinct Contributions of Astrocytes and Pericytes to
1129 Neuroinflammation Identified in a 3D Human Blood-Brain Barrier on a Chip. *PLOS ONE*
1130 **11**, e0150360, doi:10.1371/journal.pone.0150360 (2016).
1131 42 Musah, S. *et al.* Mature induced-pluripotent-stem-cell-derived human podocytes
1132 reconstitute kidney glomerular-capillary-wall function on a chip. *Nature biomedical*
1133 *engineering* **1**, 0069, doi:10.1038/s41551-017-0069 (2017).
1134 43 Agarwal, A., Goss, J. A., Cho, A., McCain, M. L. & Parker, K. K. Microfluidic heart on a chip
1135 for higher throughput pharmacological studies. *Lab on a chip* **13**, 3599-3608,
1136 doi:10.1039/c3lc50350j (2013).
1137 44 Nunes, S. S. *et al.* Biowire: a platform for maturation of human pluripotent stem cell–
1138 derived cardiomyocytes. *Nature Methods* **10**, 781, doi:10.1038/nmeth.2524
1139 https://www.nature.com/articles/nmeth.2524#supplementary-information (2013).
1140 45 Lee-Montiel, F. T. *et al.* Control of oxygen tension recapitulates zone-specific functions
1141 in human liver microphysiology systems. *Experimental Biology and Medicine* **242**, 1617-
1142 1632, doi:10.1177/1535370217703978 (2017).

1143 46 Senutovitch, N. *et al.* Fluorescent protein biosensors applied to microphysiological
1144 systems. *Experimental Biology and Medicine* **240**, 795-808,
1145 doi:10.1177/1535370215584934 (2015).
1146 47 Zhang, Y. S. *et al.* Multisensor-integrated organs-on-chips platform for automated and
1147 continual in situ monitoring of organoid behaviors. *Proceedings of the National*
1148 *Academy of Sciences* **114**, E2293 (2017).
1149 **Advanced multi-organ platform for automated control and biosensing over multiple**
1150 **days , including pH, O2, temperature, protein biomarker presence and microscopes for**
1151 **imaging. Demonstrated cardio- and hepatotoxicity from chronic and acute drug**
1152 **dosing.**
1153 48 Toepke, M. W. & Beebe, D. J. PDMS absorption of small molecules and consequences in
1154 microfluidic applications. *Lab on a Chip* **6**, 1484-1486, doi:10.1039/B612140C (2006).
1155 49 Markov, D. A., Lillie, E. M., Garbett, S. P. & McCawley, L. J. Variation in diffusion of gases
1156 through PDMS due to plasma surface treatment and storage conditions. *Biomedical*
1157 *microdevices* **16**, 91-96, doi:10.1007/s10544-013-9808-2 (2014).
1158 50 Tan, S. H., Nguyen, N.-T., Chua, Y. C. & Kang, T. G. Oxygen plasma treatment for reducing
1159 hydrophobicity of a sealed polydimethylsiloxane microchannel. *Biomicrofluidics* **4**,
1160 032204, doi:10.1063/1.3466882 (2010).
1161 51 Chuah, Y. J. *et al.* Simple surface engineering of polydimethylsiloxane with
1162 polydopamine for stabilized mesenchymal stem cell adhesion and multipotency.
1163 *Scientific Reports* **5**, 18162, doi:10.1038/srep18162
1164 https://www.nature.com/articles/srep18162#supplementary-information (2015).
1165 52 van Meer, B. J. *et al.* Small molecule absorption by PDMS in the context of drug
1166 response bioassays. *Biochemical and Biophysical Research Communications* **482**, 323-
1167 328, doi:https://doi.org/10.1016/j.bbrc.2016.11.062 (2017).
1168 53 Regehr, K. J. *et al.* Biological implications of polydimethylsiloxane-based microfluidic cell
1169 culture. *Lab on a chip* **9**, 2132-2139, doi:10.1039/b903043c (2009).

