Lab on a Chip

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HIGHLIGHT

Research highlights

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A foldable microfluidic device for point-of-care applications

Lab-on-a-chip platforms have permeated many research laboratories in the biomedical field, where they are used for proteomics, drug screening and cell and tissue culture applications. Since these platforms help reduce the amount of reagents and yield results faster than benchtop experiments, the cost associated with the operation of these devices is comparatively low. Due to the low cost nature of these devices, they are expected to find new applications in the emerging point-of-care disposable device field.[1–3] However, sample preparation and analysis on microfluidic devices often requires multiple cumbersome steps, including reagent mixing, sample purification and sample transfer, all of which require complex device elements and off-chip equipment such as syringe pumps and pressure sources. Böhringer and colleagues have recently introduced a foldable device platform that simplified device operation significantly. Govindarajan *et al.*[4] dispensed reagents onto flexible substrates and by folding these functional layers onto others were able to create a technology to introduce and transfer samples without requiring externally powered equipment.

The disposable device was fabricated and designed to conduct sample preparation at a target cost of less than $2 USD per patient. It was fabricated from a stack of sticky Mylar sheets, cellulose paper and repositionable window decal material with laser cut fluidic ports and transport channels. The cellulose paper served as the liquid wicking layer. Glued to the Mylar layer were a DNA filter, waste absorption pad, a sample loading cup, and a pad containing dried lysis and wash buffer. The operational sequence is shown in Fig. 1. First, a new DNA binding filter was added to the device, near the waste absorption pad. Then, the lysis buffer storage pad and rehydration buffer were put in place on another layer of the device.

The biological sample used in the proof-of-concept experiments was 50 µl *E. coli*, added to 250 µl pig mucin. The highly viscous sample was dispensed into a small plastic cup above the DNA binding filter. When another layer of the device was folded onto the cup, the liquid was wicked through the cellulose to the DNA filter and the lysis buffer pad. To ensure that the sample and transport channels were in good contact, a weight was placed on the folded device. Cell lysis was conducted at room temperature. After 30 min, the device was unfolded and all layers except the DNA capture filter were removed. The filter was then washed manually with 100% ethanol, and the resulting waste collected in the waste absorption pad. After waste removal, the DNA capture filter was left to dry and the DNA later eluted using a low salt buffer.

Although similar sample preparation experiments traditionally required a sterile environment where the device fabrication was conducted in cleanrooms, here the complete process was done on a benchtop, which was sterilized with alcohol. The device efficiency was comparable to traditional sample preparation, allowing for DNA amplification and detection of concentrations as low as 33 CFU *E.coli*/ml of mucin. Currently, the device yields 150 µl of concentrated nucleic acid solution, which can then be amplified and analyzed with advanced instrumentation after transport to

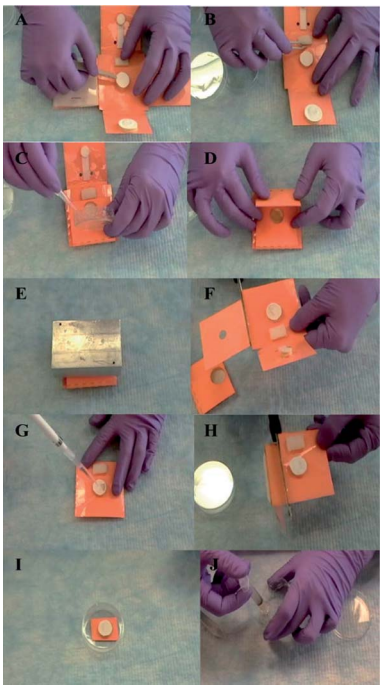

Fig. 1 Foldable point-of-care device operation: (A) insertion of DNA binding filter and (B) lysis buffer pad placement and rehydration, (C) sample loading, (D) folding of the pad containing the fluidic channel, (E) lysis, (F) removal of unwanted layers, (G) ethanol wash, (H) waste removal, (I) DNA filter drying, and (J) DNA elution. Figure reprinted with permission from the Royal Society of Chemistry from Govindarajan *et al.*[4]

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a well-equipped laboratory. The prototype developed by the researchers carries great promise to make the leap from development to application in the field, where hospitals and medical laboratories are not readily accessible to patients. Furthermore, the device is simple to fabricate and operate, such that field medical staff in rural areas can collect and prepare samples on-chip with minimal training.

