Lab on a Chip

RSC Publishing

HIGHLIGHT

View Article Online
View Journal | View Issue

Research highlights

Cite this: *Lab Chip*, 2013, **13**, 2863
DOI: 10.1039/c3lc90061d
www.rsc.org/loc

Šeila Selimović,[ab] Mehmet R. Dokmeci[ab] and Ali Khademhosseini*[abcd]

NutriChip: modeling food allergies on-chip

Some 15 million Americans suffer from food allergies – exaggerated immune responses upon exposure to certain foods, such as shellfish, peanuts, or dairy products. Additionally, the gastrointestinal (GI) tract is susceptible to food intolerance (*e.g.* Celiac disease, lactose intolerance) and chronic diseases like gastroenteritis. Hence, novel and more efficient medical treatments are paramount for addressing this epidemy and require a detailed understanding of the structure and cellular responses inside the GI tract. The highly intricate topography of the gut tissue, peristaltic fluid motion and presence of many different symbiotic bacillae pose a barrier to conducting the required studies *in vitro*. However, microfluidic technologies are excellent enabling platforms for developing biologically relevant models of the GI tract, as they offer an ability to mimic the structural and biochemical conditions of the microenvironment inside the intestine. Most recent models[1,2] focus on generating a functional tissue by incorporating a permeable membrane inside an elastomeric device, lining it with epithelial cells, and analyzing the flow-through to the opposite site of the membrane. This enabled other researchers to develop microfluidic disease models of the GI-tract.

A microfluidic chip for development of the GI-tract was recently introduced by Gijs and co-workers. Using this chip, Ramadan *et al.*[3] evaluated the immune response of *in vitro* grown intestinal tissue to dairy products. The poly(dimethylsiloxane) (PDMS) microfluidic platform used in this study – the NutriChip – was built around a confluent monolayer of epithelial cells on a polyester membrane. The epithelial (Caco-2) and immune cells (macrophages) required different dynamic culture conditions, so the latter were cultured downstream and on the opposite side of the membrane. Then, molecules released by or introduced into the epithelial layer, such as dietary proteins, permeated through the membrane and traveled towards the region containing immune cells, mimicking the *in vivo* process (Fig. 1).

The researchers were especially interested in the inflammatory response to lipopolysaccharides (LPS) as a model of a high-fat diet and to lactose-based nutrients. Hence, two sets of microfluidic structures for co-culture were incorporated into every chip for differential analysis – the pro-inflammatory LPS was added to one and anti-inflammatory digested milk to the other. The integrity of the epithelial cell layer, which can be modulated by certain cytokines or bacterial excretions, reached its maximum after one week of culture. This was demonstrated by the fact that the amount of LPS needed to provoke a measurable immune response (quantified on-chip by an immunomagnetic assay) was 1000-fold higher when added to the epithelial cell barrier than directly to the macrophages. In addition, any potentially down-regulating

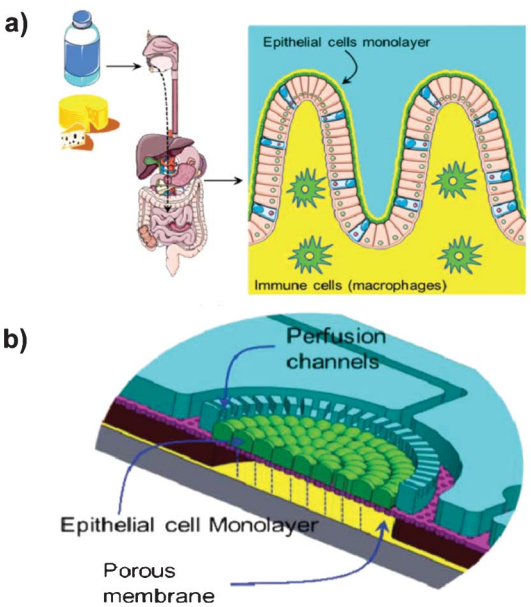

Fig. 1 Schematic of the human gastrointestinal tract at the macro- and microscales (a) and of the NutriChip cross-section. The epithelial cell monolayer can communicate with immune cells indirectly through a porous membrane. Image adapted and reprinted with permission from the Royal Society of Chemistry from Ramadan *et al.*[3]

[a]*Center for Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Cambridge, Massachusetts 02139, USA. E-mail: alik@rics.bwh.harvard.edu*
[b]*Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA*
[c]*Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, Massachusetts 02115, USA*
[d]*World Premier International – Advanced Institute for Materials Research (WPI-AIMR), Tohoku University, Sendai 980-8577, Japan*

effects of dairy products on the immune response could be determined by visually monitoring the transport and absorption of calcium in the trans-epithelial layer, using fluorescent probes.

Data obtained from tests conducted on the NutriChip could revolutionize our understanding of food sensitivities and allergies beyond those due to dairy products, and ultimately lead to new medical treatments. Furthermore, adding enteric bacillae to the NutriChip gut tissue could improve our understanding of the gut flora for prophylactic and treatment purposes. Since orally administered drugs are absorbed through the epithelial cell layer of the small intestine and can interact with the macrophages there, the proposed device could be helpful not only for nutrikinetic but also pharmacokinetic studies to screen for drug absorption efficiency and drug toxicity.