1170 54 Suntharalingam, G. *et al.* Cytokine Storm in a Phase 1 Trial of the Anti-CD28 Monoclonal
1171 Antibody TGN1412. *New England Journal of Medicine* **355**, 1018-1028,
1172 doi:10.1056/NEJMoa063842 (2006).
1173 55 Kaur, R., Sidhu, P. & Singh, S. What failed BIA 10-2474 Phase I clinical trial? Global
1174 speculations and recommendations for future Phase I trials. *Journal of pharmacology &*
1175 *pharmacotherapeutics* **7**, 120-126, doi:10.4103/0976-500X.189661 (2016).
1176 56 Fowler, S. *et al.* Microphysiological systems for ADME-related applications: current
1177 status and recommendations for system development and characterization. *Lab on a*
1178 *Chip* **20**, 446-467, doi:10.1039/C9LC00857H (2020).
1179 57 Fabre, K. *et al.* Introduction to a manuscript series on the characterization and use of
1180 microphysiological systems (MPS) in pharmaceutical safety and ADME applications. *Lab*
1181 *on a Chip* **20**, 1049-1057, doi:10.1039/C9LC01168D (2020).
1182 58 Rudmann, D. G. The Emergence of Microphysiological Systems (Organs-on-chips) as
1183 Paradigm-changing Tools for Toxicologic Pathology. *Toxicologic Pathology* **47**, 4-10,
1184 doi:10.1177/0192623318809065 (2018).
1185 59 Gerets, H. H. J. *et al.* Characterization of primary human hepatocytes, HepG2 cells, and
1186 HepaRG cells at the mRNA level and CYP activity in response to inducers and their
1187 predictivity for the detection of human hepatotoxins. *Cell biology and toxicology* **28**, 69-
1188 87, doi:10.1007/s10565-011-9208-4 (2012).
1189 60 Heslop, J. A. *et al.* Mechanistic evaluation of primary human hepatocyte culture using
1190 global proteomic analysis reveals a selective dedifferentiation profile. *Archives of*
1191 *Toxicology* **91**, 439-452, doi:10.1007/s00204-016-1694-y (2017).
1192 61 Vernetti, L. A. *et al.* A human liver microphysiology platform for investigating physiology,
1193 drug safety, and disease models. *Experimental Biology and Medicine* **241**, 101-114,
1194 doi:10.1177/1535370215592121 (2016).
1195 62 Li, X., George, S. M., Vernetti, L., Gough, A. H. & Taylor, D. L. A glass-based, continuously
1196 zonated and vascularized human liver acinus microphysiological system (vLAMPS)
1197 designed for experimental modeling of diseases and ADME/TOX. *Lab on a Chip* **18**, 2614-
1198 2631, doi:10.1039/C8LC00418H (2018).

1199 63 Jang, K.-J. *et al.* Reproducing human and cross-species drug toxicities using a Liver-Chip.
1200 *Science Translational Medicine* **11**, eaax5516, doi:10.1126/scitranslmed.aax5516 (2019).
1201 **First paper to compare rat, dog and human microfluidic multi-cellular liver chips after**
1202 **exposure to hepatotoxic compounds. A fibrotic liver model showed species**
1203 **differences highlighting species-specific differences in drug metabolism and toxicity.**
1204 64 Mathur, A. *et al.* Human iPSC-based Cardiac Microphysiological System For Drug
1205 Screening Applications. *Scientific Reports* **5**, 8883, doi:10.1038/srep08883
1206 http://www.nature.com/articles/srep08883#supplementary-information (2015).
1207 65 Ahn, S. *et al.* Mussel-inspired 3D fiber scaffolds for heart-on-a-chip toxicity studies of
1208 engineered nanomaterials. *Analytical and Bioanalytical Chemistry* **410**, 6141-6154,
1209 doi:10.1007/s00216-018-1106-7 (2018).
1210 66 Marsano, A. *et al.* Beating heart on a chip: a novel microfluidic platform to generate
1211 functional 3D cardiac microtissues. *Lab on a Chip* **16**, 599-610, doi:10.1039/C5LC01356A
1212 (2016).
1213 67 Lind, J. U. *et al.* Instrumented cardiac microphysiological devices via multimaterial three-
1214 dimensional printing. *Nature Materials* **16**, 303, doi:10.1038/nmat4782
1215 https://www.nature.com/articles/nmat4782#supplementary-information (2016).
1216 68 Capulli, A. K., MacQueen, L. A., O'Connor, B. B., Dauth, S. & Parker, K. K. Acute pergolide
1217 exposure stiffens engineered valve interstitial cell tissues and reduces contractility in
1218 vitro. *Cardiovascular Pathology* **25**, 316-324,
1219 doi:http://dx.doi.org/10.1016/j.carpath.2016.04.004 (2016).
1220 69 Goversen, B., van der Heyden, M. A. G., van Veen, T. A. B. & de Boer, T. P. The immature
1221 electrophysiological phenotype of iPSC-CMs still hampers in vitro drug screening: Special
1222 focus on IK1. *Pharmacology & Therapeutics* **183**, 127-136,
1223 doi:https://doi.org/10.1016/j.pharmthera.2017.10.001 (2018).
1224 70 Sheehy, S. P. *et al.* Toward improved myocardial maturity in an organ-on-chip platform
1225 with immature cardiac myocytes. *Experimental Biology and Medicine* **242**, 1643-1656,
1226 doi:10.1177/1535370217701006 (2017).

