Promises, challenges and future directions of μCCAs

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Micro-cell culture analogs (μCCAs) are a class of in vitro tissue analogs that combine multiple organ analogs on one microfluidic platform in physiologically correct volume ratios. The microfluidic platform also provides fluid flow rates and substance residence times close to those present in the human body. Several advantages arise from the microfluidic format that can be exploited for realistic simulations of drug absorption, metabolism and action. We envision that, together with theoretical modeling, μCCAs may produce reliable predictions of the efficacy of newly developed drugs. Advantages, challenges, and future directions of μCCAs are discussed and examples of systems are provided.
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1. Introduction

Drug discovery has greatly benefitted from the development of in vitro tissues that can be used to screen libraries of chemicals for new drug candidates. Screening experiments are typically carried out in multiwell plates, in which the cell culture based tissues are exposed to drug candidates. Such assays are easy to carry out and allow for quick testing of drug candidates. Because immortalized cells as well as isolated primary cells can change their behavior significantly when cultured in an environment outside the body, the results obtained from these tests are only an estimate of what might happen inside the body. For example, Caco-2 cell systems simulate the uptake of nutrients and drugs through the intestine with sufficient predictability for passively absorbed compounds, but the data obtained with some of these systems for carrier-mediated and paracellularly transported compounds indicate that they do not always accurately reflect the in vivo uptake of these compounds (Stewart et al., 1995). Further, the multi-cellular, three-dimensional architecture of organs necessary for authentic cell function is not present in multiwell plates. On the other end of the spectrum of drug screening tools are whole, perfused animal organs. Technologies have been developed that allow whole organs to stay intact ex vivo and maintain a viable state for a reasonable period of time (for example, see Bekersky and Colburn, 1981; Feldhoff et al., 1977). In comparison to in vitro tissues, perfused, whole organs provide testing conditions that are often much closer to those present in vivo. Their preserved three-dimensional structure, native cellular architecture, and the presence of vasculature and substances such as hormones all support a more authentic cell behavior. However, dynamic regulatory changes due to nervous and immune regulations may not be captured even with these pseudo organs (Leung, 2009). Perfused organs are also typically isolated and hence do not provide a realistic simulation of drug metabolism if several organs participate in a particular drug's metabolism.

In this paper we are reviewing a relatively new, in vitro drug screening tool that provides an intermediate between cell-based assays and experimentation with perfused organs. Micro-cell culture analogs (μCCAs) are a particular class of in vitro tissue models in which one drug can challenge a combination of several tissues arranged in physiologically correct order. Microfabrication has provided the opportunity to integrate these tissues into microfluidic devices. Because of the small dimensions of microfluidic structures, the cells cultured within μCCAs experience physiological conditions such as realistic liquid to cell ratios, fluid residence times that approach those seen in vivo, and physiological shear forces, typically below 2 dynes/cm². Assumptions made for theoretical, physiologically based pharmacokinetic (PBPK) models provide guidance to correctly design the geometry of μCCAs (see Fig. 1). According to these assumptions, organs can be represented as compartments that absorb, metabolize, and excrete substances. Differential equations describe these processes in the theoretical model. In μCCAs, which can be thought of as physical representations of PBPK models, microfluidic chambers represent each organ, fluidic channels provide in and outflow, and the cells cultured within provide the metabolic function. If the cells have retained most of their original function, they exhibit their complete metabolism while challenged with a chemical or drug. Hence they include reactions unknown to us and therefore not included in
theoretical models. The physiologic design of μCCAs with its combination of organs and microfluidic characteristics provides μCCA with advantages over other in vitro tissue models.

Since the first successful design of a μCCA (Sin et al., 2004), several other μCCAs have been developed. They demonstrate the described concept and show that complex mechanisms can be simulated with them. Here we provide a brief review of their advantages as well as an outlook into the future of μCCA development.

2. Advantages

Drug development is an expensive undertaking that benefits from screening identified drug candidates with cell culture based in vitro assays. Such assays provide data that inform researchers whether to pursue the development of a particular drug candidate or not. In addition to conventional in vitro tissue models, microcell culture analogs can provide an opportunity to test drugs in a more complex environment that includes analogs of several types of tissues. Advantages of μCCAs that derive from their microfluidic format are outlined below and examples of devices that demonstrate these are provided.