Heart-on-a-chip

Drug discovery efforts require well-controlled, high throughput experiments on biological tissue samples. This is often easier to accomplish using lab-on-a-chip platforms than on traditional well-plates which do not allow exact replication of the cellular microenvironment. Specifically in cardiovascular pharmacology, where generation of aligned, contractile cardiac fibers is important, on-chip studies can provide control of the cell environment on the micro- and even nanoscale.[6,7]

One such study has recently been demonstrated by Parker and colleagues. In this study, Grosberg *et al.*[5] utilized thin film technology to engineer anisotropic cardiac fibers on an elastomeric substrate. They spin coated a masked cover slip with a sacrificial layer of poly(*N*-isopropylacrylamide) (PIPAAm), which melts below 35 °C, then removed the mask to expose a single strip of PIPAAm. The cover slip was next coated with a thin layer of the elastomer poly(dimethylsiloxane) (PDMS). The same procedure was used to prepare batches of multiple devices at the same time, yielding 10 mm × 6 mm glass chips (Fig. 2a,b). To help organize cells – neonatal rat ventricular myocytes – into aligned, 4 μm thick tissue constructs, a fibronectin pattern was microcontact printed on the chips. The film was then placed in media and cut into two arrays of rectangular strips, each 2 mm × 4 mm in size with a 1.2 mm spacing (Fig. 2c,d), however the strips were connected to each other and were part of the same experiment. As the medium cooled to room temperature, the PIPAAm film melted, leaving thin strips of PDMS detached from the glass underneath and free to move. Finally, two Pt electrodes were placed on the opposing film strips. As the cells organized and grew into spontaneously beating fibers, their contractile force caused the PDMS film strips to bend, which was visually observed. When necessary, the myocytes were paced at 2 Hz to jump start their contractile behavior.

Tissue contractions were recorded with a video camera; the resulting extensions and bending of the PDMS strips were measured and correlated with their radii of curvature. This, in turn, was taken as a measure of the systolic and diastolic stresses, since the strips could be modeled as two-layer strain beams (the PDMS film was considered a passive layer, and the cells an active layer). Although the cell culture in this study was effectively 2D, the calculated stresses were comparable to literature values from 3D tissues, namely ~21 kPa (systolic) and ~8 kPa (diastolic), at 2 Hz and 37 °C.

In addition to measuring the contractile stresses, spontaneous beating rates were also recorded. For example, the beating rates sped up fourfold after an increase in epinephrine concentration (a positive chronotropic agent) from 10^{-9} M to 10^{-6} M. Furthermore, the chip allowed measurements of the action potential wavefronts and morphology. The tissue organization was highlighted after the contractile studies by staining the cell nuclei, actin, and sarcomeric α-actinin. All markers revealed highly aligned cytoskeletal structures on the fibronectin-patterned films. In comparison, cardiac tissue formed on isotropic films (with fibronectin coating) showed a much larger distribution of sarcomere orientation angles. Similarly, the peak systolic and diastolic stresses were much larger in aligned tissues than in isotropic ones.

Size reduction of experiments from macro- to microscale is advantageous in many ways, most of all because it offers improved control over the cellular microenvironment. In this case it also allows for a range of measurements on the generated tissue, including quantification of contractile stresses, frequencies and the action potential. In the future, devices like this could be utilized for studies of human cardiac cells, specifically to predict the toxicity of drugs and environmental agents on the human heart. This line of research might potentially lead to personalized medicine, which would allow for drug testing on patients' own cells to maximize the success of treatment.

Photoreversible biomolecular patterning

Hydrogel biomaterials are of great interest in tissue engineering and cell culture applications due to their ability to mimic the extracellular matrix in the body.[8] These materials can be spatially functionalized on different length scales to generate controlled porosities, elastic moduli and biological features. However, these modifications have to be made early in the experiment and are usually not reversible. DeForest and Anseth[9] have recently addressed this issue and developed a method to reversibly pattern biomolecules inside hydrogel structures to enable a range of new applications.

This approach is based on using two bioorthogonal photosensitive chemical reactions, one of which conjugates the biomolecule to the gel whereas the second reaction can cleave the molecule from it. The first reaction is a thiolene reaction (addition of an S–H bond *via* ionic interactions or free radicals) and is activated by illumination with visible light. In the second reaction, an *o*-nitrobenzyl ether is cleaved by UV light to produce a nitroso molecule and an acid. The researchers combined these two reactions by synthesizing a biomolecule that contained both the thiol group and the *o*-nitrobenzyl group. As a consequence, the molecule could either be bound to the hydrogel or removed from it, depending on the light signal. To ensure that this process occurred in a robust manner, the activation wavelengths for the binding and cleavage processes were designed to be different from each other.

