Lab on a Chip

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Pulmonary edema meets lung-on-a-chip

Organ-on-a-chip microdevices are designed to mimic key structures, physiological functionality, and dynamic biochemical and mechanical microenvironments found in human organs. Compared to static well-plate cell culture systems, which often lack complexity, these systems can provide stimuli that replicate conditions experienced in living systems and hence carry the potential to develop more realistic *in vitro* human disease models as well as drug screening platforms.[1] In this context, it is evident that most biomimetic chips will be exploited in the study of complex diseases which are currently difficult to study.

Pulmonary edema is a life-threatening disease of the lung that causes intravascular fluid to accumulate in the lung alveoli (hollow air sacs deep in the lung). Due to the structural and functional complexity of the human lung, very few *in vitro* models exist.[2] Ingber and co-workers have previously reported a lung-on-a-chip microdevice that reconstitutes the alveolar–capillary interface of the living human lung, as well as its integrated organ-level physiological functions such as immune responses to pathogens and environmental particulates. This was accomplished by replicating breathing movements, which cause periodic expansion of the cell layers (Fig. 1). Now, the researchers have leveraged the unique capabilities of this human lung-on-a-chip microdevice[3] to (i) develop a clinically relevant human disease model of pulmonary edema *in vitro* and (ii) use this system to identify new drug compounds and reliably evaluate their efficacy and toxicity.[4] Huh *et al.*[4] have applied this model to mimic the development and progression of pulmonary edema induced by side effects of a chemotherapeutic drug interleukin-2 (IL-2) in human cancer patients. Their novel disease model recapitulated IL-2-induced vascular leakage and subsequent fluid accumulation and fibrin clot formation in the alveolar airspace at doses and time courses similar to those observed in humans.

Studies using this system also revealed previously unknown detrimental effects of breathing-induced physiological mechanical forces on barrier permeability. For example, treatment with the IL-2 alone was sufficient to increase the permeability of the alveolar–capillary barrier, but the application of both IL-2 and cyclic mechanical strain that mimics physiological breathing motions led to 3–4-fold greater increases in barrier permeability. Interestingly, a combination of these stimuli with the endothelial intercellular junction stabilizer protein, Angiotensin-1, prevented an increase in cell barrier permeability, suggesting that changes in the intercellular junctions could be the main cause of the problem. Exploring this hypothesis, the researchers pursued the pharmacological inhibition of a specific ion channel, known to be mechanically activated. The use of a recently developed drug for controlling this ion channel[5] blocked the combined effects of IL-2 and mechanical strain. Furthermore, an experiment using an *ex vivo* mouse lung ventilation–perfusion model confirmed the same effects of IL-2 alone and in combination with mechanical strain, as well as reversibility with Angiotensin-1 treatment. This comparison between the *in vitro* model and the whole lung offered evidence that the system was robust and closely mimicked pulmonary edema.

What distinguishes this model from conventional methods is that it allows one to investigate the effects of dynamic and complex microenvironmental cues, such as breathing-induced

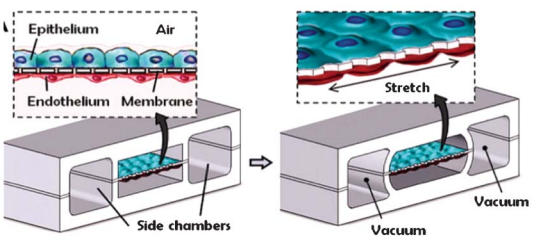

Fig. 1 Sketch of the lung-on-a-chip microdevice without (left) and with (right) an applied stretch. Figure reprinted with permission from Huh *et al.*[2]

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mechanical strain, on human disease processes in real-time and at high-resolution, which is not possible to achieve in existing culture systems. The model responds to pharmacological modulation, potentially leading to new ways to control vascular barrier permeability, and it may be used to predict the effects and safety of recently discovered drugs for personalized medicine applications. Nonetheless, there is room for improvement: reabsorption of lung fluid seen *in vivo* is not mimicked in this model, and the use of a lung alveolar cell line derived from human adenocarcinoma may lack the full features of normal cells *in vivo*. Reprogramming stem cells to the desired tissue could overcome the problem. At the same time it would allow an easier scale-up process for a lung-on-a-chip. *Ex vivo* and *in vivo* results almost overlap, indicating that the model is a step in the right direction.

Microfluidic separation of tumor-initiating cells

During metastasis some of the cancer cells from the primary tumor invade other tissues and cause secondary tumors. In fact, metastasis tends to result in more deaths than the primary tumors. Hence it is of utmost importance to isolate and study the cells with metastatic potential. Studies on the mechanical properties of cancer cells have indicated that the metastatic cells display enhanced deformability.[6,7] Despite the importance of capturing metastatic cells, their isolation from other cell types is very challenging due to the similarities in dimensions and surface properties.

To this end, Qin and collegues[8] have recently designed a microfluidic cell purification platform for generating subpopulations rich in highly deformable cells. Here, the primary breast cancer cell line SUM149 was of special interest due to the cells' stem-cell-like deformability and the potential to form mammospheres (special aggregates of mammary gland cells).

In the study performed by Zhang *et al.*,[8] SUM149 cells were filtered from a population containing three cell lines. Within the mechanical separation chip (MS-chip), microbarriers and hydrodynamic force were used to separate the deformable SUM149 cells from the remaining stiffer cells (Fig. 2). This was accomplished by perfusing all the cells through an array of constrictions and collecting them based on their ability to pass through this barrier. In the case of cell blockage, the rectangular matrix-like organization of the microbarriers allowed the more flexible cells to find alternate routes. In addition, wide channels were placed between adjacent microbarrier arrays to control the hydrodynamic pressure and prevent chip failure due to clogging.