The presence of several organ analogs in μCCAs allows us to investigate their synergistic response to a drug. Often, a drug will produce the desired therapeutic effect in the target organ, but its metabolites cause unwanted side effects in other organs. In μCCAs, the systemic circulation represented by microfluidic channels carries metabolites to other tissue compartments where cell stress can be monitored. We can reproduce such interactions in μCCAs that contain several relevant organ compartments. To demonstrate this, we have challenged these μCCAs with known toxins/drugs such as naphthalene (Viravaidya et al., 2004; Viravaidya and Shuler, 2004) and acetaminophen (Mahler et al., 2009). When the μCCA contained both liver and lung compartments, liver cells metabolized naphthalene to reactive metabolites (1,2-naphthoquinone and 1,2-naphthalenediol), which subsequently travelled to the lung compartment. Here the metabolites caused cell stress and decreased lung cell viability. Another μCCA designed with compartments for liver and gastrointestinal tract analogs, simulated the absorption of acetaminophen and its conversion within epithelial cells of the digestive system analog as well as the liver analog. The experiment replicated a dose-dependent toxic effect of acetaminophen and its metabolites on liver cell viability (Mahler et al., 2009). The results obtained with the μCCA was comparable to those published for in vivo studies with mice (Kola and Landis, 2004). These experiments have demonstrated that toxicological problems that involve two interacting tissues can be reliably addressed with μCCAs.

Since μCCAs are designed with an emphasis on recreating physiological relationships between organ compartments, and physiological fluid flow and drug residence times, it is possible to design experiments in which realistic drug concentrations can be tested. Compared to other in vitro systems, the liquid to cell ratio in μCCAs is closer to that found in vivo and the tested drugs come into contact with a realistic number of cells. Therefore, drug absorption and metabolism take place at a rate, closer to those seen in vivo. Potentially, results obtained from such experiments will let us eliminate those concentration ranges that cause harmful side effects, and estimate concentrations that are potentially of benefit to humans. Therefore the parameter space that is investigated further with animal experiments is reduced, potentially leading to fewer experiments needed to identify successful drug candidates and their effective concentrations. This is particularly useful if combinations of drugs need to be tested, as might be the case for the development of drug combinations needed to treat multidrug resistant cancer. When combining drugs, their concentrations as well as the order in which they are administered play an important role in the effect they cause. Knowledge about the effective concentrations ranges, eliminates unsuccessful drug combinations. Hence the required number of experiments could potentially be decreased.

We have also observed that testing combinations of drugs with μCCAs can reveal unexpected synergistic effects that cannot be seen in conventional multiwall plates. Experimenting with multidrug resistant (MDR) cancer cell lines (uterine cancer cell line MES-SA and its multidrug resistant variant, MES-SA/DX-5), we have found that combinations of three drugs that are not typically used in combination with each other are more effective in diminishing...
tumor cell growth than each drug would be on its own (Tatosian and Shuler, 2009). While doxorubicin is a chemotherapeutic, the MDR suppressing activity of cyclosporine, clinically used as an immune system suppressor, and nicardipine, a β-channel blocker was incidental to their normal pharmacological use. Remarkably, their toxic effect was specific to the MDR cell line. While all drugs decreased the viability of the other two cell lines present in the μCCA-HepG2/C3A, representing the liver and metabolism of drugs and a megakaryoblast cell line (MEG-01) representing cells responsible for platelet formation – the combination decreased cell death only in the MDR cell line. The concentrations used were only slightly higher than what is used clinically. The observation of a selective synergistic interaction of MDR suppressors suggests that the use of a combination of MDR suppressors, as suggested earlier by Pascaud et al. (1998) and Lehnter et al. (1991), might result in a useful therapeutic window. While we do not suggest that this combination of drugs should be used for multidrug resistant cancer treatment, we believe that the results of this experiment shows that, using μCCAs, we can investigate combinations of drugs and uncover their synergistic actions. Since μCCAs platforms are microfluidic platforms, many combination experiments can be conducted in parallel, testing their efficacy and toxicity in a rapid, inexpensive manner.