1227 71 Ronaldson-Bouchard, K. *et al.* Advanced maturation of human cardiac tissue grown from
1228 pluripotent stem cells. *Nature* **556**, 239-243, doi:10.1038/s41586-018-0016-3 (2018).
1229 **OoC used to show enhanced maturation of stem cell-derived cardiac tissues in 3D**
1230 **when subject to electrical stimulation protocols, addressing a key challenge in cardiac**
1231 **stem cell differentiation.**
1232 72 Zhao, Y. *et al.* A Platform for Generation of Chamber-Specific Cardiac Tissues and
1233 Disease Modeling. *Cell* **176**, 913-927.e918,
1234 doi:https://doi.org/10.1016/j.cell.2018.11.042 (2019).
1235 73 Mills, R. J. *et al.* Functional screening in human cardiac organoids reveals a metabolic
1236 mechanism for cardiomyocyte cell cycle arrest. *Proceedings of the National Academy of*
1237 *Sciences* **114**, E8372, doi:10.1073/pnas.1707316114 (2017).
1238 74 Giacomelli, E. *et al.* Human-iPSC-Derived Cardiac Stromal Cells Enhance Maturation in
1239 3D Cardiac Microtissues and Reveal Non-cardiomyocyte Contributions to Heart Disease.
1240 *Cell Stem Cell* **26**, 862-879.e811, doi:10.1016/j.stem.2020.05.004 (2020).
1241 75 Jang, K.-J. *et al.* Human kidney proximal tubule-on-a-chip for drug transport and
1242 nephrotoxicity assessment. *Integrative Biology* **5**, 1119-1129, doi:10.1039/C3IB40049B
1243 (2013).
1244 76 Kim, S. *et al.* Pharmacokinetic profile that reduces nephrotoxicity of gentamicin in a
1245 perfused kidney-on-a-chip. *Biofabrication* **8**, 015021, doi:10.1088/1758-
1246 5090/8/1/015021 (2016).
1247 77 Weber, E. J. *et al.* Development of a microphysiological model of human kidney proximal
1248 tubule function. *Kidney International* **90**, 627-637, doi:10.1016/j.kint.2016.06.011
1249 (2016).
1250 78 Weber, E. J. *et al.* Human kidney on a chip assessment of polymyxin antibiotic
1251 nephrotoxicity. *JCI Insight* **3**, doi:10.1172/jci.insight.123673 (2018).
1252 79 Kim, H. J. & Ingber, D. E. Gut-on-a-Chip microenvironment induces human intestinal cells
1253 to undergo villus differentiation. *Integrative Biology* **5**, 1130-1140,
1254 doi:10.1039/C3IB40126J (2013).

1255 80 Workman, M. J. *et al.* Engineered human pluripotent-stem-cell-derived intestinal tissues
1256 with a functional enteric nervous system. *Nature Medicine* **23**, 49, doi:10.1038/nm.4233
1257 https://www.nature.com/articles/nm.4233#supplementary-information (2016).
1258 81 Williamson, I. A. *et al.* A High-Throughput Organoid Microinjection Platform to Study
1259 Gastrointestinal Microbiota and Luminal Physiology. *Cellular and Molecular*
1260 *Gastroenterology and Hepatology* **6**, 301-319,
1261 doi:https://doi.org/10.1016/j.jcmgh.2018.05.004 (2018).
1262 82 Shah, P. *et al.* A microfluidics-based in vitro model of the gastrointestinal human–
1263 microbe interface. *Nature Communications* **7**, 11535, doi:10.1038/ncomms11535
1264 https://www.nature.com/articles/ncomms11535#supplementary-information (2016).
1265 83 Workman, M. J. *et al.* Enhanced Utilization of Induced Pluripotent Stem Cell–Derived
1266 Human Intestinal Organoids Using Microengineered Chips. *Cellular and Molecular*
1267 *Gastroenterology and Hepatology* **5**, 669-677.e662,
1268 doi:https://doi.org/10.1016/j.jcmgh.2017.12.008 (2018).
1269 84 Tovaglieri, A. *et al.* Species-specific enhancement of enterohemorrhagic E. coli
1270 pathogenesis mediated by microbiome metabolites. *Microbiome* **7**, 43,
1271 doi:10.1186/s40168-019-0650-5 (2019).
1272 85 Jalili-Firoozinezhad, S. *et al.* A complex human gut microbiome cultured in an anaerobic
1273 intestine-on-a-chip. *Nature Biomedical Engineering* **3**, 520-531, doi:10.1038/s41551-
1274 019-0397-0 (2019).
1275 86 Benam, K. H. *et al.* in *3D Cell Culture: Methods and Protocols* (ed Zuzana Koledova) 345-
1276 365 (Springer New York, 2017).
1277 87 Benam, K. H. *et al.* Matched-Comparative Modeling of Normal and Diseased Human
1278 Airway Responses Using a Microengineered Breathing Lung Chip. *Cell Systems* **3**, 456-
1279 466.e454, doi:10.1016/j.cels.2016.10.003 (2016).
1280 88 Blundell, C. *et al.* Placental Drug Transport-on-a-Chip: A Microengineered In Vitro Model
1281 of Transporter-Mediated Drug Efflux in the Human Placental Barrier. *Advanced*
1282 *Healthcare Materials* **7**, 1700786, doi:10.1002/adhm.201700786 (2018).

