Lab on a Chip

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HIGHLIGHT

Research highlights

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Engineering a human "gut-on-a-chip"

The development of engineered tissues inside microfludic systems is an emerging field that combines microfabrication technologies with tissue engineering to create *in vitro* tissues with the ultimate goal of mimicking human physiology for aiding the drug discovery process.[1,2] Despite the development of various types of tissues on a chip (spleen, lung, marrow, liver, muscle, cardiac, *etc*), it has been difficult to generate a functional gut epithelium. A major reason for this is the difficulty in adequately mimicking the native gastrointestinal environment, including the intricate topography of the intestines, the flow of liquids, cellular deformation and the presence of microbes.[3–5] Ingber and coworkers have recently developed a microfluidics-based technology that mimics the architecture of the human intestine. Namely, the device by Kim *et al.*[6] utilizes continuous perfusion of the developing tissue with cyclic stretching to mimic the intestinal peristaltic motion.

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The device was fabricated from poly (dimethylsiloxane) (PDMS) and included a main fluidic channel and a cell culture channel, which was separated into two sections, an upper and a lower channel. The two sections were separated by a perforated PDMS membrane (pore size: 10 μm). The upper channel was first coated with a mixture of collagen and Matrigel and subsequently seeded with human intestinal epithelial cells (Caco-2 cells), such that the cells proliferated on the surface of the membrane (Fig. 1b). Adjacent to the main channel were two vacuum chambers. When vacuum was applied to these chambers, the main channel was stretched laterally and strain was applied to the membrane (Fig. 1c). After the cells formed a monolayer on the membrane, both a cyclic stretch (0.15 Hz and 10% strain) and flow of the perfusion medium were applied simultaneously. For comparison, static culture experiments were conducted in transwell plates (Fig. 1a). In addition, continuous perfusion studies were performed without the dynamic stretching component.

At an applied flow rate of 30 μl h^{-1}, the cells experienced a shear stress comparable to those observed under physiological conditions. Here, the cells formed polygonal epithelial layers and close junctions within 3 days of culture. In the static case, the same cellular morphology was observed after 3 weeks. Furthermore, the cells cultured inside the microchannels developed into vertical columnar structures, similar to *in vivo*, but the cells that were cultured inside the transwells remained flat (Fig. 1a–c). The application of continuous shear stress to the cells was thought to be responsible for this development, however, a change in cell shape was only observed while varying the flow rate from 0 to 30 μl h^{-1}. Above a flow rate of 30 μl h^{-1}, the cells did not show accelerated changes in morphology.

Even more interesting were the effects of the cyclic strain applied to the cells. Specifically, the cells cultured under this condition exhibited paracellular permeability that was several times larger than the zero-strain case. Furthermore, the cells began to organize spontaneously into villous structures after 7 days of cyclic stretch, while no villi were observed in the absence of the applied stretch. Importantly, when a *Lactobacillus rhamnosus*, a human gut microbe, was introduced after a few days of culture, both the Caco-2 cells and the microbes remained attached to the stretched substrate. In fact, the presence of the microbes helped improve the integrity of the intestinal epithelial barrier. Also, due to the continuously flowing medium, excess

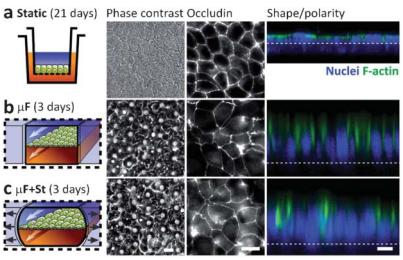

Fig. 1 The differences in the formation of the gut epithelium between a static culture system (a), culture inside a microfluidic channel with flow (b) and with the addition of cyclic strain (c). For each case a schematic of the bioreactor is shown, in addition to phase contrast, occludin fluorescence, and confocal fluorescence images of the epithelium. The confocal images display a cross-section of the cell layer, indicating the cell shape and polarity. Figure adapted and reprinted with permission from the Royal Society of Chemistry from Kim *et al.*[6]

microbes were easily removed. Thus, the microbe population was kept in balance with the intestinal cells. This was in stark contrast to static culture experiments, in which fast growing microbe populations could not be removed, leading to a strong decrease in the solution pH causing death of epithelial cells.

