Organ-on-Chip Devices towards Applications in Drug Development and Screening: A Review

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Abstract

As a necessary pathway to man-made organs, organ-on-chips which simulate the activities, mechanics and physiological responses of a real organs have attracted plenty of attention over the past decade. As the maturity of 3D cell-culture models and microfluidics advances, the study of organ-on-chips has made significant progress. This review article provides a comprehensive overview and classification of organ-on-chip microfluidics. Specifically, the review focuses on organ-on-chip systems capable of being used in pre-clinical drug screening and development. Additionally, the review highlights the strengths and weaknesses of each organ-on-chip system towards the goal of improved drug development and screening. The various organ-on-chip systems investigated throughout the review include, blood vessel, lung, liver, and tumor systems and the potential benefits which each provides to the growing challenge of high-throughput drug screening. Published organ-on-chip systems have been reviewed over the past decade (2007-2018) with focus given mainly to more recent advances and improvements within each organ system. Each organ-on-chip system has been reviewed on how closely and realistically it is able to mimic its physiological counterpart, the degree of information provided by the system towards the ultimate goal of drug development and screening, how easily each system would be able to transition to large scale high-throughput drug screening, and what further improvements to each system would help to improve the functionality, realistic nature of the platform, and throughput capacity. Lastly, a summary is provided of where the broad field of organ-on-chips appears to be headed in the near future along with suggestions on where future efforts should be focused for optimized performance of organ-on-chip systems in general.

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Introduction

Over the past decade, organ-on-chip (OOC) systems have gain popularity in the biomedical and pharmaceutical analysis fields[1–4]. Demand for a more reliable understanding of expected drug performance early in the development and screening process has been established in order to better inform down-stream decision processes[5,6]. Introduction of OOC systems engineered to enhance the predictive capabilities of *in vivo* drug performance at an early stage, have attempted to reduce wasted resources and time on non-viable drug candidates[1–4,7–9,9–19]. The increase in OOC system development has additionally been facilitated by improvements in device fabrication and advanced cell/tissue culturing techniques[8,10,20–26]. Recent advances in OOC systems have mainly focused on mimicking physiologically relevant conditions, experienced within the given organ, which influence drug delivery or performance *in vivo*[7–9,27–37]. The organs which often receive the most attention in this field include blood vessels, the lungs, the liver, and tumor environments[10–18,27–30,32–45]. In addition, large scale and high throughput testing have become necessities in the field of therapeutic development, and as such OOC systems must give consideration to the volume of various drug candidates which can be simultaneously tested[46,47]. Without achieving high-throughput screening capabilities, OOC systems have no chance to enter into mainstream pharmaceutical development[46,47]. Previous reviews of OOC systems designed for drug discovery have been conducted which have focused on many systems such as the lungs, liver, blood vessels, cancer, heart, intestine, and kidneys[3,48–52]. From these past reviews, we draw inspiration for the review of more current OOC systems which have been developed with focus on improvements made which facilitate improved drug performance analysis and screening. Throughout this review of OOC systems, we highlight some of the recent advances that have taken place in the field over the past decade[9–18,27–40,42–45,53–55]. Here we focus on reviewing the capabilities, functionality, physiological relevance, and throughput capacity of each OOC system[9–18,27–40,42–45,53–55]. As a conclusion, we predict where the future of OOC research and development are headed in the near future while noting future advances required to help transition OOC work out of academic research labs and into commercial settings. A wider-scale acceptance and application of OOC technologies will be required to facilitate healthcare advances in the future[1–6,8,9,19]. This review supports the improvement of future healthcare and medicine with OOC technologies being developed around the world.

Organ-On-Chip Micro- & Nano-Fabrication

Fabrication of organ-on-chip devices relies on micro (10^{-3}m – 10^{-6}m) and nano (10^{-6}m – 10^{-9}m) fabrication techniques to produce environments which contain appropriate micro and nano features of the given biological system being mimicked[23,24]. These small features are generated and enclosed within microfluidic systems which are designed with highly defined geometries, allowing for controlled flows of

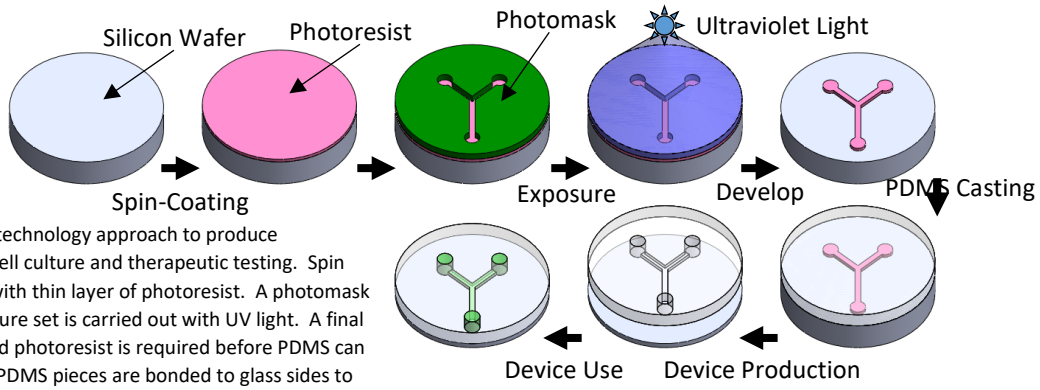

Fig. 1. Photolithography technology approach to produce microfluidic devices for cell culture and therapeutic testing. Spin coating of silicon wafer with thin layer of photoresist. A photomask is positions and an exposure set is carried out with UV light. A final development of the cured photoresist is required before PDMS can be cast from the wafer. PDMS pieces are bonded to glass sides to produce devices which can then be used for testing after appropriate sterilization.

various solutions[8]. A few typical fabrication techniques employed on these size scales include photolithography, focus ion beam (FIB) milling, 3D bio-printing and deep reactive ion etching (DRIE)[7,20–22]. Such techniques function via additive (photolithography & 3D bioprinting) or subtractive (FIB & DRIE) processes in order to produce casting molds or to directly produce microfluidic chips. Additive fabrication techniques such as photolithography and 3D bioprinting function by depositing and building-up material on a base substrate[7,20]. In the case of photolithography, the features which are additively produced are used as a replicating molds as depicted in Fig. 1, while 3D bioprinting can be directly used to produce a microfluidic structure in an additive manner as in Fig. 2 [7,20]. On the other hand, the use of subtractive fabrication techniques remove material from an initial bulk material, often to produce replicating molds or to produce smaller nano-scale features within larger micro-scale channels or resivours.[21,22,56]. Because many of the fabrication techniques produce replicating molds as opposed to directly producing a microfluidic chip, a secondary casting process must be performed to produce a microfluidic system[21,22]. Most common microfluidic systems are comprised of a polymer base infrastructure onto which various biological components can be added in order to mimic the function and structure of an organ[8,24]. Such microfluidic systems often utilize the inherent ability of the system to handle fluid or gas flow to further replicate biologically relevant conditions experienced within various organs[8,9]. Such flow is often established through the use of syringe pumps, peristaltic pumps, concentration gradients, pressure differentials or gravity driven systems. The fabrication techniques chosen for any specific microfluidic chip are done so in order to produce an environment within the

system which mimics the conditions of the real organ as closely as possible. As such, it is not uncommon for researchers to employ several different techniques to accomplish various features of varying size and complexity. The use of nanofabrication in conjunction with microfabrication can occur to improve the functionality of an organ-on-chip system such as having the ability to produce nanopore/nanopillar arrays within a large microfluidic channel, or through the incorporation of nano-scale materials such as proteins, nanoparticles or nanofibers to assist in directing cell behavior or growth within such larger systems[19,46,57–59]. By directly mimicking *in vivo* organ conditions within microfluidic chips a more realistic representation of expected results and outcomes can be achieved. In the field of therapeutic development, such organ-on-chip systems function to provide insight to guide future decisions regarding the *in vivo* viability of potential drug candidates. Given the versatility of microfluidics, only a sub-section of the organ systems mimicked using such chips will be explored in this review (blood vessel, lung, liver and tumor systems).