1283 89 Yin, F. *et al.* A 3D human placenta-on-a-chip model to probe nanoparticle exposure at
1284 the placental barrier. *Toxicology in Vitro* **54**, 105-113,
1285 doi:https://doi.org/10.1016/j.tiv.2018.08.014 (2019).
1286 90 Sobrino, A. *et al.* 3D microtumors in vitro supported by perfused vascular networks.
1287 *Scientific Reports* **6**, 31589, doi:10.1038/srep31589 (2016).
1288 91 Barrile, R. *et al.* Organ-on-Chip Recapitulates Thrombosis Induced by an anti-CD154
1289 Monoclonal Antibody: Translational Potential of Advanced Microengineered Systems.
1290 *Clinical Pharmacology & Therapeutics* **104**, 1240-1248, doi:10.1002/cpt.1054 (2018).
1291 92 Cook, D. *et al.* Lessons learned from the fate of AstraZeneca's drug pipeline: a five-
1292 dimensional framework. *Nat Rev Drug Discov* **13**, 419-431, doi:10.1038/nrd4309 (2014).
1293 93 Horton, R. E. *et al.* Angiotensin II Induced Cardiac Dysfunction on a Chip. *PLOS ONE* **11**,
1294 e0146415, doi:10.1371/journal.pone.0146415 (2016).
1295 94 Hinson, J. T. *et al.* Titin mutations in iPS cells define sarcomere insufficiency as a cause of
1296 dilated cardiomyopathy. *Science* **349**, 982-986, doi:10.1126/science.aaa5458 (2015).
1297 95 Nesmith, A. P., Agarwal, A., McCain, M. L. & Parker, K. K. Human airway musculature on
1298 a chip: an in vitro model of allergic asthmatic bronchoconstriction and bronchodilation.
1299 *Lab on a Chip* **14**, 3925-3936, doi:10.1039/C4LC00688G (2014).
1300 96 van der Meer, A. D., Orlova, V. V., ten Dijke, P., van den Berg, A. & Mummery, C. L.
1301 Three-dimensional co-cultures of human endothelial cells and embryonic stem cell-
1302 derived pericytes inside a microfluidic device. *Lab on a Chip* **13**, 3562-3568,
1303 doi:10.1039/C3LC50435B (2013).
1304 97 Cruz, N. M. *et al.* Organoid cystogenesis reveals a critical role of microenvironment in
1305 human polycystic kidney disease. *Nature Materials* **16**, 1112, doi:10.1038/nmat4994
1306 https://www.nature.com/articles/nmat4994#supplementary-information (2017).
1307 98 Faal, T. *et al.* Induction of Mesoderm and Neural Crest-Derived Pericytes from Human
1308 Pluripotent Stem Cells to Study Blood-Brain Barrier Interactions. *Stem Cell Reports* **12**,
1309 451-460, doi:https://doi.org/10.1016/j.stemcr.2019.01.005 (2019).
1310 99 Rooney, G. E. *et al.* Human iPS Cell-Derived Neurons Uncover the Impact of Increased
1311 Ras Signaling in Costello Syndrome. *The Journal of Neuroscience* **36**, 142 (2016).

1312 100 Atchison, L., Zhang, H., Cao, K. & Truskey, G. A. A Tissue Engineered Blood Vessel Model
1313 of Hutchinson-Gilford Progeria Syndrome Using Human iPSC-derived Smooth Muscle
1314 Cells. *Scientific Reports* **7**, 8168, doi:10.1038/s41598-017-08632-4 (2017).
1315 101 Wang, G. *et al.* Modeling the mitochondrial cardiomyopathy of Barth syndrome with
1316 induced pluripotent stem cell and heart-on-chip technologies. *Nat Med* **20**, 616-623,
1317 doi:10.1038/nm.3545
1318 http://www.nature.com/nm/journal/v20/n6/abs/nm.3545.html#supplementary-information
1319 (2014).
1320 **Rare pediatric disease modeled on OoC and mechanism of pathology uncovered using**
1321 **gene editing techniques.**
1322 102 Ben Jehuda, R., Shemer, Y. & Binah, O. Genome Editing in Induced Pluripotent Stem
1323 Cells using CRISPR/Cas9. *Stem Cell Reviews and Reports* **14**, 323-336,
1324 doi:10.1007/s12015-018-9811-3 (2018).
1325 103 Nguyen, D.-H. T. *et al.* Biomimetic model to reconstitute angiogenic sprouting
1326 morphogenesis in vitro. *Proceedings of the National Academy of Sciences* **110**, 6712-
1327 6717, doi:10.1073/pnas.1221526110 (2013).
1328 104 Montanez-Sauri, S. I., Sung, K. E., Berthier, E. & Beebe, D. J. Enabling screening in 3D
1329 microenvironments: probing matrix and stromal effects on the morphology and
1330 proliferation of T47D breast carcinoma cells. *Integrative Biology* **5**, 631-640,
1331 doi:10.1039/c3ib20225a (2013).
1332 105 Zervantonakis, I. K. *et al.* Three-dimensional microfluidic model for tumor cell
1333 intravasation and endothelial barrier function. *Proceedings of the National Academy of*
1334 *Sciences* **109**, 13515, doi:10.1073/pnas.1210182109 (2012).
1335 106 Jeon, J. S. *et al.* Human 3D vascularized organotypic microfluidic assays to study breast
1336 cancer cell extravasation. *Proceedings of the National Academy of Sciences* **112**, 214-
1337 219, doi:10.1073/pnas.1417115112 (2015).
1338 107 Clark, A. M. *et al.* A Model of Dormant-Emergent Metastatic Breast Cancer Progression
1339 Enabling Exploration of Biomarker Signatures. *Molecular & Cellular*
1340 *Proteomics* **17**, 619, doi:10.1074/mcp.RA117.000370 (2018).