Another combination of drugs studied with μCCAs is that of Tegafur and uracil (Sung and Shuler, 2009a). In the human body as well as on the μCCA, P450 monoxygenases, residing in the liver, convert Tegafur, a produg, to 5-fluorouracil (5-FU). 5-FU is released from the liver compartment and reaches the “colon cancer” cell line HT-116 via the systemic circulation of the μCCA. The compound inhibits DNA and RNA synthesis as well as the activity of the enzyme thymidylate synthetase and thereby prevents the cancer cells from proliferating. The enzyme dihydrouracil dehydrogenase (DPD) degrades 5-FU, a process that can be inhibited by its competitive inhibitor uracil. The results for combinations of Tegafur and uracil observed on the μCCA were analogous to those observed in clinical applications where the growth of colon cancer could be suppressed. Further, the μCCA experiments predicted that hematological toxicity would be a more important side effect than hepatotoxicity. The outcome of this experiment shows that clinical results can be simulated using μCCAs.

While theoretical simulations and animal experiments can predict the outcome of clinical trials with humans, μCCAs might provide an additional tool that can be employed for this purpose. Results from μCCA may not simply be replications of those obtained with animal experiments. Micro-CCAs can be operated with human cell lines and potentially simulate human metabolism better than animals. Data from animals cannot, without hesitation, be transferred to humans. This is due to the difference in human and animal physiology and metabolism and is reflected in the fact that only 10% of drugs tested on animals perform as expected in clinical tests with humans (Kola and Landis, 2004). Experimenting with μCCAs could complement current drug testing methods, contribute to identifying the most successful drug candidates, and potentially reduce the number of animal experiments needed to develop a new drug.

3. Challenges

Supplying a drug directly to the μCCA simulates intravenous drug administration, which is suitable for testing the therapeutic actions of many new drug candidates. However, often drugs are administered orally, through inhalation, or through application to tissues such as the skin or buccal mucosa of the cheek. These tissues pose barriers to the drug it must be able to pass to enter systemic circulation. Its chemical structure should allow its absorption through the tissue but still permit its full therapeutic function. Experimenting with μCCAs that contain models of barrier tissues would allow the simulation of absorption of drugs and their subsequent effects. Models of barrier tissues in microfluidic format have been developed recently. A few of these models have been used in combination with μCCAs. For example, models of the gastrointestinal tract were combined with liver tissue analogs to simulate first pass metabolism toxins and drugs such as acetaminophen (Mahler et al., 2009).

The inclusion of barrier tissues into μCCAs is challenging, because the cells making up the barrier tissue must be cultured on porous membranes that give support to the cells and allow metabolites to transfer from one side of the tissue to the other. Commercially available multiwall plates with membrane inserts have been used (Mahler et al., 2009; Choi et al., 2004a, 2004b), but their standardized sizes and shapes limit the geometric options for designing gastrointestinal tissue chambers. This problem can be addressed by constructing membranes directly on microfluidic platforms so that barrier tissue chambers can be incorporated into the general μCCAs design. An additional challenge lies in operating systems with such barrier tissue models. The apical side of the gastrointestinal tract model, for example, requires a second fluidic circuit used to simulate nutrient supply. This additional circuit needs to be carefully balanced with respect to the pressure applied to the basolateral/systemic circulation circuit so that no unnatural mechanical forces act on the cells. Despite this challenge, the presence of fluidic flow might benefit to the overall function of barrier tissues since the application of shear stress on cells capability has been shown to support the establishment of tight junction complexes (Chien, 2007).

Another barrier tissue of interest to drug developers is the blood–brain barrier (BBB). The blood–brain barrier consists of tightly connected endothelial cells that line the capillaries of the brain. The endothelial lining prevents 100% of large molecule drugs and 98% of small molecule drugs from entering the brain (Pardridge, 2001). Experimenting with μCCAs that include a physiologically authentic model of the blood–brain barrier would enable the prediction BBB permeability of drugs that aim to treat brain diseases as well as the neurotoxicity of drugs that were developed to treat other diseases in the body. However, developing a model of the BBB is challenging because most brain endothelial cells form only incomplete tight junctions when cultured outside the body (Rist et al., 1997). The environmental, mechanical, and chemical cues that exist in the brain are vitally important to the cell’s behavior. It has been suggested that the tight junction functionality in vitro cultures could be improved by applying glial factors, phosphodiesterase inhibitors, compounds that increase intracellular cAMP (Rubin et al., 1991), and hydrocortisone (Franke et al., 1999). However, these factors could cause the barrier to develop non-physiological characteristics. Other studies suggest that culturing endothelial cells in close contact with astrocytes/astrogliomal cells helps to restore the barrier function (Bauer and Bauer, 2000; Hayashi et al., 1997). For this purpose, the two cell types are often cultured on opposite sides of porous membranes. Commercially available membranes have a maximum porosity of 15%, are relatively thick (10 μm), and only allow limited contact between the two cell types. Microfabricated membranes that are thinner and have a higher porosity such as those fabricated by Harris and Shuler (2003) might allow for a better contact between the cells.