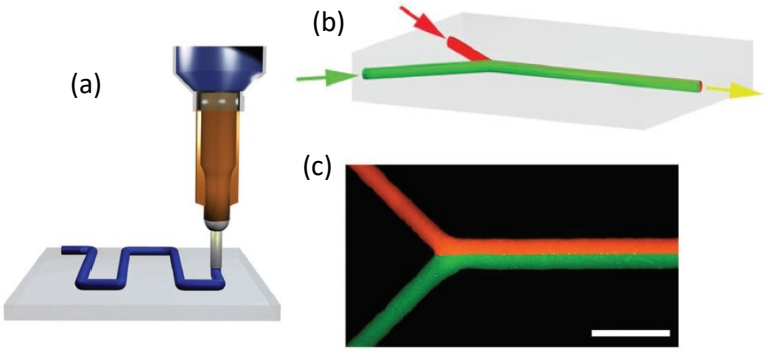

Fig. 2. Microvascular scaffold fabrication and mixing experiment. (a) Robotic deposition of fugitive organic ink (blue) through a cylindrical nozzle onto a moving *x*–*y* stage. (b) Schematic representations of microfluidic device mixing experiment, where two fluids (red and green) are mixed at *Re*=30.6 to produce the yellow (mixed) output. The arrows indicate the flow direction. The two fluids meet at a Y-junction where they enter a 17-mm straight microchannel. (c) Fluorescent microscope image of microfluidic device mixing experiment in Y-junction. Scale bare = 0.5 mm. Reproduced from [20] with permission.

Hydrogel Based Organ-On-Chip Fabrication

Besides traditional techniques for producing microfluidic systems such as photolithography, newer approaches have been developed which allow for improved functionality through incorporation of extracellular matrix (ECM) components[60–64]. The incorporation of such ECM components often in the form of hydrogels assists in recreating the complex nature of *in vivo* systems[60–64]. Many studies have shown the cell-matrix interaction for many organoids is important in creating viable organ-on-chip systems capable of producing realistic microenvironments as would be found *in vivo*[60–64].

In particular a study by Yamada *et al* has been conducted with hepatocytes, where alginate hydrogel was gelled within a gelation channel to produce a hepatic cord-like tissue[64]. In this case, the alginate hydrogel functions as a support structure to facilitate the formation of a hepatocyte-3T3 organoid[64]. Once the organoid becomes developed, the alginate hydrogel can be removed via

enzymatic degradation[64]. The process of forming the alginate hydrogel is facilitated by mixing alginate solution with a chloride salt solution within a microfluidic channel via flow[64]. Incorporation of the hepatocytes and 3T3 cells into the alginate gel was accomplished by flowing both cell types into the microfluidic channel from different ports[64]. Using this approach, the group was able to form hepatic micro-organoids over the course of 7 to 30 days which showed physiologically relevant structure and function as a result of the growth within the alginate hydrogel[64]. However, there are drawbacks associated with using such an approach. The use of a hydrogel to encapsulate the growing cells required an additional procedural step to enzymatically digest the hydrogel[64]. Besides increasing the complexity of the experimental procedure, the required enzymatic degradation of the hydrogel could potentially influence the phenotype, behavior and performance of the hepatocytes and 3T3 organoids[64]. Additionally, the use of a hydrogel means that nutrient and waste exchange within the system is purely driven by diffusion as flow conditions are not possible to establish through the solidified gels[64]. While Yamada *et al* claim that the scale of their organoids were within the usual limitations of thickness, the use of hydrogels poses the issue of limited or restricted nutrient delivery and waste removal as a result of relying purely on diffusion[64]. Despite such drawbacks, the use of hydrogel materials have been shown to improve the physiological nature of organ-on-chip systems[60–64]. Besides alginate, other types of hydrogel materials have been utilized to facilitate organoid formation such as agarose, methacrylated gelatin (GelMA), star poly(ethylene glycol-co-lactide) (SPELA), poly(ethylene glycol) dimethacrylate (PEGDMA) and poly(ethylene glycol) diacrylate (PEGDA)[63].

Work conducted by Bertanssoni *et al* has utilized agarose gel in an attempt to produce vascularized tissue constructs using a bioprinting method[63]. In their work, agarose gel was bioprinted in order to produce vascular channels within a larger hydrogel construct[63]. The printed agarose channels were encased within a range of GelMA hydrogels and removed after photopolymerization leaving behind a network of vascular channels as can be seen in Fig. 3[63]. The group utilized a range of GelMA hydrogels including methacrylated gelatin (GelMA), star poly(ethylene glycol-co-lactide) (SPELA), poly(ethylene glycol)

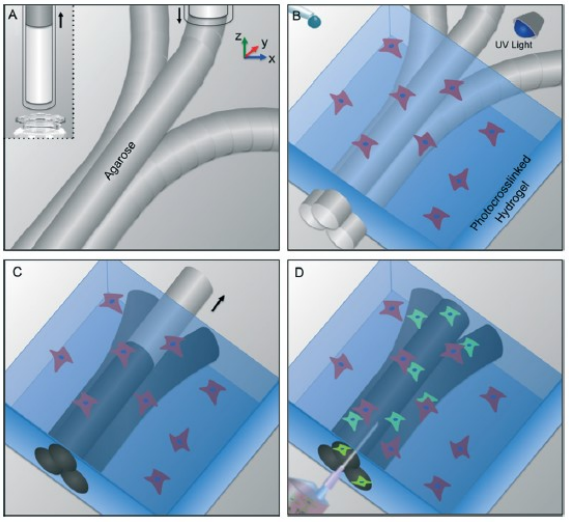

Fig. 3. Schematic representation of bioprinting of agarose template fibers and subsequent formation of microchannels via template micromolding. a) A bioprinter equipped with a piston fitted inside a glass capillary aspirates the agarose (inset). After gelation in 4 °C, agarose fibers are bioprinted at predefined locations. b) A hydrogel precursor is casted over the bioprinted mold and photocrosslinked. c) The template is removed from the surrounding photocrosslinked gel. d) Fully perfusable microchannels are formed. Reproduced from [63] with permission.

dimethacrylate (PEGDMA) and poly(ethylene glycol) diacrylate (PEGDA) in order to demonstrate improvements in mass transport, cell viability and differentiation as a result of the fabricated vascular network[63]. The ability of utilizing hydrogel materials combined in such a manner allows for vascularization of organoid constructs to be developed early in the fabrication process as opposed to relying on biological cues and long durations to time to facilitate vascularization through angiogenesis[60,62,63]. As such, more complex and intricate organoid models can be developed and tested in short periods of time while mimicking *in vivo* conditions[63]. Despite the benefits provided by the use of the hydrogel materials, the fabrication of the vascular channels via bioprinting techniques introduces limitations. Specifically, the ability to recreate more complicated vascular structures such as bifurcations is very difficult to do in a seamless manner[63]. In addition, the use of an extrusion based system often places a limit on the minimum feature size which can be achieved[63]. In their work, Bertanssoni *et al* achieved, channels on the order of several hundred micrometers in diameter[63]. Such limitations in achieving small feature sizes often results from physical characteristics of the hydrogels being used[63]. The extrusion of hydrogel materials through small openings generates large pressures and requires large extrusion forces[63]. In order to combat this issue, the size of the nozzles used in extrusion systems are often large or the process of hydrogel gelation is performed after the extrusion step in order to reduce the force required for extrusion[63].

As a comparison, work performed by Chan *et al* utilized a combination of collagen I and alginate to produce a 3D capillary bed which formed as a result of natural angiogenic endothelial sprouting[62]. In their approach, a bed of alginate beads was formed and encased with collagen I within a microfluidic system[62]. The collagen I was allowed to cure after which endothelial cells were introduced into channels which ran along the side of the hydrogel zone[62]. The endothelial cells were allowed to grow into the collagen I and alginate bead hydrogel matrix with the help of VEGF to facilitate angiogenesis[62]. Using this approach, small vascular channels were produced in the spaces between the alginate beads as the endothelial cells grew into the collagen I gel[62]. Highly interconnected and dense vascular networks were made in 3D to demonstrate the improved transport capabilities within the organoid system without having to rely entirely on diffusion of materials through large sections of tissue[62]. While such, capabilities are beneficial, the system has some inherent drawbacks which can limit the application. To start, the system is able to produce dense networks of vessels, however, there is very little control over the final vascular orientation and geometry[62]. The only feature which can be tuned is the size of the alginate beads in order to influence the final vascular geometry, however this approach does not allow for predetermined vessel orientations and networks to be formed[62]. Additionally, the system relies

heavily on the use of extracellular cues in the form on growth factors to drive angiogenesis and sprouting of the new vessels[62]. Such growth factors like vascular endothelial growth factor (VEGF) are often expensive and require long periods of time to influence the growth of vessels within an organoid system[62]. In their work, Chan *et al*, required nearly 1 week to facilitate the growth of a vascular network where Bertanssoni *et al* was capable of producing a vascularized organoid system within one day[62,63]. However, despite the increased time required to produce a vascular network, the approach adopted by Chan *et al* does have the benefit of producing more organic vessel structures within their hydrogel[62]. In both cases, the research mainly focused on the production of the vascular network and not on the additional integration of surrounding tissues within the organoid[62,63]. This next step can be seen in the work conducted by Agarwal *et al* where a hydrogel components are utilized in the formation of a tumor vascularized network[60].