To demonstrate the utility of this approach, poly(ethylene glycol) (PEG) hydrogels that had a homogeneous distribution of alkene molecules (as anchoring sites for the thiolene reactions) were used. Functional peptides were subsequently delivered to the hydrogel and induced to anchor to the gel network *via* irradiation with visible light. To generate spatially confined regions of patterned peptides, the light exposure was restricted to specific regions through a transparency mask. As expected, the degree of binding was shown to be dependent on the total light exposure. Thus, at light exposure of 10 mW cm^{-2} for up to two minutes, peptide concentrations were generated that were on the order of

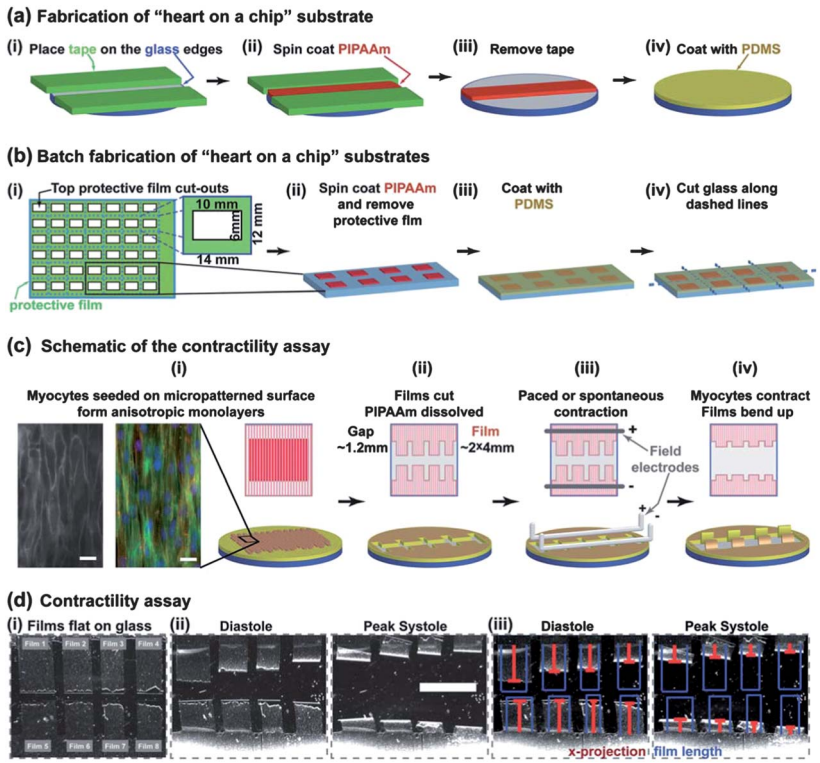

Fig. 2 Fabrication of a single (25 mm diameter) (a) and batch (b) heart-on-a-chip platform. Schematic of cell seeding and contractility tests, scale bar: 20 μm (c). Brightfield images of contractile heart fibers on bendable film strips (d). Figure reprinted with permission from the Royal Society of Chemistry from Grosberg *et al.*[5]

1 mM or less. At these concentrations, many peptides are known to affect integrin binding and ultimately control cell behavior. Upon binding of the peptide, selected gels were irradiated with UV for different time periods (5–20 mW cm^{-2} for up to 10 min) to remove different amounts of the biomolecules.

To demonstrate the spatial patterning of desired peptides, 200 μm lines of fluorescently conjugated peptide molecules were generated. By rotating the mask and removing the exposed peptides, a checkered pattern with a 1 μm resolution was formed. A more complex double-helix structure was also generated by illuminating the gels through a multiphoton microscope. In addition, it was shown that both discrete and continuous peptide concentration gradients could be formed by controlling the light exposure to various regions in the hydrogel network. Since the dimension of these patterns was comparable to cells, this process could be of potential use in regulating the cellular microenvironment. This was demonstrated by selectively inducing the adhesion of NIH-3T3 fibroblasts on adhesive patterns on the hydrogels. Furthermore, upon selective removal of the peptides, cells were detached from the exposed areas and washed away.

The proposed hydrogel chemistry and the related patterning process provides a significant improvement compared to the existing approaches and gives researchers control not only over spatial, but also over temporal changes in the hydrogel properties. Given its ability to conjugate biofunctional molecules inside gels, this approach can be used to generate microenvironments that can be used to study the dynamics of the cellular microenvironment as well as form tissue structures for various regenerative medicine and drug discovery applications.

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