The human "gut-on-a-chip" is a promising development in the application of microtechnologies to create biomimetic organs on a chip. Since the fabrication and operation of the device is relatively simple, it carries a strong potential for rapid translation to clinical research. Studies that would greatly benefit from the application of this device include those focusing on absorption anomalies such as gluten intolerance (Celiac disease) and sucrose or lactose intolerance, as well as structural problems, *e.g.* Blind Loop Syndrome, in which part of the intestine is obstructed.

Origami for microfluidics

In the last few years, the field of microfluidics has permeated many research disciplines. However, microfluidic devices often have a single experimental purpose and require skill in their design, fabrication and operation, which inhibit their widespread use. More recently, efforts have been made to simplify device fabrication and handling through the use of paper microfluidics. The advantages of using water-wicking paper channels for fluid flow instead of externally powered designs include portability and low manufacturing cost. Hence, paper microfluidic chips are particularly well suited for chemical assays (*e.g.* in diagnostic applications) in developing or resource scarce regions.[7–8]

Liu and Crooks have recently introduced a particularly simple method of fabricating paper microfluidic chips.[7] Instead of individually patterning several layers of paper with photoresist and then gluing them together, they patterned a single sheet of 100 μm thick chromatography paper and subsequently folded it several times to form a 3D microfluidic channel. All paper folds (up to 9 folds in certain devices) were in contact with each other, enabling vertical movement of liquids. For stability, the folded paper device was inserted into an aluminium box with access holes. Thus, a multi-layer device was fabricated in only a few minutes in three simple steps.

Aqueous solutions were introduced into reservoirs (1.25 mm radius) through openings in the aluminium box and followed the fluidic channels (900 μm wide) without any leaks. They ultimately reached reservoirs at the bottom of the folded device, which had been loaded with analyte suspensions and dried prior to folding (Fig. 2). The introduced solutions dissolved and reacted with the analytes, generating reaction products that could be visualized upon unfolding of the device. Among the tested analytes were glucose and bovine serum albumin (BSA), both of which were suitable for colorimetric analysis. Here, the concentration of the respective molecule could be determined from the color of the reaction byproducts. The researchers also conducted four independent BSA assays on a single device utilizing a fluorescent, BSA-sensitive dye. Upon unfolding, a fluorescence imager was used to capture the signal indicating the presence of a reaction. The detection limit was determined to be on the order of 0.1 μM.

The paper-folding approach to generating microfluidic devices could potentially become the preferred fabrication method for diagnostic chips in point of care applications. It should be straightforward to extend the number of folds in order to allow for complex fluidic paths, without adding extra complexity to the fabrication process. However, for this class of devices to be useful for a variety of applications including DNA assays *etc.*, issues involving environmental robustness of the dried reagents (*e.g.* against changes in temperature and humidity) still need to be evaluated.

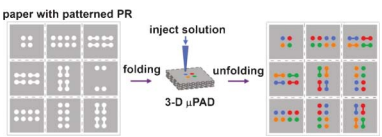

Fig. 2 Schematic of the 3D paper origami: A paper microfluidic device is fabricated by patterning with photoresist, folded, loaded with aqueous solutions, and unfolded to reveal specific reservoirs filled with the solutions. Figure reprinted with permission from the Journal of the American Chemical Society from Liu and Crooks.[7]