In their work, Agarwal *et al*, produced microtumors within collagen and alginate constructs which were then organized into a 3D structure within a microfluidic device[60]. Subsequent addition of stromal and endothelial cells into the microfluidic system allowed for the generation of a vascular network to grow between the beads containing the microtumors as depicted in Fig. 4[60]. As with the previous approach by Chan *et al*, the growth of the endothelial cells within the collagen network required the presence of growth factors[60,62]. The difference with the approach taken by Agarwal *et al* was that the microtumors served as the source of the growth factors instead of requiring the external introduction of VEGF[60]. As such, the combination of the hydrogel and a more physiologically relevant source of VEGF allowed for

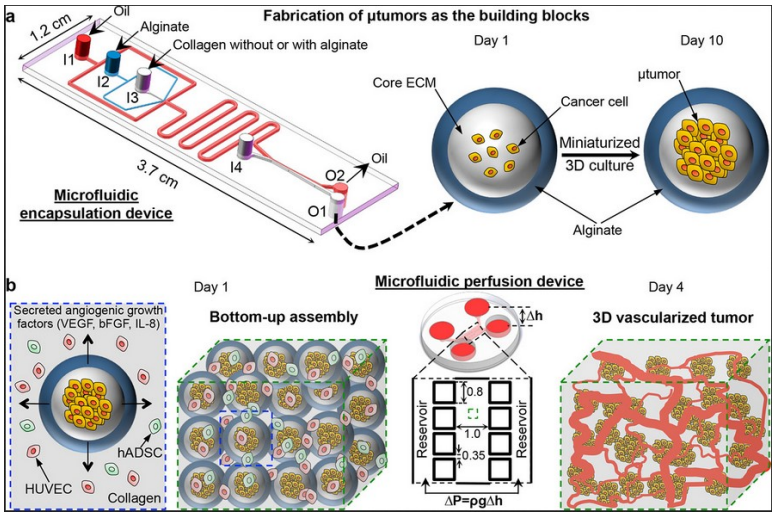

Fig. 4. (a) A nonplanar microfluidic encapsulation device is used for encapsulating cancer cells in core–shell microcapsules, and the cells are cultured in the microcapsules for 10 days to form microtumors (μtumors, < ~200 μm in radius). Mineral oil infused with calcium chloride, aqueous sodium alginate solution (to form the microcapsule shell), aqueous collagen solution (with or without cells) to form the microcapsule core, and aqueous extraction solution are pumped into the device via inlets I1, I2, I3, and I4, respectively. The aqueous phase (containing core–shell microcapsules) and oil exit the device from outlets O1 and O2, respectively. (b) A microfluidic perfusion device is used to assemble the μtumors and stromal cells including endothelial cells for perfusion culture to form 3D vascularized tumor. The μtumors in core–shell microcapsules are assembled together with human umbilical vein endothelial cells (HUVECs) and human adipose-derived stem cells (hADSCs) in collagen hydrogel in the microfluidic perfusion device. The alginate shell of the microcapsules is dissolved to allow cell–cell interactions and the formation of 3D vascularized tumor in the microfluidic perfusion device under perfusion driven by hydrostatic pressure. Units for the dimensions of micropillars and sample chamber: mm; P: pressure; ρ: density; g: gravitational acceleration; and h: height of medium column linked to the reservoirs. Reprinted with permission from Agarwal P, Wang H, Sun M, et al. Microfluidics Enabled Bottom-Up Engineering of 3D Vascularized Tumor for Drug Discovery. *ACS Nano*. 2017;11(7):6691-6702. doi:10.1021/acsnano.7b00824. Copyright (2018) American Chemical Society.

the production of the vascularized tumor network[60]. However as indicated previously, the use of the hydrogel and growth factor in the production of vasculature requires long periods of time[60,62]. In such systems, the use of hydrogel is a trade-off between developing organoids which behave very closely to their *in vivo* counterparts as a result of the external cues and support provided by the presence of the hydrogels and the time required to establish the model[60,62]. As such, a balance is required to generate physiologically relevant and realistic models while not taking too long or becoming too complicated to setup and control[60,62,63].

In some cases, the need to a high degree of physiological mimicry is needed in order to properly facilitate *in vivo* processes in an *in vitro* setting[61]. One example is the differentiation of pluripotent stem cells, where strict control over external ques is required to properly achieve a desired differentiation[61]. As such, additional work conducted by Agarwal *et al* has focused on encapsulation of pluripotent stem cells in alginate hydrogel[61]. Their work has demonstrated the importance of the alginate in protecting the cells from a host immune system, shielding them from extreme conditions of external forces such as shear when introduced into microfluidic channels and can assist in the regulation of nutrients, oxygen and waste[61]. As such, high degrees of cell viability and physiologically relevant levels of cellular expression can be achieved[61]. Beyond this scope, there are other techniques and considerations which are given to design and function of microfluidic systems in order to better recreate organoid structures in an *in vitro* environment.

Recreating *In Vivo* Environments

As touched upon previously, the ability to recreate certain features of biological systems is key in producing viable organ-on-chip systems[2,7,19,65–75]. Beyond the scope of incorporating hydrogel materials in organ-on-chip systems, there exist factors such as protein coatings, shear stress, cyclic stretching, chemical concentration gradients, and variations in elastic moduli which can also play a large role in the performance of the system[2,7,19,65–75]. In all of these cases, the factors have the largest influence on the cells being grown within the devices[2,7,19,65–75]. The utilization of each factor is designed to improve the behavior of the cells in order to establish a model which closely mimics *in vivo* phenotypical responses[2,7,19,65–75].

To start, the use of protein coatings within organ-on-chip systems is applied in order to better facilitate cell adhesion to synthetic microfluidic surfaces and to facilitate or direct cellular

growth[2,7,19,68,75]. This approach can take to form of fully coating the entire surface of a microfluidic channel with an ECM molecule such as fibronectin or collagen[2,7,19,68,75]. Other methods for utilizing proteins within microfluidics is often in the form of introducing growth factors or cytokines in solution or laden within hydrogel materials to direct cell growth or to induce disease conditions such as inflammation[2,7,19,36,38,39,68,75]. Along a similar line with protein coatings, the use of chemical concentration gradients occurs within hydrogel materials where gradients are developed to vary cellular response across a microfluidic channel[2,7,19,68–72]. Additionally such gradients can be utilized in the fabrication of the microfluidic devices themselves in order to tune the mechanical properties of the synthetic material used in device fabrication such as PDMS[2,19,68,75]. The establishment of concentration gradients within hydrogels is often achieved by varying the rate at which various components are flown into a microfluidic channel over time[2,19,68–71,75]. Similar approaches can be used when producing microfluidic devices of varying elastic moduli or a variety of culture grade materials from soft substrates such as hydrogels to rigid substrates such as polycarbonate or polystyrene can be combined to produce a single[19,65,66,68,71–75]. Additional factors which can be controlled within organ-on-chip systems include the ability to mimic physiologically relevant shear stresses and stretching[2,7,19,65–68,70,72,75]. The establishment of appropriate shear stress within microfluidic channels improves the physiological relevance and phenotypic response of endothelial cells[36,38,39,55,68,70]. Sear stress is typically achieved with the use of a syringe or peristaltic pump which allows for the exact shear stress to be accurately tuned based on the physiology being mimicked[36,38,39,55,68,70]. The use of peristaltic pumps adds in an additional feature encountered *in vivo* which is the pulsatile flow of blood through the vascular system[36,38,39,55,68,70]. Lastly as an example, the ability to produce physiologically relevant stretching within organ-on-chip systems mimicking the lungs blood vessels can be accomplished through the increase or decrease of pneumatic pressure in chambers within the microfluidic devices or through the use of peristaltic pumps[2,7,19,65,67,68,75]. The cyclic stretching of the lung epithelium and vascular endothelium promote more phenotypical behavior and responses within microfluidic systems[2,7,19,65,67,68,75].