A key challenge in mimicking complex multi-organ interactions in μCCAs is to achieve authentic cell function. Each cell type should function like the cells of the corresponding organ in vivo. For example, a liver chamber in a μCCA should perform the role of biotransformation to a level that is comparable to that of the liver. Cells from immortalized liver cell lines, however, often contain low levels of metabolizing enzymes, compared to the cells of the actual liver (Wilkening et al., 2003). Primary hepatocytes retain metabolic functionality after separation, but only for a limited time.
(Gomez-Lechon et al., 2003). Further, as normal tissues are composed of many different cell types with different functions, in vitro cell layers should also consist of several cells types. For example, a pure Caco-2 cell model behaves different than a Caco-2/HT29-MTX co-culture model in which the epithelial cell layer is covered with a mucous layer. The mucous layer has been shown to play a role in metal ion absorption and has had an effect on iron absorption (Rubin et al., 1991; Franke et al., 1999).

In the mammalian tissue environment, cells reside in a complex three-dimensional structure, which consists of neighboring cells and the extracellular matrix (ECM) that provides structural support. This 3-D organization provides cells with biochemical and mechanical signals, which often play a vital role in their growth and differentiation (Abbott, 2003). When cells are cultured in vitro, they typically grow on flat surfaces, in 2-D monolayers. An increasing number of studies show that the behavior of cells is drastically different when they are cultured as two-dimensional layers or three-dimensional constructs. For example, breast cancer cells can revert to their original non-cancerous state when treated with antibodies against the cell surface receptor β1-integrin when grown in 3-D, but not when cultured in 2-D layers (Weaver et al., 1997). Three-dimensional cell culture also enhances the hematopoietic efficacy of embryonic stem cells (Liu and Roy, 2005). These studies suggest that cells cultured in a three-dimensional construct within μCCAs will behave more authentically than the cells grown in 2-D monolayers.

Given the importance of 3-D cell culture, a significant amount of effort has been directed towards the development of 3-D scaffolds that can provide similar biochemical and mechanical cues as found in native tissues (see Lee et al., 2008 for a review). Many of these scaffolds (but not all) are hydrogels (Cushing and Anseth, 2007). One of the most popular hydrogels is Matrigel, which is a natural hydrogel derived from mouse tumor cells (Kleinman et al., 1982). Another similar type of hydrogel is collagen, which is actually most prevalent protein in ECM. The advantage these natural hydrogels have in comparison to synthetic hydrogels, is that they provide various biochemical signals that are present in native tissue, promoting cellular interactions. However, being a natural product, the complexity and batch-to-batch variability often hinders accurate and quantitative characterization of cellular behavior in these matrices. Cells can also be cultured in synthetic hydrogels, such as polyethylene glycol (PEG). The advantage of synthetic hydrogels is that it is better characterized, more consistent and it is easier to manipulate their mechanical properties than natural hydrogels. On the other hand, they do not provide functional sites for cellular interactions, although it is possible to link biomimetic cues, such as a RGD peptide, with the backbones of synthetic hydrogels to render them more ‘cell-friendly’ (Benton et al., 2009).

When 3-D cell culture is combined with a microfluidic environment the spatial and temporal control of molecules allows one to create a physiologically realistic environment. For example, the oxygen concentration is known to play an important role in modulating the metabolic functions of liver cells, and it has been demonstrated that the oxygen gradient generated in microfluidic devices can enhance the metabolic function of cultured liver cells (Allen et al., 2005). Furthermore, the flow inside microfluidic systems can be manipulated easily to give cells proper mechanical stimuli, which often affects cell function (Nerem et al., 1998; Keane et al., 2003; Sivaraman et al., 2005) conducted a study that demonstrates the capability of the microfluidic environment to elicit authentic cell function. Gene expression, protein expression and biochemical activity of primary rat hepatocytes, cultured in a microscale 3-D cell culture system with medium perfusion, were closer to those observed in cells of the in vivo liver than in cells cultured in conventional in vitro environments, such as collagen sandwich culture or Matrigel culture.