Mulberry leaf scaffolds for vascular networks

Progress in engineering complex tissues *in vitro* is chiefly limited by the lack of robust microscale methods for fabricating vascular networks. These networks are crucial for the survival of cells inside thick tissue constructs, as they enable an efficient distribution of nutrients and gases deep into the constructs. For this purpose, various molding and printing approaches have been developed, all of which begin with a computer-generated vascular design based on theoretical flow predictions.[4] But what if one did not try to copy nature, but instead used nature-engineered vascular networks straight from the environment?

That is precisely what the team led by Dichen Li has recently accomplished. He *et al.*[5] exploited the vascular structure (venation) of a mulberry leaf by using it as a master for molding PDMS and hydrogels. They selected an object from nature as their master, the mulberry leaf, as nature has already ''engineered'' a highly efficient vascular network for nutrient distribution. Importantly, it has been shown that the fluid transport obeys the same fluid dynamics principles in plants as does blood in animals, making leaves an appropriate choice for this experiment. In this paper, the soft tissue of the leaves was initially digested, exposing the venation skeleton. This network was then coated with a chrome layer which was utilized as a photomask for patterning a photosensitive polymer on a silicon wafer, such that a negative venation pattern was generated. The thickness of this polymer layer determined the height of the resulting venation features (in this case: 150 μm). In the following step, the wafer was coated with a thin layer of chrome, and then the photoresist was dissolved in acetone, this time leaving a positive venation pattern on the wafer. Finally, a mold of this pattern was generated in PDMS, which was in turn used to produce an agarose-based microfluidic device with a positive vascular structure (hollow vessels). Ultimately, flat agarose slabs were used to seal the open venation networks. Images of the resulting devices, containing vascular channels between 30 μm and 1 mm in diameter, are shown in Fig. 2.

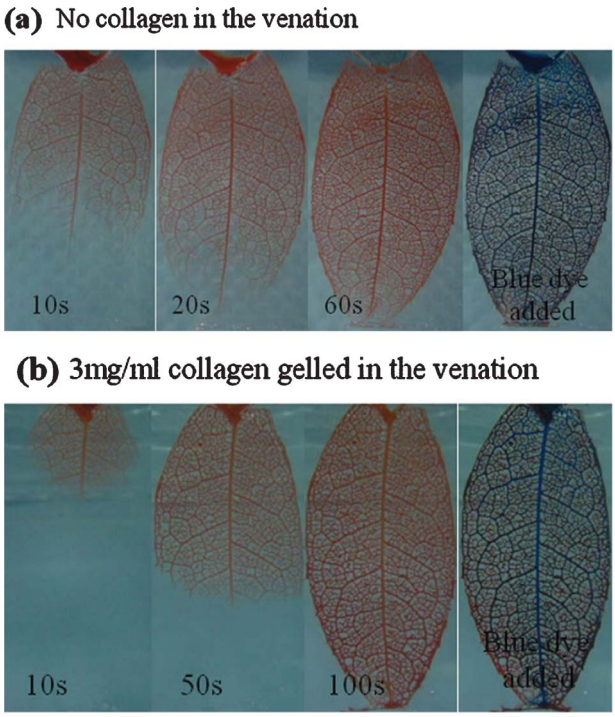

Fig. 2 Agarose-based microfluidic networks molded from a mulberry leaf as the master. The network can quickly be filled with an aqueous solution (red) and then perfused with another liquid (blue), whether the venation is empty (a) or filled with a collagen-based hydrogel (b). Figure adapted and reprinted with permission from He *et al.*[5]

To test the utility of the generated microfluidic platform for cell-based experiments, the team encapsulated HepG2 cells in agarose and introduced endothelial cells suspended in cell media into the microvessels. The liquid filled the microfluidic leaf device within ~1 min, driven by capillary force, and could subsequently be fully replaced by a second fluid (Fig. 2a). In another experiment, endothelial cells were first suspended in a low concentration collagen solution, then introduced into the leaf veins and the gel was crosslinked. In this case the device could also be fully perfused, albeit slightly more slowly (Fig. 2b), indicating that the collagen gel did not clog any channels. All cell types flourished inside the pump-free perfusion system.

Borrowing a microfluidic design from nature – as shown here with a mulberry leaf – has two main advantages. First, the time intensive computer-based design of the microfluidic vascular structure is eliminated, and so are the flow simulations that serve as a basis for the design. Second, each mulberry leaf becomes a new, independent photomask, making the mask printing process obsolete. Currently, the major limitation of the proposed process is the restriction to 2D patterns, but it is likely that a potential solution exists at another location in the natural environment.

Fluid flow engineering

The realm of microfluidics is commonly confined to laminar flows. In certain cases, *e.g.* when rapid mixing of two or more fluids is desired, structural features can be incorporated into a microfluidic device to give rise to chaotic flows.[6] However, the space between these two extremes (laminar and turbulent flows), namely, orderly fluid transformations, has only recently begun to awake interest.