Overall, all of the additional factors incorporated into organ-on-chip microfluidics are designed to improve the predictive performance of the systems[2,7,19,65–75]. The implementation of each factor from the inclusion of special chemical species to establishment of physical physiological stimuli requires a unique approach within microfluidic systems[2,7,19,65–75]. The ability to include such factors ensures that the physiological relevance of the models being constructed and the data being collected from them, can be translated to *in vivo* conditions for improved drug discovery and analysis[2,7,19,65–75].

Blood Vessel-On-Chip

The delivery or transport of therapeutic agents often involves the use of the vascular system as it is highly integrated throughout the entire body[27,28]. As such, understanding how therapeutics and therapeutic carriers behave within the vascular system is of great importance. The portion of the vasculature often mimicked is the endothelial lining which functions as a biological barrier. As a biological barrier, the endothelium must often be traversed for proper drug delivery[29–31]. Vessel-on-chip systems in turn typically incorporate the growth of endothelial cells either as a monolayer or hollow-tube structure[36,38,39,55]. Such systems can be set up in microfluidic devices which are produced from a master template or they can be extruded using a syringe based system utilizing hydrogel materials. Out of the two fabrication methods, the use of microfluidic device casting is more common, where vascular geometries are utilized to produce the mimicked vessels[36,38,39,55]. Prabhakarpandian *et al* has performed blood vessel work utilizing cast microfluidics[32].

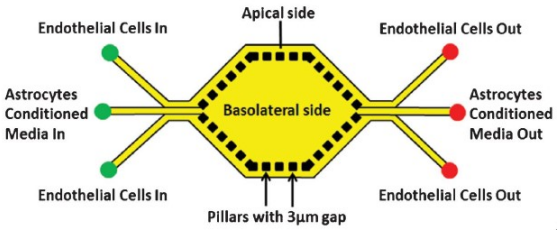

Fig. 5. SyM-BBB model. Concept showing the apical and basolateral sides separated by 3 mm gaps formed by microfabricated pillars. Apical side contains endothelial cells while basolateral side contains astrocytes conditioned media. The design is based on the idealized concept of the microvasculature comprising of diverging and converging bifurcations. Reproduced from [32] with permission.

A microfluidic based testing platform was utilized by Prabhakarpandian *et al* to produce a blood brain barrier vessel model as depicted in Fig. 5 [32]. The system involved basolateral and apical regions which were separated by an array of pillars spaced 3µm apart. Immortalized rat brain endothelial cells were grown in the apical regions to mimic brain microvasculature with astrocyte conditioned media being flown through the basolateral region[32]. This particular microfluidic system utilized physiologically relevant channel dimensions and shear rates while also managing to achieve the growth of endothelial cells directly on the devices. As such, the Prabhakarpandian *et al* system provides a suitable platform for the analysis of dye permeation, tight junction occurrence and Rhodamine 123 efflux[32]. While the system is capable of providing cellular based information regarding the condition of the cultured endothelium, the simplified model is far from mimicking the full nature of the blood brain barrier experienced *in vivo*. Specifically, the current state of the system only allowed for the use of a single cell type which was grown as a monolayer instead of a three-dimensional tube with accompanying astrocytes[32]. Overall, the system produced by Prabhakarpandian *et al* serves as reasonable system capable of recreating several physiologically relevant factors making it ideal for simple therapeutic/dye binding and delivery studies[32]. Additionally,

given the nature in which the system was produced, the possibility to scale up such testing capabilities could be readily achieved by utilizing large microfluidic arrays. Coupling such arrays with automated liquid handling, imaging, and other data collection systems could prove to provide valuable information regarding therapeutic performance in a high-throughput manner. Improving system capabilities and physiological relevance would further increase the value of the data which could be collected from such a system. Use of three dimensional culturing systems is one way the previous system might be able to advance.

As an example Gao *et al* was capable of creating three dimensional blood vessel structures which contained three different cell types[25]. The approach used to produce three dimensional blood vessel structures was a combination of bio-printing and cell seeding techniques as seen in Fig. 6. The formation of the tubular structures was accomplished with a custom built extrusion system utilizing a coaxial nozzle. The coaxial nozzle incorporated into the system allowed for simultaneous extrusion of sodium-alginate mixed with fibroblasts and a calcium chloride solution[25]. The formation of hollow tubes was made possible by reacting the alginate solution with the calcium chloride to produce an alginate gel. The gelation of the alginate tubes resulted in the encapsulation of the fibroblasts present in the sodium-alginate solution and were used as the basis for building large vessel structures. Extrusion of the hollow tubes around rods to form large hollow coils allowed for subsequent endothelial cell seeding onto the lumen surface after collagen coating. Utilizing this system, a vessel model was generated which incorporated fibroblasts (L929), smooth muscle (MOVAS), and endothelial (HUVEC) cells within the same microenvironment which is an important consideration required for studying cellular interaction during therapeutic delivery[25]. The three-dimensional structure also provides improved physiological relevance when compared to most systems utilizing microfluidics. However, despite the advantages this system holds over microfluidic based models, the approach of tube extrusion is usually limited in the size of the vessels capable of being fabricated. Specifically, the vessel models generated using extrusion based methods such as bio-printing tend to be very large, on the scale of several hundred micrometers up into the millimeter scale[25]. Such

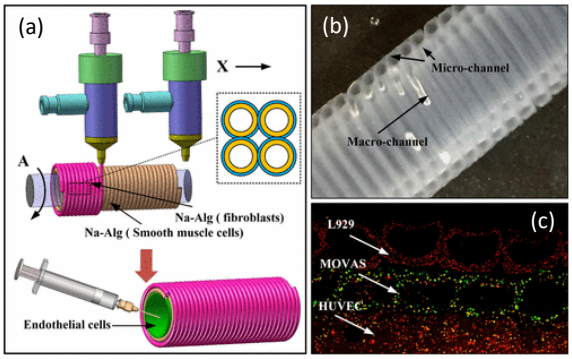

Fig. 6. 3D hydrogel-based vascular structures with multilevel fluidic channels fabricated by extrusion-based three-dimensional (3D) bioprinting. (a) Printing a layer of smooth muscle cell-laden structure over a rod (inset on the right: cross-section of the selected area) and seeding endothelial cells into the inner wall of the structure. (b) Longitudinal section of the double-layer structure under different magnification. (c) Printed vessel-like structure containing three kinds of vascular cells with three colors: red-L929, green-MOVAS, and orange-HUVEC. Reproduced from [25] with permission.

large vessels in turn have difficulties mimicking conditions experienced in small microvasculature and capillaries where the majority of nutrient and therapeutic exchange occurs between tissues. As such, the vessel extrusion system utilized by Gao *et al* produced "microvessels" around 800 micrometers in diameter and larger vessels on the scale of 6mm. In addition to the relatively large size of typical vessels produced using extrusion methods, the process as a whole is not conducive for high-throughput screening because of the time and complicated setup required to produce viable samples[25]. The inability to produce large quantities of individual tubes coupled with complex testing setups means that the current stage of bio-printed vessels would likely not be able to cope with the demand for high-throughput testing in the pharmaceutical industry. With improvements to the fabrication process and size of the produced vessels, such extrusion based system could prove to provide key physiologically relevant conditions such as the use of several cell types in a three-dimensional environment. In order to achieve these goals, advancements in the printing/extrusion technology will likely be required which may possibly include the incorporation of automation to help expedite the fabrication and testing of such blood vessel systems.

Lastly, the work performed by Zheng *et al* functions to incorporate features of the two previous systems. In their work, Zheng *et al* combine the use of microfluidics and patterned hydrogel materials to produce microvessels in three dimensions[37]. Through these efforts, microvessels were molded using microstructures within a type 1 collagen gel[37]. The resulting microvessels were then able to be seeded with HUVECs which were fed with nutrients from a PDMS reservoir[37].