1341 108 Shirure, V. S. *et al.* Tumor-on-a-chip platform to investigate progression and drug
1342 sensitivity in cell lines and patient-derived organoids. *Lab on a Chip* **18**, 3687-3702,
1343 doi:10.1039/C8LC00596F (2018).
1344 109 Regier, M. C. *et al.* Transitions from mono- to co- to tri-culture uniquely affect gene
1345 expression in breast cancer, stromal, and immune compartments. *Biomedical*
1346 *Microdevices* **18**, 70, doi:10.1007/s10544-016-0083-x (2016).
1347 110 Marturano-Kruik, A. *et al.* Human bone perivascular niche-on-a-chip for studying
1348 metastatic colonization. *Proceedings of the National Academy of Sciences* **115**, 1256,
1349 doi:10.1073/pnas.1714282115 (2018).
1350 111 Kim, S., Lee, H., Chung, M. & Jeon, N. L. Engineering of functional, perfusable 3D
1351 microvascular networks on a chip. *Lab on a Chip* **13**, 1489-1500,
1352 doi:10.1039/C3LC41320A (2013).
1353 112 Miller, C. P., Tsuchida, C., Zheng, Y., Himmelfarb, J. & Akilesh, S. A 3D Human Renal Cell
1354 Carcinoma-on-a-Chip for the Study of Tumor Angiogenesis. *Neoplasia* **20**, 610-620,
1355 doi:https://doi.org/10.1016/j.neo.2018.02.011 (2018).
1356 113 Hassell, B. A. *et al.* Human Organ Chip Models Recapitulate Orthotopic Lung Cancer
1357 Growth, Therapeutic Responses, and Tumor Dormancy In Vitro. *Cell Reports* **21**, 508-
1358 516, doi:https://doi.org/10.1016/j.celrep.2017.09.043 (2017).
1359 114 Lee, J.-H. *et al.* Microfluidic co-culture of pancreatic tumor spheroids with stellate cells
1360 as a novel 3D model for investigation of stroma-mediated cell motility and drug
1361 resistance. *Journal of Experimental & Clinical Cancer Research* **37**, 4,
1362 doi:10.1186/s13046-017-0654-6 (2018).
1363 115 Jeong, S.-Y., Lee, J.-H., Shin, Y., Chung, S. & Kuh, H.-J. Co-Culture of Tumor Spheroids and
1364 Fibroblasts in a Collagen Matrix-Incorporated Microfluidic Chip Mimics Reciprocal
1365 Activation in Solid Tumor Microenvironment. *PLOS ONE* **11**, e0159013,
1366 doi:10.1371/journal.pone.0159013 (2016).
1367 116 Rizvi, I. *et al.* Flow induces epithelial-mesenchymal transition, cellular heterogeneity and
1368 biomarker modulation in 3D ovarian cancer nodules. *Proceedings of the National*
1369 *Academy of Sciences* **110**, E1974, doi:10.1073/pnas.1216989110 (2013).

