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HIGHLIGHT

Research highlights

Šeila Selimović[ab] and Ali Khademhosseini*[abcd]

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Zebrafish-on-chip

There is a need in biology for animal models that are cheap, amicable for real-time analysis as well as high-throughput screening and have a short reproduction cycle.[1] Zebrafish fulfill these requirements. Richardson and colleagues have recently developed a microfluidic device designed to function as a high-throughput zebrafish embryo incubator, thereby further enhancing the use of zebrafish as a model in drug screening and biological studies.[2]

The chip consisted of three layers of borosilicate glass, the middle of which contained fluidic channels connecting several embryo growth chambers in parallel. The embryos were dispensed directly into the wells, and the device was closed with the top glass sheet. Adjacent to the main channel and fully separated from it was a temperature control line. This channel was filled with preheated water to spatially regulate the temperature. A perfusion structure for delivery of fresh buffer to the embryos was also implemented, an improvement in zebrafish culture compared to well-plate experiments which required static buffer replacement.

[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*

Average embryo viability after a 5-day culture on-chip reached 88% at certain buffer flow rates, although up to a 100% viability was observed on individual chips (Fig. 1). This was comparable to survival rates in 96-well plates. In the microfluidic device, the main factor influencing the embryo survival was the flow rate, while well size, well shape and oxygenation levels did not have a measurable effect.

Interestingly, the chip-grown embryos had on average a shorter body length than those grown on standard well-plates, although the body length was shown to increase with applied perfusion rate. Further tests revealed that the zebrafish embryos developing in microfluidic devices had higher stress levels, which were expressed in stronger pigment and slight deformations of the yolk sac. The rates of occurrence of other malformations were similar on- and off-chip. Aside from these observations, the authors also showed that it was possible to affect the embryo development in all microfluidic wells simultaneously by introducing chemicals into the buffer stream. For example, they exposed the embryos for 1 h to a 10% ethanol solution and noticed a measurable increase in the rate of occurrence of severe deformities.

This work is of importance for biomedical research, as it offers a working, reliable microfluidic chip for animal culture. The low-cost nature of this approach increases its potential to revolutionize both academic and industrial research.

Nanoscale features for microscale cell culture

Cell morphology, gene expression and differentiation can be affected by a host of microenvironmental factors. An emerging area of research is to mimic these native cell microenvironments inside microfluidic devices. Substrate topography as a microenvironmental factor is relatively easy to control using microscale technologies, as most device fabrication methods utilize lithography and micromolding, albeit often only with microscale precision.[3] Kam W. Leong and colleagues[4] have advanced this area by developing a microfluidic cell culture platform that incorporates nanometre-size features that mimic the extracellular matrix and serve as fluidic paths for nutrients and other molecules (Fig. 2).

The microchannels in the PDMS device were fabricated using standard photolithographic protocols, utilizing SU8-coated silicon wafers. The master for the second PDMS layer containing nanogratings (350 nm wide and 280 nm deep) was an electron beam lithography treated PMMA thin film. The two layers were bonded in one instance *via* microtransfer assembly that utilizes a thin, uncured PDMS film as glue. In this case, the glue entered and filled the nanogratings. In another instance, oxygen plasma was used to assemble the device; here, the nanogratings were preserved.

Beyond using the patterned PDMS layers to assemble a single device, the authors showed that they could also apply them as masters for pattern transfer *via* hot embossing of a thin polymer film.

To demonstrate the utility of this platform, the researchers cultured human mesenchymal stem cells on the nanograting under both static and dynamic conditions. The nanoscale topography of the substrate had a clearly observable effect on cell alignment. Cells best aligned with the nanogrooves in static culture and when the nanogrooves were parallel to the perfusion flow. On smooth surfaces,