The functionality of the Zheng *et al* system in turn allowed for studies of angiogenesis into the surrounding collagen gel, perivascular interaction in a co-culture system, as well as thrombosis within the mimicked vessels[37]. The ability of the system to form hollow EC microvessels allows for the use of whole blood within the system which facilitates thrombosis studies[37]. Besides being able to consistently from well-defined microvessels, the system was shown to be capable of monitoring vascular permeability throughout the application of various culturing conditions and platelet adhesion in thrombosis studies[37]. In both cases, the ability of the system to allow for microscale imaging of ECs, pericytes and platelets facilitates a superior testing platform for therapeutic studies involved with treatment of vascular permeability and thrombosis[37]. Despite all of these advantages, the system does lack one key feature experienced within microvasculature networks. Specifically, the system lacks the ability to have constant driven flow across a large range as it relies on gravity driven flow to transport blood and media[37]. Ideally, a more precise and continuous means of controlling flow within the system

with a syringe pump would provide a more dynamic range of experimental conditions to test under, especially when larger degrees of shear stress are desired. Additionally, high throughput devices for screening are more desirable to simultaneously facilitate larger scale therapeutic testing.

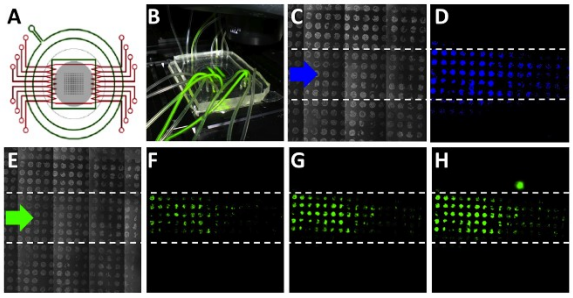

Fig. 7. Figure 5. Coupling of the cell array with a microfluidic platform. **A** - Schematic representation of the microfluidic platform containing 8 microfluidic channels for media perfusion (in red) and containing a membrane-vacuum system (in green), acting as a suction pad for reversible sealing and which delimits the culture chamber. Cell array is represented in grey. **B** - Images of the assembled microfluidic platform under fluorescent light on the microscope stage. Two channels deliver a fluorescein solution. **C–D** - Validation of the coupled system using a nuclear dye (HOECHST). Phase contrast (C) and fluorescence image (D) of the entire cell array show how the HOECHST signal could be detected only on the area selectively exposed to the fluid stream containing the nuclear dye (blue arrows). **E–H** - Validation of the coupled system using adenoviral vectors for EGFP delivery. (E) Phase contrast of the entire cell array and (F–H) fluorescence images of the temporal sequence showing an increased EFGP expression at 16 h (F), 22 h (G), 26 h (H) post-infection. The viral transduction is clearly compartmentalized on the area selectively exposed to the fluid stream containing the viral particles. Reproduced from [77] with permission.

High throughput systems have been developed for the vascular system where cardiomyocytes are utilized for pharmacological studies[76,77]. In these studies, large arrays of cardiomyocytes are grown within perfusion chambers and in turn can be subjected to therapeutic agents[76,77]. The adverse influence of therapeutic agents on the contractility and protein expression within the myocytes is used as a gauge to determine drug safety on a large scale[76,77]. Systems designed for such testing typically contain 40 to 400 individual cultures of myocytes for large scale testing simultaneously as can be seen in Fig 7[77].

Moving beyond the vascular system, therapeutic screening tends to focus on organs and tissues which heavily interact with circulating therapeutics. As such, the next organ-on-chip systems which will be reviewed are geared towards mimicking tissues found within the lungs, liver and tumors.

Lung-On-Chip

Within the lungs exist a large number of capillary beds required to facilitate the exchange of gasses in and out of blood[33,34,53]. Increased rates of exchange between the lungs and associated blood vessels leads to increased interaction between therapeutic agents also present within the vascular network. In addition, therapeutics delivered via inhalation often times must cross the lung epithelial/vessel endothelial barrier to be further distributed throughout the body[33,34,53]. As such, research focus on the fabrication of lung-on-chip devices is of great importance in screening process of therapeutics and therapeutic carriers.

Therapeutic testing in such lung-on-chip devices typically focuses on the transport of such therapeutics across epithelial cells found within alveoli. Beyond the study of therapeutic transport across biological barriers, many lung models incorporate physiologically relevant mechanical forces to further mimic therapeutic delivery and transport in the lungs. One such example of this approach to studying therapeutic transport has been developed by Huh *et al*, where a microfluidic platform was developed to culture lung epithelial cells, vascular endothelium, and immune cells[9]. In addition to providing a multi-cell culturing platform, the microfluidic system allows for cyclic mechanical stretching of the cultured cells through use of a vacuum as depicted in Fig. 8 [9]. Inclusion of all such physiologically relevant conditions into a single platform has allowed for improved therapeutic testing, however, there are further capabilities which the system as a whole would benefit from. Specifically, the incorporation of three-dimensional culturing capabilities, especially for the vascular portion of the device would better mimic *in vivo* interactions between lung epithelium and vascular endothelium. Despite this drawback, the system has been shown to allow for the quantification of nanoparticle translocation under different conditions[9]. Specifically, it has been shown that the addition of stain (10%) improves the rate of nanoparticle translocation from the lung over to the vascular side of the microfluidic system. Beyond just quantifying nanoparticle translocation, the system also allows for direct assessment of cellular ICAM-1 expression along with Occludin and VE Cadherin[9]. Being able to directly investigate how the expression of such proteins changes over time and as a result of various therapeutic treatments makes the platform a useful tool for predicting expected cellular responses *in vivo*. Additionally, the microfluidic nature of the platform allows for the potential of high-throughput screening. As noted previously for the vascular microfluidic systems, the ability to produce microfluidic arrays provides the capability to run large numbers of therapeutic compounds simultaneously for screening purposes. Such

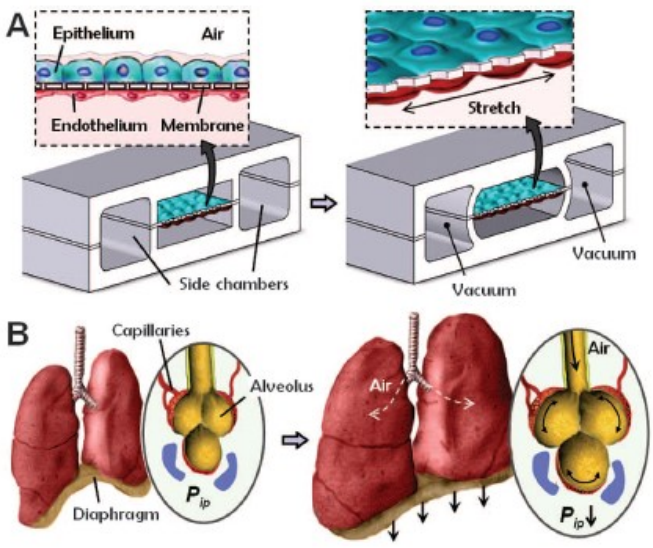

Fig. 8. Biologically inspired design of a human breathing lung-on-a-chip microdevice. (a) The microfabricated lung mimic device uses compartmentalized PDMS microchannels to form an alveolar-capillary barrier on a thin, porous, flexible PDMS membrane coated with ECM. The device recreates physiological breathing movements by applying vacuum to the side chambers and causing mechanical stretching of the PDMS membrane forming the alveolar-capillary barrier. (b) During inhalation in the living lung, contraction of the diaphragm causes a reduction in intrapleural pressure (Pip), leading to distension of the alveoli and physical stretching of the alveolar-capillary interface. Reproduced from [9] with permission.

a system could function to provide improved therapeutic performance insight while capturing many features of the respiratory and vascular systems. However, other lung-on-chip systems tend to not be quite as elaborate.