In our studies, we have used Matrigel as an encapsulating matrix for colon tumor and liver cells separately, and alginate to encapsulate myeloblasts. We utilized the contraction of Matrigel upon gel formation to create space for medium flow on top of the matrix, while alginate in a cylindrical shape was inserted into a chamber with medium flow around the gel. Inclusion of Tegafur in the circulating media caused a drop in the cell viability, but only in the presence of liver cells. In a comparison study using 96-well plates and the same drugs, cytotoxicity of 5-FU was observed, but Tegafur was not toxic to tumor cells because of the lack of metabolic conversion to 5-FU. A similar gel insertion method was utilized to assess the P450 enzyme activity of HepG2/C3A cells cultured in a μCCA (J.H. Sung et al., 2009). A comparison with the same cells cultured in 96-well plates showed that the cells cultured in the μCCA exhibited a higher P450 enzyme activity than the cells in the 2-D, static environment. To further improve the usability of μCCA devices with 3-D cell cultures, their design was modified so that the cell culture chambers were separated from the medium supplying fluidic channels. A PDMS layer containing fluidic channels is stacked on top of a silicon layer with holes into which hydrogel-encapsulated cells can be inserted. Using this method, various thicknesses and sizes of cell-hydrogel matrices can be inserted into the device (Sung et al., 2010).

While 3-D cell cultures are advantageous in terms of eliciting authentic cell functions, culturing cells in three-dimension creates challenges, especially if the constructs are to be located inside microfluidic systems. Inserting a 3-D matrix of cells into a microscale device poses a significant challenge and requires elaborate schemes. During the last five years, a number of novel methods have been developed to address this challenge. One of the earliest attempts was made by Leclerc et al. (2003), who cultured the hepatoma cell line HepG2 in a microfluidic device made of two stacked layers of PDMS. However, the cells were simply confined in 3-D structures, but the confinement did not enhance cellular interactions. Tan and Desai (2004) used surface modifications to immobilize cell-collagen matrices inside microfluidic devices. By repeating the procedure, several cell-matrix-layers could be stacked on top of each other. In another proof-of-concept study, vascular cell types were encapsulated in a hydrogel matrix to construct biomimetic blood vessels (Tan and Desai, 2005).

To localize cells in microfluidic culture systems the gelling mechanism of Matrigel has been exploited (Frisk et al., 2005). At low temperatures, Matrigel maintains its liquid state and the material, mixed with cells, can be injected into a microfluidic device. The cell-Matrigel matrix that fills up the chamber contracts upon temperature increase, providing space for medium perfusion. By applying two parallel flow streams on opposite sides of the matrix, stable concentration gradients can be formed (Frisk et al., 2007). Several other studies have shown that laminar flow can be used to incorporate 3-D hydrogel cell cultures into microfluidic devices. Kim et al. (2007) used a microfluidic device to hydrodynamically focus the self-assembling peptide Puramatrix, and to immobilize cells in 3-D configuration. Wong et al. (2008) developed a microfluidic device that utilizes laminar flow to divide a fluidic channel into multiple sub-channels of hydrogel matrix containing cells. Using this device, the authors created a co-culture of two cell types within a slab of hydrogel that enabled intercellular communication.

Despite these novel methods, more systematic and in-depth studies are required to achieve 3-D cell cultures in which cells can be maintained for a prolonged periods of time as well as to understand the physical properties of the gels that influence gelling and cellular behavior within the matrices. In a recent study by Huang et al., several factors affecting the process of confining 3-D hydrogel matrices in microfluidic environments were examined with mathematical modeling. Surface tension, hydrophobic interactions, and spatial geometry were listed as important factors (Huang et
al., 2009). Multiple gel types were patterned side-by-side encapsulating different cell types, and invasion of one cell type into neighboring gels was observed. K.E. Sung et al. (2009) examined the polymerization of collagen in a microfluidic environment, and compared the viability and growth of cells in collagen matrices formed in a conventional multiwell format and in a microfluidic environment. Such studies by Huang and Sung will facilitate more consistent and reproducible incorporation of 3-D hydrogel cell matrix into microfluidic systems.