One of the teams researching related fluid phenomena, led by Stone and Di Carlo, has recently developed simulations and experiments to understand changes in fluid flow due to a series of microscale pillars. In this work, Amini *et al.*[7] utilized a simple PDMS device containing a wide, but shallow (10 : 1 width : height ratio) channel with a series of randomly positioned cylindrical posts. The channel walls could be visualized with a red fluorescent dye, which permeates into PDMS, while the fluid of interest contained a different color dye.

In Stokes' flow (flow without inertia) the fluid simply flows around obstacles, preserving fore–aft symmetry; that is, the streamlines before the barrier look the same way as after the barrier has been passed. (This is due to the linear equations of motion, which yield mirror-symmetry after time reversal.) However, in cases with non-negligible inertia, secondary flows are generated near obstacles in the channel, which lead to a deformation of the main applied flow and ultimately to a breakdown of this fore–aft symmetry (Fig. 3). Furthermore, the exact shape of the secondary flows and therefore their net effect on the main flow were shown in simulations to depend on the barrier position (*e.g.* in the center of the channel *vs.* close to the channel walls).

The researchers conducted numerical simulations, corroborated by experiments, to determine the value of the Reynolds number, Re, at which the secondary flows (eddies) are created. They showed that above the region of laminar flow (up to Re $\sim$ 1), the higher the Re, the more likely eddies were to occur. The ratio of inertial to viscous forces to cause an onset of eddies was calculated as Re = 100. Aside from the Reynolds number, some parameters that affected the flow pattern were pillar diameter, number, and placement. Then, when an array of micropillars was incorporated into the microfluidic channel, the simulated effects of each individual micropillar could be added to predict the total change to the flow.

There are numerous practical applications of this work. Aside from using well-controlled flow patterns for creating combinatorial mixtures of solutions, one could ''program'' flow to *e.g.* translate cells from a culture solution to a buffer, or to enhance or suppress a chemical reaction occurring at the interface of two streams. In engineering applications, the proposed flow control may enable the generation of complex 3D structures, *e.g.* if the liquid is a cross-linkable polymer. Nonetheless, this study is worthwhile on its own, as it highlights the breadth of fluid dynamics phenomena that have yet to be understood.

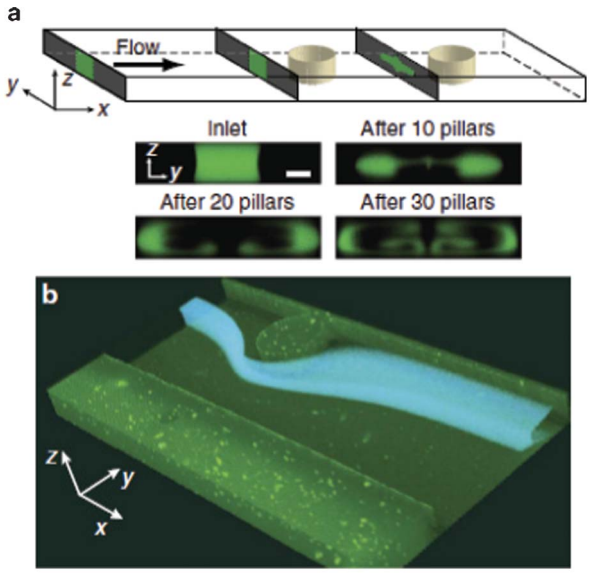

Fig. 3 Schematic of flow deformations due to a series of microstructures (a, top), and experimental images (a, bottom). The flow was visualized with fluorescent dye. Confocal representation of a blue fluid (b), surrounded by undyed liquid and displaced due to a rounded microstructure in its path. Here, the channel walls and the micropillar were visualized using fluorescent dye. The symmetry of flow before and after the microstructure is broken. Figure adapted and reprinted with permission from Amini *et al.*[7]

References

1 H. J. Kim, D. Huh, G. Hamilton and D. E. Ingber, *Lab Chip*, 2012, **12**, 2165–2174.
2 J. H. Sung, J. Yu, D. Luo, M. L. Shuler and J. C. March, *Lab Chip*, 2011, **11**, 389–392.
3 Q. Ramadan, H. Jafarpoorchekab, C. Huang, P. Silacci, S. Carrara, G. Koklu, J. Ghaye, J. Ramsden, C. Ruffert, G. Vergeres and M. A. M. Gijs, *Lab Chip*, 2013, **13**, 196–203.
4 J. T. Borenstein, E. J. Weinberg, B. K. Orrick, C. Sundback, M. R. Kaazempur-Mofrad and J. P. Vacanti, *Tissue Eng.*, 2007, **13**, 1837–1844.
5 J. He, M. Mao, Y. Liu, J. Shao, Z. Jin and D. Li, *Adv. Healthcare Mater.*, 2013, DOI: 10.1002/adhm.201200404.
6 H. A. Stone, A. D. Stroock and A. Ajdari, *Annu. Rev. Fluid Mech.*, 2004, **36**, 381–411.
7 H. Amini, E. Sollier, M. Masaeli, Y. Xie, B. Ganapathysubramanian, H. A. Stone and D. Di Carlo, *Nat. Commun.*, 2013, **4**, 1826.