Microfluidic models such as those created by Douville *et al* and Tavana *et al* have incorporated the growth of lung epithelial cells to show the importance of establishing a liquid-air-interface[44,78]. Establishment of such an interface when studying the lung epithelium produces physiologically relevant responses from the cells as depicted in Fig. 9. As such, these features of the microfluidic systems are ideal to include in the lung-on-chip systems. However, despite being able to replicate a liquid-air-interface, such models only utilize a single cell line[44,78]. Ideally, such models would include vascular endothelium along with additional stimuli in order to better mimic *in vivo* conditions. The application of mechanical strain in the system developed by Tavana *et al* in addition to the shear and compressive forces would have functioned to better condition the lung epithelium grown within their microfluidic chip[44]. However, despite these drawbacks, the simplified microfluidic systems did function to provide a means of quantifying the influence of shear and pressure on the viability of lung epithelium[44]. The ability to achieve such direct cellular measurements is crucial for understanding how potential therapeutic options influence cell behavior and responses to stimuli. In addition to ease of direct cellular measurements, these simplified microfluidic systems offer a means of easily scaling up testing capabilities. While use of a single cell line detracts from the physiological relevance of these systems, it also make large scale testing setups more simplified and easier to achieve towards the goal of high-throughput screening[44,78]. Overall, current lung-on-chip systems have been successful in capturing many of the physiologically relevant features of the lung epithelium, with the more advanced models capable

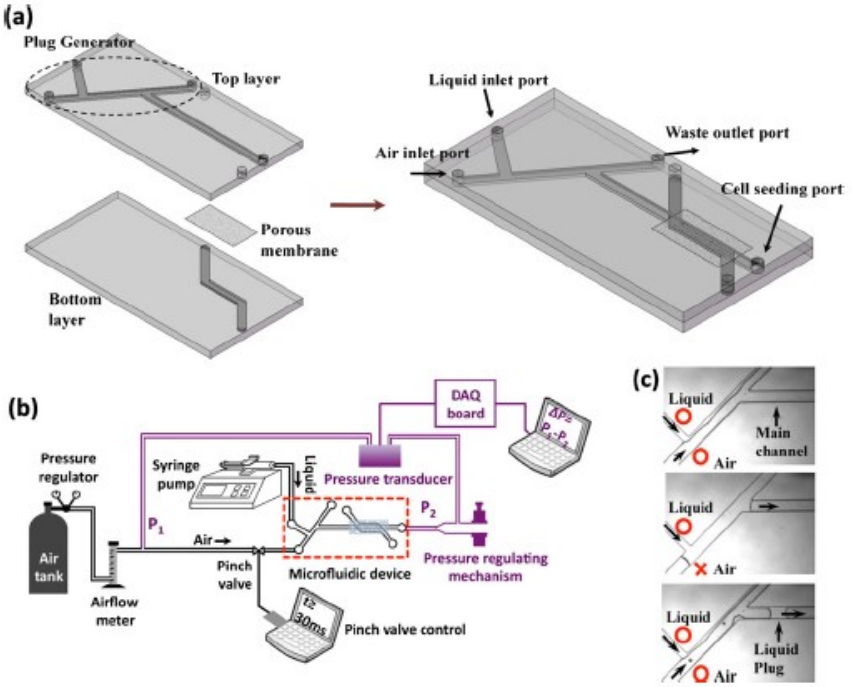

Fig. 9. (a) Schematic of the microfluidic airway model. (b) Schematic of components of the experimental setup. (c) The process of liquid plug generation from air and liquid streams. Reproduced from [44] with permission.

of incorporating vascular endothelium[9,44,78]. Further improvements to lung-on-chip systems could potentially include improved applications of mechanical forces in more realistic manners, such as the use of equiaxial strain to better mimic the expansion and stretching of the lung epithelium as air fills the lungs. In addition, the ability to establish more physiologically relevant architectures and geometries will help to improve the predictive capabilities of the systems with regards to the expected *in vivo* performance of therapeutics and therapeutic carriers.

Besides the lung, another organ of interest when considering therapeutic interactions with the body is the liver due to role it plays in waste removal from the circulatory system. As such, we will next shift focus onto liver-on-chip systems developed to assist in therapeutic screening and development.

Liver-On-Chip

When considering liver-on-chip systems, often times the main goals are to understand the degree of therapeutic toxicity (hepatotoxicity) within a model which accurately reproduces the typical phenotype of key cells found in the liver and their physiological metabolic functions[12–15]. The key cell type focused on in such systems are hepatocytes which comprise the majority of the liver. Liver-on-chip systems have been developed to utilize such cells in

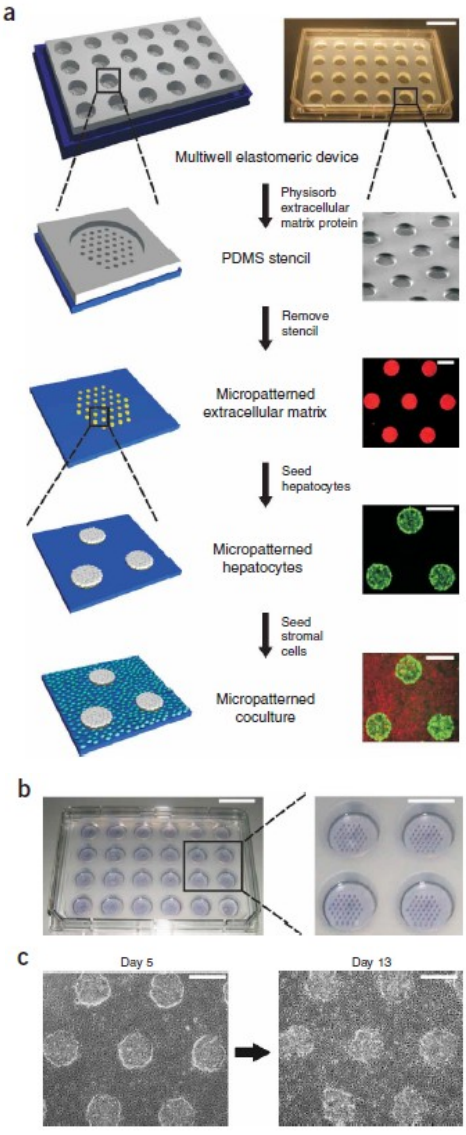

Fig. 10. Soft lithographic process to fabricate microscale liver hepatocyte cultures in a multiwell format. (a) Schematic of the process flow aside photomicrographs taken at each step. A reusable PDMS stencil is seen consisting of membranes with through-holes at the bottom of each well in a 24-well mold. To micropattern all wells simultaneously, one seals the device under dry conditions to a culture substrate. A photograph of a device (scale bar represents 2 cm) sealed to a polystyrene omni-tray is seen along with an electron micrograph of a thin stencil membrane. Each well is incubated with a solution of extracellular matrix protein (ECM) to allow protein to adsorb to the substrate via the through-holes. The stencil is then peeled off leaving micropatterned ECM protein on the substrate (fluorescently labeled collagen pattern). A 24-well PDMS 'blank' lacking membranes is then sealed to the plate before cell seeding (not shown here). Primary hepatocytes selectively adhere to matrix-coated domains, allowing supportive stromal cells to be seeded into the remaining bare areas (hepatocytes labeled green and fibroblasts orange; scale bar is 500 mm). (b) Photograph of a 24-well device with repeating hepatic microstructures (37 colonies of 500-mm diameter in each well), stained purple by MTT. Scale bars, 2 cm and 1 cm for enlargement. (c) Phase-contrast micrographs of micropatterned cocultures. Primary human hepatocytes are spatially arranged in B500-mm collagen coated islands with B1, 200 mm center-to-center spacing, surrounded by 3T3-J2 fibroblasts. Images depict pattern fidelity over several weeks of culture. Scale bars, 500 mm. Reproduced from [10] with permission.

physiologically relevant architectures towards to goals of understanding therapeutic and carrier metabolism and toxicity.

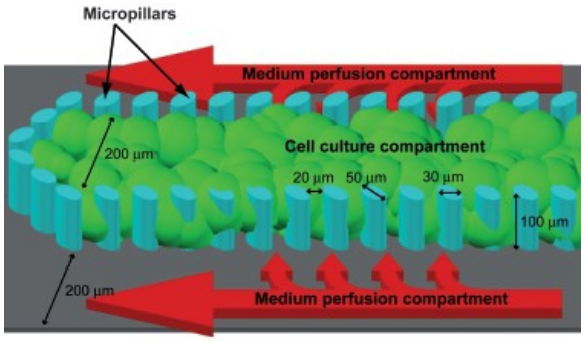

Fig. 11. Magnified view of a single cell culture channel of the multiplexed cell culture chip. An array of 30 _ 50 mm micropillars separated the channel into 3 compartments: a central cell culture compartment and 2 side media perfusion compartments. Reproduced from [12] with permission.