1370 117 Li, R. *et al.* Macrophage-Secreted TNFα and TGFβ1 Influence Migration Speed and
1371 Persistence of Cancer Cells in 3D Tissue Culture via Independent Pathways. *Cancer*
1372 *Research* **77**, 279, doi:10.1158/0008-5472.CAN-16-0442 (2017).
1373 118 Wang, N. *et al.* 3D microfluidic in vitro model and bioinformatics integration to study the
1374 effects of Spatholobi Caulis tannin in cervical cancer. *Scientific Reports* **8**, 12285,
1375 doi:10.1038/s41598-018-29848-y (2018).
1376 119 Low, L. A. & Tagle, D. A. Tissue chips to aid drug development and modeling for rare
1377 diseases. *Expert Opin Orphan D* **4**, 1113-1121, doi:10.1080/21678707.2016.1244479
1378 (2016).
1379 120 Shik Mun, K. *et al.* Patient-derived pancreas-on-a-chip to model cystic fibrosis-related
1380 disorders. *Nature Communications* **10**, 3124, doi:10.1038/s41467-019-11178-w (2019).
1381 121 Shahjalal, H. M., Abdal Dayem, A., Lim, K. M., Jeon, T.-i. & Cho, S.-G. Generation of
1382 pancreatic β cells for treatment of diabetes: advances and challenges. *Stem Cell*
1383 *Research & Therapy* **9**, 355, doi:10.1186/s13287-018-1099-3 (2018).
1384 122 Takebe, T., Zhang, B. & Radisic, M. Synergistic Engineering: Organoids Meet Organs-on-
1385 a-Chip. *Cell Stem Cell* **21**, 297-300, doi:https://doi.org/10.1016/j.stem.2017.08.016
1386 (2017).
1387 123 Park, S. E., Georgescu, A. & Huh, D. Organoids-on-a-chip. *Science* **364**, 960,
1388 doi:10.1126/science.aaw7894 (2019).
1389 124 Takebe, T. *et al.* Vascularized and Complex Organ Buds from Diverse Tissues via
1390 Mesenchymal Cell-Driven Condensation. *Cell Stem Cell* **16**, 556-565,
1391 doi:https://doi.org/10.1016/j.stem.2015.03.004 (2015).
1392 125 Zhang, Y. S. *et al.* Bioprinting 3D microfibrous scaffolds for engineering endothelialized
1393 myocardium and heart-on-a-chip. *Biomaterials* **110**, 45-59,
1394 doi:https://doi.org/10.1016/j.biomaterials.2016.09.003 (2016).
1395 126 Park, D., Lee, J., Chung, J. J., Jung, Y. & Kim, S. H. Integrating Organs-on-Chips:
1396 Multiplexing, Scaling, Vascularization, and Innervation. *Trends in Biotechnology*,
1397 doi:https://doi.org/10.1016/j.tibtech.2019.06.006 (2019).

1398 127 Edington, C. D. *et al.* Interconnected Microphysiological Systems for Quantitative
1399 Biology and Pharmacology Studies. *Scientific Reports* **8**, 4530, doi:10.1038/s41598-018-
1400 22749-0 (2018).
1401 **Multi-organ microfluidic "physiome-on-a-chip" platform modeling up to 10 organs for**
1402 **4 weeks with pharmacokinetic analysis of diclofenac metabolism, noting general**
1403 **design and operational principles for multi-organ platforms.**
1404 128 Novak, R. *et al.* Robotic fluidic coupling and interrogation of multiple vascularized organ
1405 chips. *Nature Biomedical Engineering* **4**, 407-420, doi:10.1038/s41551-019-0497-x
1406 (2020).
1407 **Multi-organ linked system for up to 10 OoC for 3 weeks in automated culture and**
1408 **perfusion machine capable of medium addition, fluidic linking, sample collection and**
1409 ***in situ* microscopy.**
1410 129 Shim, M. K. *et al.* Carrier-free nanoparticles of cathepsin B-cleavable peptide-conjugated
1411 doxorubicin prodrug for cancer targeting therapy. *Journal of Controlled Release* **294**,
1412 376-389, doi:https://doi.org/10.1016/j.jconrel.2018.11.032 (2019).
1413 130 Al-Malahmeh, A. J. *et al.* Physiologically based kinetic modeling of the bioactivation of
1414 myristicin. *Archives of Toxicology* **91**, 713-734, doi:10.1007/s00204-016-1752-5 (2017).
1415 131 Schurdak, M. E. *et al.* in *Phenotypic Screening: Methods and Protocols* (ed Bridget
1416 Wagner) 207-222 (Springer New York, 2018).
1417 132 Oliver, C. R. *et al.* A platform for artificial intelligence based identification of the
1418 extravasation potential of cancer cells into the brain metastatic niche. *Lab on a Chip*,
1419 doi:10.1039/C8LC01387J (2019).
1420 133 Satoh, T. *et al.* A multi-throughput multi-organ-on-a-chip system on a plate formatted
1421 pneumatic pressure-driven medium circulation platform. *Lab on a Chip* **18**, 115-125,
1422 doi:10.1039/C7LC00952F (2018).
1423 134 Boos, J. A., Misun, P. M., Michlmayr, A., Hierlemann, A. & Frey, O. Microfluidic
1424 Multitissue Platform for Advanced Embryotoxicity Testing In Vitro. *Adv Sci (Weinh)* **6**,
1425 1900294-1900294, doi:10.1002/advs.201900294 (2019).