In addition to the technical challenge of constructing 3-D cell cultures, the challenge of characterizing and monitoring cellular status arises when working with 3-D cell cultures. Methods to measuring cell viability in two and three-dimensions have been discussed by Ng et al. (2005), with a comparison of four different approaches. The most widely used method to characterize 3-D cell constructs is confocal microscopy with fluorescence labeling. However, observing cells in a running microfluidic system with a confocal microscope is difficult. We are therefore developing a small, custom-made optical detection system that can measure fluorescence across the depth (z-axis) of 3-D matrix and that can be easily coupled with a microfluidic system inside a cell culture incubator. Various approaches have been developed for either coupling an optical detection system with a microfluidic device, or directly integrating optical components into a microfluidic device (Kuswandhi et al., 2007; Mogensen and Kutter, 2009). These approaches will potentially be useful for better characterization of cellular status inside microfluidic systems.

4. Future directions

The fabrication of μCCA devices is relatively inexpensive and can be optimized so that generic devices are available to health care providers. This makes it feasible to consider their use for providing individualized health care. Since the level of enzymes and other metabolites can differ from person to person, everyone responds differently to drugs. For routine use, we envision that the devices could be prepared with in vitro tissue samples or tissue slices that can be stored until the devices are needed. Preparation of such tissue samples and their storage is an important research area that needs to be investigated more.

Micro-CCA systems are small and require only a small amount of reagents and cell mass to conduct a test. It is conceivable that a small tissue sample could be taken from a patient (biopsy) and tested for the efficacy of a mixture of drugs. Testing drugs on a patient’s tissue sample in a μCCA is a potential tool to individualize treatment when the patient is fragile and in need of effective treatment without high risk of side effects. The best options for treatment could also be determined with μCCAs when individuals suffer from diseases such as cancer where the individual response to a particular drug regime may be highly variable.

In order for μCCAs to be used by health care providers it would be beneficial if the devices contained a variety of sensors that would enable the continuous monitoring of the physiology of each cell type in addition to measuring cell viability. The sensors must be reliable and easy to read. Cell stress in general and the presence of particular metabolites would indicate the actions of drugs in the μCCA. For example, an oxygen sensor such as that utilized by Sin et al. (2004), could be used to measure the rate of oxygen consumption by liver cells on the chip as a measure of their metabolic activity. Since the field of micro-biosensors has grown immensely during the past few years, a large number of sensors that are suitable for monitoring μCCAs are now available (for a review see Erickson et al., 2008). Among these are sensors that record optical, electrical, and electrochemical signals. The performance of current μCCA systems would be enhanced and their operation in routine diagnostic laboratories would be easier if a variety of such sensors would be installed on the platforms.

At the same time, computerized control of medium flow and pressure within the microfluidic circuit and built-in pumps would add to the simplicity of use of μCCA systems. Aiming to simplify the μCCA operation, Sung et al. have developed a bubble trap (Sung and Shuler, 2009b) as well as a fluid “pump” mechanism based on gravity driven flow (J.H. Sung et al., 2009) that allows the devices to be operated without the relatively bulky peristaltic pumps routinely used. Such tubing-less pump mechanisms add to the suitability of the devices for point of care use.

The operation of μCCAs would also benefit greatly if the devices could be operated for prolonged periods of time. Currently, μCCA devices are capable of operating for 72 h without loss of function. After 72 h metabolic waste products accumulate and decrease the viability of cells. Efforts to increase the lifetime of μCCAs will require a focus on both, the removal of metabolic waste products and on replenishing the amount of consumed nutrients in the blood surrogate. On-chip the metabolic waste products might be removed from the blood surrogate stream using a microfluidic dialysis process. Replenishing nutrients requires the removal of depleted medium and replacing it with fresh medium. Both processes would require the insertion of additional microfluidic loops, again emphasizing the need for precise pressure and fluid flow controls.

5. Conclusions

Experimenting with μCCAs is much less expensive than experimenting with animals and allows a larger experimental space to be explored. The microfluidic format and the presence of several interconnected tissue chambers afford the devices several advantages compared to others in vitro drug screening models. The biggest challenges when developing μCCAs lie in recreating authentic cell behavior on the microfluidic platform and in developing sensors that can monitor the physiology of cells within three-dimensional tissue constructs. Our vision for μCCAs is that they could be used as drug screening devices that provide additional information to researchers who must decide whether to invest effort and resources in a particular drug candidate. Individualized patient care may also be possible with μCCAs that might be used to test biopsy samples for their reactions to drugs or mixtures of drugs. To achieve this goal the devices would need to be designed so that their operation would be easy and reliable. Tissue constructs that could be stored and revitalized on the devices would be ideal for this application.

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