Hepatotoxicity is of great interest and concern when new therapeutics are developed and screened[12–15]. As such, careful studies of IC_{50} or TC_{50} values are carried out which indicate at what concentration a therapeutic produces a 50% inhibitory effect or 50% decrease in mitochondrial activity of hepatocytes[12–15]. Following these standards of testing, micro-culturing platforms have been developed which utilize hepatocytes to identify the toxicity of therapeutic compounds before moving into more complicated testing models as depicted in Figs. 10 and 11. Specifically, Khetani *et al* and Toh *et al* have produced systems capable of growing hepatocyte clusters while allowing for introduction of various therapeutic agents and direct hepatocyte visualization[10,12]. The systems developed by both groups are capable of growing hepatocytes in a confined space which are designed to mimic the general structure of liver sinusoids which can be chemically challenged with therapeutics. In particular, the system developed by Khetani *et al* is particularly well adapted for high throughput screening, where a total of 888 individual tests can be run simultaneously on 24 different drug compounds as depicted in Fig. 10[10]. Due to the nature of the systems, direct cell imaging and access to the chemical contents of the devices, quantification is possible for many proteins produced by hepatocytes such as albumin, urea, and CHC, 4-MUG along with quantification of gene expression[10,12]. In addition to the wide range of quantification possible with such devices, therapeutic testing has been proven possible for many compounds, where mitochondrial activity or cell viability is monitored to determine IC_{50} and TC_{50} values[10,12]. In particular, Toh *et al* has shown good correlation between IC_{50} values obtained from their microfluidic device and LD_{50} values obtained from studies performed in rats[12]. The microfluidic system developed by Toh *et al* also has the key distinction of being able to facilitate physiologically relevant flow or perfusion as would be experineced *in vivo*. The pefusion of drugs through the system as opposed to a static incubation allows for more reliable and realisitc hepatotoxicity perdictions to be made. The presence of the perfusion influences the system in two major and distinct ways. The first being that the hepatocytes grown within the system are subjected to flow and as a result maintain a morphology which is closer to that found *in* vivo. The second influence provided by perfusion within the system is that the drugs being tested are

interacting with hepatocytes in a manner which is very close to *in vivo* conditions. In addition to the capabilities of perfusion within the Toh *et al* system, the cappabilities of estalbishing drug concentration gradients provides a means of uderstanding dose-dependant responses to drugs being tested. However, despite the success of such systems, they tend to lack additional supporting cell types which function alongside hepatocytes in the liver[10,12]. As such these systems would benefit from improved design in order to incorporate such additional cells as Kupffer, stellate, and sinusoidal endothelium in improved structural architectures mimicking that of the real liver. In order to achieve more physiologically relevant structures, a group has turned toward utilizing live liver slices directly integrated into a microfluidic system as seen in Fig. 12.

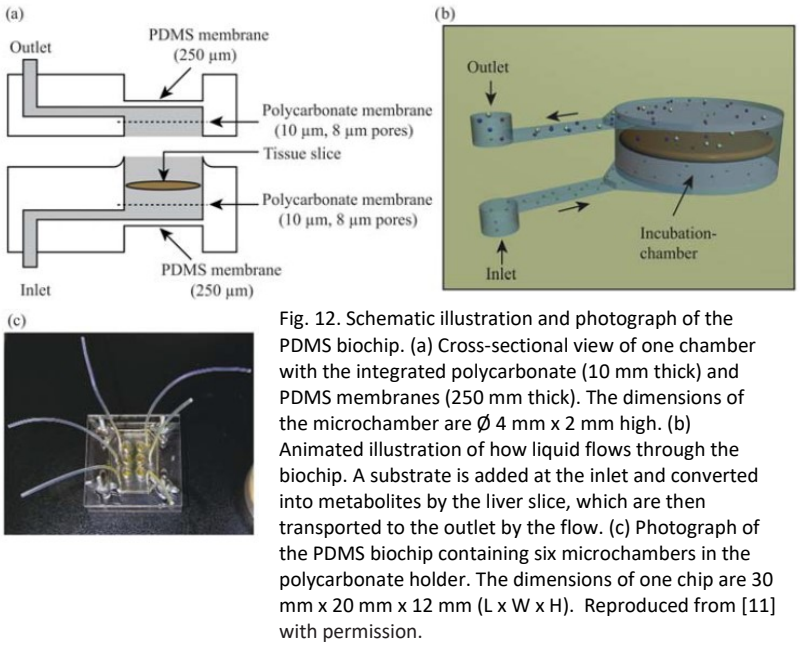

Fig. 12. Schematic illustration and photograph of the PDMS biochip. (a) Cross-sectional view of one chamber with the integrated polycarbonate (10 mm thick) and PDMS membranes (250 mm thick). The dimensions of the microchamber are Ø 4 mm x 2 mm high. (b) Animated illustration of how liquid flows through the biochip. A substrate is added at the inlet and converted into metabolites by the liver slice, which are then transported to the outlet by the flow. (c) Photograph of the PDMS biochip containing six microchambers in the polycarbonate holder. The dimensions of one chip are 30 mm x 20 mm x 12 mm (L x W x H). Reproduced from [11] with permission.

The production of a microfluidic system directly incorporating live slices of liver tissue has been accomplished by van Midwood *et al* which has distinct advantages over the previous two liver-on-chip system discussed[11]. Specifically, the use of liver slices ensures that realistic tissue structure and morphology of hepatocytes is achieved. Liver slices were produced and sealed within microfluidic devices to allow for the flow of therapeutic compounds as a means of studying metabolism[11]. The microfluidic system allowed for the quantification of metabolite formation for the liver slices. The observed results from their work showed that the liver slices grown in the microfluidic system performed similarly to slices grown in standard well plates[11]. The group in turn was able to conclude that the developed microfluidic system was capable of being utilized for metabolism based studies while maintaining high levels of viability[11]. Such a system has the potential to be utilized with various drug and therapeutic targets where resulting metabolites from the liver can be identified and quantified in a continuous flow setup. Further advances for such a system would be the improvement of viability time for the liver slices. Maintaining a continuously stable metabolic rate similar to that of the organ *in vivo* for more than 24 hours would prove to be a large help in further increasing the

capabilities of the system for long term studies of therapeutic metabolism and subsequent metabolite production.

Overall, the current state of liver-on-chip microfluidic systems function well to re-create the metabolism of various compounds and production of various proteins and metabolites. From this information, the viability and function of hepatocytes and slices of liver can be understood and challenged through the addition of various therapeutics and drugs[10–12]. Further advances which would greatly assist many of the liver-on-chip systems are improved microfluidic structures and culturing techniques to more closely mimic liver tissue structure and function. This is possible through the direct incorporation of harvested liver slices, however issues of long-term viability then become an issue[11]. Improved culturing capabilities may one day facilitate the growth of a system which closely mimics liver form and function for prolonged periods of time allowing long-term toxicity studies over many days or weeks. Such capabilities would be beneficial for studying therapeutics designed to target cancerous tumor environments which are typically treated over very long durations. As such, we will next transition focus over to tumor-on-chip systems developed to study drug delivery capabilities and therapeutic effects.

Tumor-On-Chip

Tumor-on-chip systems are often designed to allow for the growth of tumor models in physiologically relevant manners while allowing for the assessment of various therapeutics to observe how effectively they are able to treat the developed cancerous model[17,41,79]. Many of the tumor-on-chip devices employ the use of 3D cell culturing techniques to recreate the architecture and structural relations of solid tumors and neighboring tissues such as blood vessels[39,16,26,35,40,42]. Such microfluidic models aim to provide a means of studying certain aspects of treating a cancerous region within a model which is simplified when compared to the *in vivo* environment[16,18,26,42,43,54].