The fluidic channels near the entrance ports were designed to be several times wider than the diameter of cells. This resulted in an initial coarse separation of flexible and stiff cells: the stiffer cells were frequently trapped along the flow channels while the more flexible cells traveled easily through the gaps between the microbarriers. This resulted in a varying ratio of SUM149 and other breast cancer cells along the length

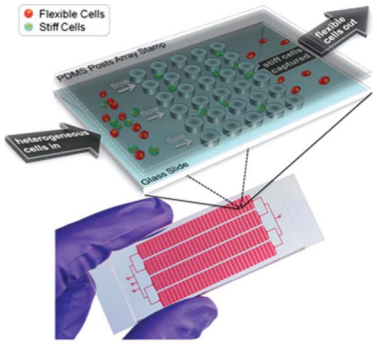

Fig. 2 Schematic (top) and photo (bottom) of the MS-chip. Figure adapted and reprinted with permission from the Proceedings of the National Academy of Sciences in Zhang *et al.*[8]

of the MS-chip. After separation, the cells were lysed, their RNA was extracted, and genome-wide gene-expression analysis was performed. Based on cell numbers and RNA quantification, it was determined that ~10% of all cells had passed through the MS-chip and 90% were retained inside the channels.

Previous studies have shown that overexpression of multiple genes active in cancer cell metastasis and motility is correlated with the flexible phenotype. Thus, Zhang *et al.*[8] examined the expression of cytoskeletal and nucleoskeletal filament protein genes to assess whether the filtered cell populations had the potential to metastasize. The expression profile of the enriched flexible SUM149 cell subpopulation indicated relatively large flexibility and a weakly differentiated state, suggesting a strong metastatic potential. Furthermore, the expression of certain Tumor-Initiating Cell (TIC) markers driving the colony forming capacity in mammosphere culture revealed that the filtered cell population was rich in cells with those markers, although mammospheres had not yet been formed.

Research in a multitude of cancer cell types will continue to advance with the use of TIC-enrichment approaches, and the MS-chip has the potential to become an important microfluidic cell-sorting platform. The MS-chip could also be utilized to purify stem cells and TICs based on their mechanical characteristics, enabling identification of molecular signatures and genes in tumor causing cells. Ultimately, we envision the application of the MS-chip in cancer studies for which TIC biomarkers have not yet been found.

Detection and size measurement of a single virus

Detection and sizing of individual RNA viruses is an emerging field of research and is leading the development of biosensors that can detect individual bio-nanoparticles in aqueous solutions. Early detection of ultra-low concentrations of viruses is crucial in identification and elimination of pathogens. For example, individual Influenza A virus particles have recently been identified using spherical optical microcavities,

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but the effectiveness of this mode of detection is limited by the signal/noise ratio.[9]

To address this challenge, Arnold and colleagues have recently developed a label-free method to detect and measure the size of MS2, an RNA virus that attacks *E. coli* bacteria.[10,11] A whispering gallery mode (WGM)/nanoplasmonic hybrid sensor made up of a spherical dielectric microsphere and a nanoscale plasmonic receptor was fabricated by Dantham *et al.*[11] Whispering gallery modes are types of waves that can travel on a concave surface (*e.g.* the equator of a sphere), so a gold nanoparticle serving as the nanoplasmonic receptor (detector of quanta of plasma oscillations) had to be placed at the equator. This was accomplished by utilizing light forces of the WGM to attract the gold particle to the microsphere. Then, when the dielectric sphere was placed in a solution containing the virus particles, the attachment of a single virus on the sphere would affect plasmonic oscillations in the near infrared wavelengths, thereby significantly altering the output signal of the WGM. This approach was shown to enhance the signal strength by a factor of 70 compared to standard WGM detection methods.

The entire experiment was conducted in a poly(dimethylsiloxane) (PDMS) microfluidic device, consisting of a straight channel for flow of the viral suspension and a detection region, which housed the silica microsphere. Laser light at 780 nm was directed onto the microsphere and transmitted to a photodiode connected to a data acquisition board. The initial WGM output signal was observed as a dip in the transmitted waveform, and the attachment of a single virus particle was detected as a shift in the wavelength at which the dip occurred.

An analytical theory of plasmonic enhancement of a dipole by a nanoshell was described by the authors and was used to present and interpret the experimental results. The MS2 virus particles were generated by inoculating *E. coli* bacteria with viable virus stock. Next, the amplified number of viruses was harvested and placed in a saline solution. Dynamic light scattering showed no evidence of clustering of viruses inside the solution, which made it possible to detect and measure individual virus particles. Contrary to inorganic nanoparticles such as polystyrene or gold, the genetic programming of MS2 results in viruses of a single size. This results in a single wavelength shift of the observed WGM signal and a relative measurement of the diameter of the virus.

In most applications in biology and chemistry, the capability of the detection platform to deliver absolute, rather than relative size measurements is a notable advantage. In addition, a modification of the system to allow for label-free single protein detection would make the WGM/nanoplasmonic hybrid potentially the method of choice for applications requiring precise measurements on the nanoscale, such as quantification of protein release during metabolic activity of cells. Finally, the signal/noise ratio could be further improved by different nanostructures including nanorods where many other opportunities exist.

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