1426 135 Vunjak-Novakovic, G., Bhatia, S., Chen, C. & Hirschi, K. HeLiVa platform: integrated
1427 heart-liver-vascular systems for drug testing in human health and disease. *Stem Cell*
1428 *Research & Therapy* **4**, 1-6, doi:10.1186/scrt369 (2013).
1429 136 Vernetti, L. *et al.* Functional Coupling of Human Microphysiology Systems: Intestine,
1430 Liver, Kidney Proximal Tubule, Blood-Brain Barrier and Skeletal Muscle. *Scientific Reports*
1431 **7**, 42296, doi:10.1038/srep42296
1432 http://www.nature.com/articles/srep42296#supplementary-information (2017).
1433 137 Brown, J. A. *et al.* Recreating blood-brain barrier physiology and structure on chip: A
1434 novel neurovascular microfluidic bioreactor. *Biomicrofluidics* **9**, 054124,
1435 doi:10.1063/1.4934713 (2015).
1436 138 Brown, J. A. *et al.* Metabolic consequences of inflammatory disruption of the blood-
1437 brain barrier in an organ-on-chip model of the human neurovascular unit. *Journal of*
1438 *Neuroinflammation* **13**, 306, doi:10.1186/s12974-016-0760-y (2016).
1439 139 Maoz, B. M. *et al.* A linked organ-on-chip model of the human neurovascular unit
1440 reveals the metabolic coupling of endothelial and neuronal cells. *Nature Biotechnology*
1441 **36**, 865, doi:10.1038/nbt.4226
1442 https://www.nature.com/articles/nbt.4226#supplementary-information (2018).
1443 140 Chen, W. L. K. *et al.* Integrated gut/liver microphysiological systems elucidates
1444 inflammatory inter-tissue crosstalk. *Biotechnology and Bioengineering* **114**, 2648-2659,
1445 doi:10.1002/bit.26370 (2017).
1446 141 Esch, M. B., Ueno, H., Applegate, D. R. & Shuler, M. L. Modular, pumpless body-on-a-
1447 chip platform for the co-culture of GI tract epithelium and 3D primary liver tissue. *Lab*
1448 *on a Chip* **16**, 2719-2729, doi:10.1039/C6LC00461J (2016).
1449 142 Loskill, P., Marcus, S. G., Mathur, A., Reese, W. M. & Healy, K. E. μOrgano: A Lego®-Like
1450 Plug & Play System for Modular Multi-Organ-Chips. *PLOS ONE* **10**, e0139587,
1451 doi:10.1371/journal.pone.0139587 (2015).
1452 143 Xiao, S. *et al.* A microfluidic culture model of the human reproductive tract and 28-day
1453 menstrual cycle. *Nature Communications* **8**, 14584, doi:10.1038/ncomms14584
1454 http://www.nature.com/articles/ncomms14584#supplementary-information (2017).

1455 **Human female reproductive system and cycle recreated on 5-organ platform with**
1456 **inclusion of endocrine signaling to mimic hormonal markers of pregnancy as a tool for**
1457 **female reproductive toxicity assessment.**
1458 144 Skardal, A., Shupe, T. & Atala, A. Organoid-on-a-chip and body-on-a-chip systems for
1459 drug screening and disease modeling. *Drug Discovery Today* **21**, 1399-1411,
1460 doi:https://doi.org/10.1016/j.drudis.2016.07.003 (2016).
1461 145 Skardal, A. *et al.* Multi-tissue interactions in an integrated three-tissue organ-on-a-chip
1462 platform. *Scientific Reports* **7**, 8837, doi:10.1038/s41598-017-08879-x (2017).
1463 146 Oleaga, C. *et al.* Investigation of the effect of hepatic metabolism on off-target
1464 cardiotoxicity in a multi-organ human-on-a-chip system. *Biomaterials* **182**, 176-190,
1465 doi:https://doi.org/10.1016/j.biomaterials.2018.07.062 (2018).
1466 147 Sakolish, C. *et al.* Technology Transfer of the Microphysiological Systems: A Case Study
1467 of the Human Proximal Tubule Tissue Chip. *Scientific reports* **8**, 14882-14882,
1468 doi:10.1038/s41598-018-33099-2 (2018).
1469 148 Roberts, R. A. *et al.* Reducing attrition in drug development: smart loading preclinical
1470 safety assessment. *Drug Discovery Today* **19**, 341-347,
1471 doi:https://doi.org/10.1016/j.drudis.2013.11.014 (2014).
1472 149 Livingston, C. A., Fabre, K. M. & Tagle, D. A. Facilitating the commercialization and use of
1473 organ platforms generated by the microphysiological systems (Tissue Chip) program
1474 through public–private partnerships. *Computational and Structural Biotechnology*
1475 *Journal* **14**, 207-210, doi:http://dx.doi.org/10.1016/j.csbj.2016.04.003 (2016).
1476 150 Schurdak, M. *et al.* Applications of the microphysiology systems database for
1477 experimental ADME-Tox and disease models. *Lab on a Chip* **20**, 1472-1492,
1478 doi:10.1039/C9LC01047E (2020).
1479 151 Lee, J. *et al.* Recent advances in genome editing of stem cells for drug discovery and
1480 therapeutic application. *Pharmacology & Therapeutics*, 107501,
1481 doi:https://doi.org/10.1016/j.pharmthera.2020.107501 (2020).