There are many approaches adopted by tumor-on-chip devices to grow cancerous cells for various testing applications. Both 2D and 3D models have been developed including, cancer cell sheets, multicellular spheroids, multicellular layers, and hollow fibers, with each offering various advantages and disadvantages[16,18,26,42,43,54]. Two dimensional culturing limits the physiological relevance of certain microfluidic systems, however offers improved capabilities when considering direct cellular imaging such as in Fig. 13 [40]. Three dimensional growth systems are capable of more accurately recreating tumor architectures and cellular interactions, processes such as imaging and cell maintenance can be troublesome[16,26,42]. Examples of some three dimensional growth microfluidic devices involve the capture of cancer cells in isolated regions where 3D spheroid formation can occur over time or through the addition of various growth factors[16,26,42]. Microfluidic devices developed by Wu *et al*, Hsiao *et al*, and Ong *et al* have utilized structures designed into the microfluidic devices to capture and retain cancer cells in order to facilitate three dimensional growth[16,26,42]. Such systems demonstrate spheroid

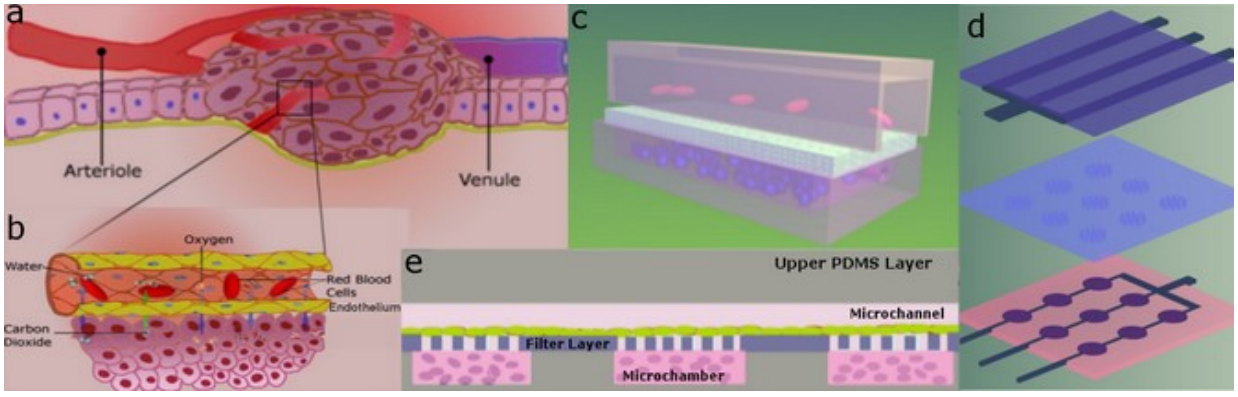

Fig. 13. Schematic drawings of tumor microenvironment and 3D microfluidic cell array (μFCA). (a) Tumor microenvironment including cancer cells, surrounding stromal cells, venules, and arterioles; (b) nutrient and gas transport between microvessels and tumor cells; (c) engineering 3D microenvironment by a layered structure; (d) schematics of each layer of 3D μFCA; and (e) cross-section view of 3D μFCA. The bottom layer has microchambers with cancer cells embedded in hydrogel. The middle layer is a permeable membrane with clustered pores. The upper layer has microchannels with seeded endothelial cells to simulate blood microvessels. Reproduced from [40] with permission.

heterogeneity which is a defining factor of most *in vivo* tumor cases while still allowing for media and therapeutic flow[16,26,42]. Flows of single or combinatory therapeutics can be introduced in order to test the effectiveness of treatments. However, despite such capabilities, such models often lack additional biological barriers typically encountered when delivery anti-cancer therapeutics *in vivo*[16,26,42]. Incorporation of associated surrounding tissues and vasculature would serve to greatly improve the capabilities and predictive nature of such platforms. It is of course recognized that doing so, often greatly increases the complexity of the system. Despite this drawback, the current systems are capable of quantifying the therapeutic effect on cancerous cells by monitoring cell viability and death, 3D cellular morphology, and protein production (albumin and 4-MUG)[16,26,42].

Future capabilities of such tumor-on-chip microfluidic system would benefit from the addition of common biological barriers encountered during drug delivery to tumor sites *in vivo* such as vascular endothelium as seen in Fig. 14 [16,35]. In addition, the timeframes required to establish such system for optimized therapeutic analysis need to occur faster in order to facilitate expedited testing. Current microfluidic system often require several days (5-7 days) to achieve tumor spheroids which are ready to be challenged with therapeutics[16,18,26,42,43,54]. Achieving similar results in a more expedited fashion will open up the possibilities for use of the microfluidic systems in patient-specific applications where personalized therapeutic treatment options can be explored in order to quickly identify an optimal treatment plan. Early treatment of many cancers *in vivo* in turn would lead to improved patient health and survival. Likewise, the application of high throughput testing in these microfluidic devices is beneficial in order to screen large drug libraries against patient derived cancers. One example of a high throughput microfluidic system was developed by Ye *et al* which has shown that large quantities of cancer populations can be grown within a single device for individual testing within 192 cell culturing reservoirs[47]. In addition to being able to test a large quantity of cell populations simultaneously, the system employs a concentration gradient generator which provides a means of testing 8 different drugs each at 8 different concentrations[47]. Such high throughput testing in turn has the potential to improve patient survival via large scale screening of viable therapeutic compounds.

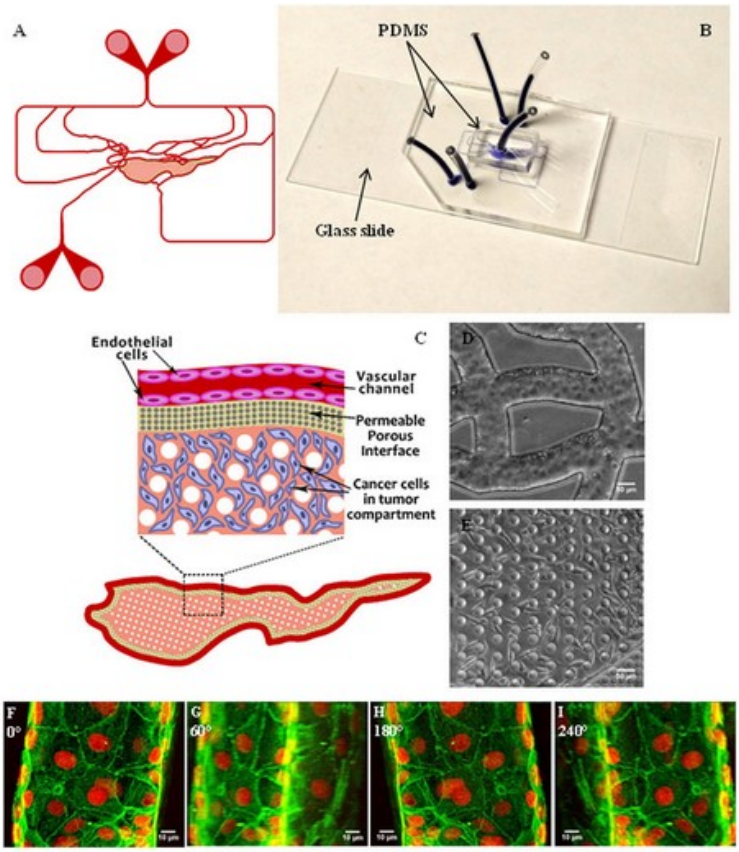

Fig. 14. Schematic of the bMTM (**A**) with magnified view of the vascular compartment, vascular-tumor compartment interface and the tumor compartment (**B**). Optical image of the bMTM (**C**) with HBTAEC cultured in the vascular compartment (**D**) and MDA-MB-231 cultured in the tumor compartment (**E**). HBTAEC cultured under flow in the vascular compartment of bMTM form a complete lumen as shown with 3D reconstruction of confocal images of HBTAEC cultured in bMTM stained with f-actin (green) and Draq5 (red) after 4 days in culture maintained under flow of 0.05 µL/min (**F–I**); images are shown with a Y-axis rotation of 0, 60, 180 and 240 degrees in (**F**, **G**, **H** and **I**) respectively. Reproduced from [35] with permission.

Future Organ-On-Chip Prospects

As the field of organ-on-chip microfluidics continues to expand and mature, the predicative capabilities of such system may one day begin to rival those of their *in vivo* counterparts. Future advances in three dimensional cell growth allowing for highly specified cellular architectures and interactions will aid in improving microfluidic based therapeutic analysis. Such advancements coupled with methods for easily and rapidly imaging and screening such platforms will allow for the automation or semi-automation of running therapeutic analysis. As noted previously, one particular example of high-throughput drug screening produced by Ye *et al* has shown that large quantities of cancer populations can be grown within a single device for individual testing within 192 cell culturing reservoirs[47]. In addition to being able to test a large quantity of cell populations simultaneously, the system employs a concentration gradient generator which provides a means of testing 8 different drugs each at 8 different concentrations[47]. Examples such as the Ye *et al* microfluidic system show that large scale therapeutic screening is possible and serve as inspiration for other organ-on-chip systems in order to improve *in vitro* drug analysis. Overall, improved therapeutic development and delivery is possible through the application of microfluidic testing platforms in the future. The development of more and more highly specialized testing platforms for a broader range of physiological issues and diseases influencing the various organs of the body will help to improve the manner in which diseases are treated in the future. The goal of achieving improved patient health and wellbeing through the applications of microfluidic therapeutic discovery and analysis continues to drive progress and inspire the next generation of researchers in the future.

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