Predictive microfluidic modeling of vaso-occlusive processes

Though biomarkers have proven invaluable in disease diagnostics and the development of novel therapeutics, the discovery of such markers that correlate reliably with clinical outcomes has proven particularly elusive for some diseases. One such disorder is sickle cell disease (SCD) where oxygen-deprived misshapen red blood cells (RBCs) block capillary blood flow in a painful and damaging process known as vaso-occlusion. While SCD can be detected through molecular and genetic testing, there is currently no reliable method to predict the clinical response of a patient with the data from existing screening techniques. This predictive task is further complicated by the fact that SCD involves multiple processes spanning a variety of length and time scales, including hemoglobin polymerization, morphological changes of the RBCs, and increases in apparent viscosity of the blood, none of which fully correlate with vaso-occlusion rates when considered individually. Diagnostic approaches that integrate many of these interdependent processes into a single *in vitro* model will likely have much more success in predicting clinical outcomes.[8]

By developing a microfluidic device that simulates RBCs in capillary flow over physiologically relevant length and time scales and recapitulates and integrates several key parameters of the native environment, Wood *et al.* have recently determined a biophysical indicator capable of predicting the severity of vaso-occlusion in patients with SCD.[9] In their approach, blood specimens were transported under constant pressure through synthetic 10 μm × 15 μm "capillaries" within a PDMS-based device, where the amount of oxygen present in the system was varied dynamically. As the blood cells become deoxygenated in response to user-controlled atmospheric gas makeup, their hemoglobin polymerized resulting in stiff misshapen cells characteristic of SCD which showed a resistance to capillary flow. The authors quantified this oxygen-dependent resistance to flow in the form of blood conductance (defined as velocity per unit pressure drop), and observed that the rate at which this conductance changed in response to oxygen removal served as a

unique biophysical parameter that could be used to predict the severity of SCD in a patient.

Interestingly, the authors used the measured rate of conductance decrease to identify "benign" *versus* "severe" samples successfully (23 severe, 6 benign), without having to rely on more traditional and less accurate methods based on hematocrit levels and white blood cell (WBC) counts. They also demonstrated that the rate of conductance decrease was reduced when the cells from the patients were treated with a small molecule that decreases sickle haemoglobin polymerization. This further validates this biophysical parameter as a predictor for vaso-occlusive risk and suggests that this *ex vivo* microfluidic-based test could be used to study the response of a specific patient to existing therapeutics (*i.e.*, personalized medicine) or to more broadly screen the efficacy of novel drug candidates.

In a separate study by Lam and coworkers, a similar PDMS-based microfluidic microvascular model was developed to examine the pathophysiological process of vaso-occlusion.[10] Although this work also sought to investigate how biophysical alterations lead to microvascular occlusion in patients with SCD, a key difference lied in their introduction of "endothelialized" capillaries (*i.e.*, channels that have been lined with endothelial cells). By flowing a solution of endothelial cells through their device and allowing them to attach to all sides of the channel and grow to confluency prior to introducing whole blood, they created a lumen-containing construct uniformly lined with an endothelial monolayer. This captures another critical aspect of the native microcirculation environment and enables the researchers to identify specific pathophysiological characteristics related to the interactions between blood and endothelial cells in a geometrically-controlled environment.

In their proof-of-concept experiment highlighting the platform's ability to investigate pathological processes that involve both blood and endothelial cells, Tsai *et al.* compared the levels of microchannel occlusion in response to different treatments of TNF-α, an inflammatory cytokine known to cause upregulation of cell surface expression of adhesion molecules.[10] Here, the highest level of channel occlusion was observed when both blood cells and the endothelial cells were treated with TNF-α as compared with just TNF-α-activated endothelial cells and nonactivated blood or fully untreated cells. This suggests that there may be a synergistic role between WBC activation and cytokine-mediated endothelial adhesion in the pathophysiology of inflammatory disorders.

While the platform developed by Lam and coworkers enables studies to be performed inside capillaries with an endothelium lining, the system by Bhatia permits the oxygen content within the system to be dynamically regulated in a user-controlled manner. Taken together, these complementary approaches each represent major advances in the development of an *in vitro* culture platform that will be beneficial for studying and predicting the effects of vaso-occlusive diseases. Nevertheless, unique aspects of both systems must be combined into a single platform to gain a full understanding of SCD.

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