1482 152 Franzen, N. *et al.* Impact of organ-on-a-chip technology on pharmaceutical R&D costs.
1483 *Drug Discovery Today* **24**, 1720-1724, doi:https://doi.org/10.1016/j.drudis.2019.06.003
1484 (2019).
1485 153 Peterson, N. C., Mahalingaiah, P. K., Fullerton, A. & Di Piazza, M. Application of
1486 microphysiological systems in biopharmaceutical research and development. *Lab on a*
1487 *Chip* **20**, 697-708, doi:10.1039/C9LC00962K (2020).
1488 154 Hardwick, R. N. *et al.* Drug-induced skin toxicity: gaps in preclinical testing cascade as
1489 opportunities for complex in vitro models and assays. *Lab on a Chip* **20**, 199-214,
1490 doi:10.1039/C9LC00519F (2020).
1491 155 Ainslie, G. R. *et al.* Microphysiological lung models to evaluate the safety of new
1492 pharmaceutical modalities: a biopharmaceutical perspective. *Lab on a Chip* **19**, 3152-
1493 3161, doi:10.1039/C9LC00492K (2019).
1494 156 Peters, M. F. *et al.* Developing in vitro assays to transform gastrointestinal safety
1495 assessment: potential for microphysiological systems. *Lab on a Chip* **20**, 1177-1190,
1496 doi:10.1039/C9LC01107B (2020).
1497 157 Phillips, J. A. *et al.* A pharmaceutical industry perspective on microphysiological kidney
1498 systems for evaluation of safety for new therapies. *Lab on a Chip* **20**, 468-476,
1499 doi:10.1039/C9LC00925F (2020).
1500 158 Baudy, A. R. *et al.* Liver microphysiological systems development guidelines for safety
1501 risk assessment in the pharmaceutical industry. *Lab on a Chip* **20**, 215-225,
1502 doi:10.1039/C9LC00768G (2020).
1503 159 Yeung, C. K. *et al.* Tissue Chips in Space—Challenges and Opportunities. *Clinical and*
1504 *Translational Science* **0**, doi:10.1111/cts.12689 (2019).
1505 160 Kim, K. *et al.* Epigenetic memory in induced pluripotent stem cells. *Nature* **467**, 285-290,
1506 doi:10.1038/nature09342 (2010).
1507 161 Wagner, J. A. *et al.* Application of a Dynamic Map for Learning, Communicating,
1508 Navigating, and Improving Therapeutic Development. *Clinical and Translational Science*
1509 **11**, 166-174, doi:10.1111/cts.12531 (2017).

1510 162 Ronaldson-Bouchard, K. & Vunjak-Novakovic, G. Organs-on-a-Chip: A Fast Track for
1511 Engineered Human Tissues in Drug Development. *Cell Stem Cell* **22**, 310-324,
1512 doi:https://doi.org/10.1016/j.stem.2018.02.011 (2018).
1513 163 McAleer, C. W. *et al.* Multi-organ system for the evaluation of efficacy and off-target
1514 toxicity of anticancer therapeutics. *Science Translational Medicine* **11**, eaav1386,
1515 doi:10.1126/scitranslmed.aav1386 (2019).
1516 164 Sances, S. *et al.* Human iPSC-Derived Endothelial Cells and Microengineered Organ-Chip
1517 Enhance Neuronal Development. *Stem Cell Reports* **10**, 1222-1236,
1518 doi:https://doi.org/10.1016/j.stemcr.2018.02.012 (2018).
1519 **Increased calcium transients and mature gene expression seen in spinal motorneurons**
1520 **and brain microvascular endothelial cells derived from induced pluripotent stem cells**
1521 **when cultured on 3D OoC versus 96-well plate.**
1522 165 Mulholland, T. *et al.* Drug screening of biopsy-derived spheroids using a self-generated
1523 microfluidic concentration gradient. *Scientific Reports* **8**, 14672, doi:10.1038/s41598-
1524 018-33055-0 (2018).
1525 166 Schutgens, F. *et al.* Tubuloids derived from human adult kidney and urine for
1526 personalized disease modeling. *Nature Biotechnology* **37**, 303-313, doi:10.1038/s41587-
1527 019-0048-8 (2019).
1528

Contributions

LAL wrote and edited the manuscript and created the figures; CM, BB, DAT and CA reviewed and edited the manuscript.

Competing Interests

There are no competing interests.

Table of contents blurb

Organs-on-chips (OoCs) could be useful at various stages of drug discovery and development; providing insight regarding human organ physiology in both normal and disease contexts, as well as accurately predicting developmental drug safety and efficacy. This Review discusses the advances that have enabled OoCs to demonstrate physiological relevance, and the challenges and opportunities that need to be tackled to tap the full potential of OoC utility for translational research.