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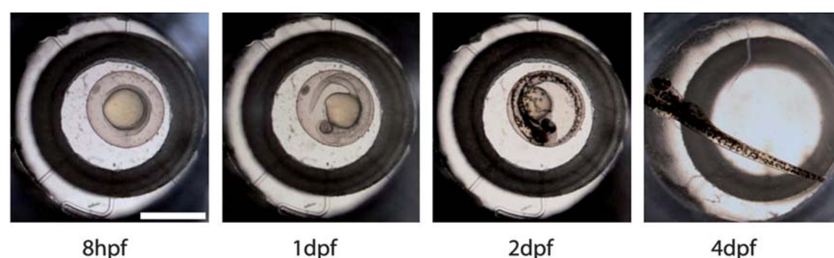

Fig. 1 From left to right: A zebrafish embryo developing in a 1.8 mm microfluidic well, shown at 8 h post fertilization (hpf) and 1, 2, and 4 days post fertilization (dpf). Figure adapted and reprinted with permission from the Royal Society of Chemistry from Wielhouwer *et al.*[2]

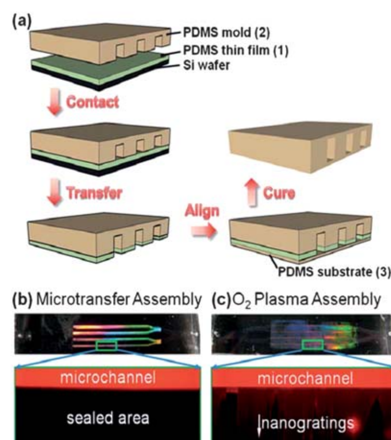

Fig. 2 PDMS device assembly process (a), complete device with microchannels (b) and complete device with micro- and nanochannels (c). Figure reprinted with permission of the Royal Society of Chemistry from Yang *et al.*[4]

however, cellular alignment was isotropic, regardless of culture condition.

Microfluidic cell culture platforms are starting to replace traditional well-plates in laboratories, among others because of the flexibility in studying cell interactions with substrate topographies. The present device is noteworthy because it not only incorporates novel nanoscale grooves and channels, but simultaneously utilizes these features for cell culture perfusion. By combining these two functions, this device provides a platform that can be used to better study the complex features of the cellular microenvironment.

Metamaterials on paper

Increasingly, paper is being used as the material of choice for developing cheap and disposable microfluidic devices for diagnostic and point-of-care applications.[5] In many cases, the feature size of paper fluidics is on the order of several hundred microns and even millimetres and the readout is optical, which limits its usefulness. Omenetto and colleagues[6] have now fabricated a new paper-based device for high-precision quantitative biochemical analysis by incorporating a metamaterial on the paper surface.

Metamaterials are artificial materials whose physical characteristics depend on their structure. Here, the metamaterial consisted of gold particles deposited onto smooth photopaper through a shadow mask using electron beam evaporation. The resulting gold features were 150 nm thick and formed electric resonators with a feature size of 10 μm. The electromagnetic resonance of metamaterials stems from the oscillations of electrons in the strongly conducting metal. The resonance frequencies depend on the feature size and here were on the order of THz. The most common feature, the split-ring-resonator, behaves like an LC-circuit, whose resonant frequency is determined by the inductance (due to the current path) and the capacitance (due to the resonator gap size and properties of the dielectric paper). The presence of any foreign molecule (*e.g.* the biochemical sample) alters the capacitance, a feature which was exploited in this device for analyte detection.

The paper–metamaterial device was used to detect different glucose concentrations, simulating low (3 mM) and high (30 mM) physiological glucose levels. The resonators were coated with 100 μl glucose solution, which was then allowed to dry on the open device. The glucose molecules residing on the resonators modified the resonator capacitance and ultimately the transmission spectrum of the driving electric field, such that a high glucose concentration resulted in a much lower transmission frequency compared to the control, and a small concentration produced only a minor difference. The minimum detection level was determined as 0.35 mM glucose, which is more precise than most commercial glucose measurement systems. In experiments on urea detection very different transmission shifts were observed, although similar concentrations were applied.

The high sensitivity of this paper microfluidic device as well as its unique response to different molecules like glucose and urea make it a promising platform for quantitative, high-precision analysis of biochemical samples in point-of-care applications.

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