

Research highlights

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Self-similar topographies on shrink-film for cell culture

Understanding the cellular response to chemical and physical factors is important for various biological and biomedical studies. Among the many parameters that are tested in this context on micro-fabricated platforms are substrate topographical features such as size and shape of surface features.^{1,2} While micro-fabricated platforms offer a controlled way to study the effects of highly specific topographies on cells (*e.g.*, periodic nano- and microgrooves), they are often difficult and expensive to fabricate. More importantly, due to the innate lack of feature randomness, these platforms do not faithfully emulate the diversity of feature size and placement that is present *in vivo* in the extracellular matrix. To address this shortcoming, Khine and colleagues developed a shrink-film based cell culture platform that incorporates a variety of nano- and microfeatures with extended size ranges and periodicities.

Chen *et al.*³ began with prestressed polyethylene (PE), a commonly available shrink film, and oxidized it by exposure to oxygen plasma for up to 15 min; this process formed a thin stiff layer on the top surface of the film. They then fixed two

opposing edges of the film on a substrate and heated it to 150 °C for 3.5 min. The polymeric film is known to shrink uniformly at this temperature. However, due to the difference in stress between the top layer and the bulk PE film, buckling of the film occurred, resulting in minor and major uniaxial ridges perpendicular to the fixed edges (Fig. 1).

The spacing and depth of the ridges was shown to depend on the duration of exposure to the oxygen plasma, and therefore on the thickness of the oxidized, stiff top layer of the film. The width of the major wrinkles (distance between two adjacent deep ridges) increased from 1 to 7 μm with longer plasma treatment. Positioned on top of a major wrinkle were several minor wrinkles, with widths in the order of 100 nm, and a minimum width of 60 nm after 5 min of plasma exposure. On average, the ridge depth was between 150 and 300 nm, with minimum depths of 2 nm and maximum depths of up to 1.5 μm. The feature dimensions formed after a 5 min plasma exposure match best those of fibrils in most extracellular matrices (ECM) (60 nm to a few μm). This was highlighted in several cell alignment experiments, in which mouse embryonic fibroblasts, aortic smooth muscle cells and human embryonic stem cells preferentially aligned parallel to the ridges and wrinkles. The cells also had better alignment on the devices treated for 5 min with oxygen plasma than others, as the resulting topographies mimicked better the *in vivo* substrates.

The ability to fabricate these shrink-film substrates in under 10 min and with a substantially lower cost than possible with standard microscale techniques makes many biological experiments more accessible. For example, using such substrates' topographies to guide stem

cell differentiation could eliminate the need for chemical differentiation factors. Further, the similarity between the PE platforms and real ECM might lead to differentiated cells that are functionally as potent as their *in vivo* counterparts. Until then, it would seem prudent to study the effects of such multi-scale topography on cell movement and proliferation.

Optically adjustable, dynamic microfluidic chips

Microfluidic devices are inherently static, in that the design has to be chosen and

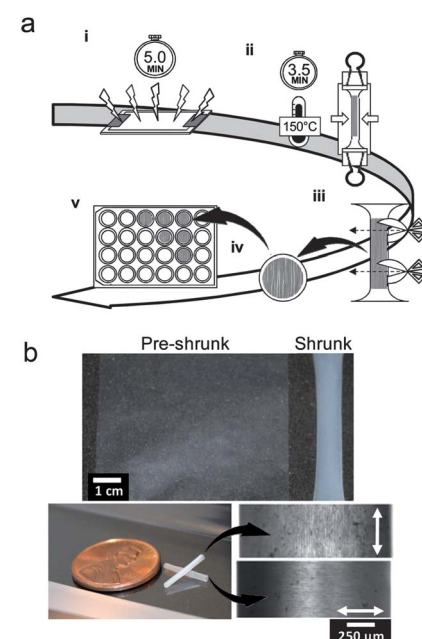


Fig. 1 (a) Shrink-film substrate fabrication: (i) oxygen plasma treatment, (ii) film shrinking during heating, (iii) cutting of the film to the desired size and shape, (iv) cell culture on substrate in well plates. (b) Images of pre-shrunk and shrunken (planar and rolled up) polyethylene film. Figure adapted and reprinted with permission from Chen *et al.*³

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geometry fabricated before an experiment. Any structural modifications require a redesign and a repetition of all fabrication steps, and potentially a modification of the interfaces between the device and external equipment. Thus, the development of a basic chip geometry can take several design and fabrication cycles, all of which is time consuming and can be costly, depending on the fabrication method. To address this shortcoming, researchers have explored various optofluidic solutions to manipulate microfluidic structures, including holographic optical tweezers and light induced electric fields for particle trapping.^{4,5} Krishnan and Erickson have recently introduced another approach to reconfiguring microscale flows, by applying a polymer that undergoes a phase change from liquid to gelatinous upon being heated by light.⁶

The researchers built a simple poly(dimethylsiloxane) (PDMS) device, whose thermal absorbance they increased by incorporating carbon black particles. The device contained a single fluidic chamber with one input channel and several output channels. An aqueous solution of Pluronic F127 (a triblock copolymer consisting of a poly(propylene oxide) base and two poly(ethylene oxide) arms) was continuously flowed through the chamber at flow speeds of up to $1500 \mu\text{m s}^{-1}$. Light was shone on the chip through a dynamic photomask, such that selected regions of the flowing polymer inside the fluidic chamber were exposed to the light, and others were not. The carbon black absorbed the light, converting it to thermal energy, which heated a local field of the chip (photothermal conversion). Then, the exposed regions of the polymer underwent a thermo-rheological conversion, in which the low viscosity liquid turned into a high yield strength gel. This gel then acted as an effective barrier to flow, in the form of walls and valves (Fig. 2). The unheated regions of the polymer continued to flow through the chamber and around the gelled features.

Temperatures above the gelation point were reached within a few seconds and the rheological response was in the order of ms. Upon removal of the excitation light the polymer returned to its original liquid phase within 2 s, confirming that the phase change response of the Pluronic was reversible—over many cycles.

The smallest gelation region was circular with a radius of $\sim 150 \mu\text{m}$. Regions imaged through a smaller mask could not be sufficiently heated to undergo a phase change. Surprisingly, large features ($\sim 700 \mu\text{m}$) did not form single gelled regions, either. Illuminating a very large region heated the polymer to a higher, secondary transition temperature, at which the polymer again became a low viscosity liquid.

The authors showed that multiple complex light patterns could be used simultaneously to reconfigure the polymeric structure inside the microfluidic chamber. Furthermore, they succeeded in confining 48 kb long DNA as well as nanoparticles to trapping wells, which were low flow sections of the chamber. The reduced average displacement of both species was used to calculate their effective diffusivities. This was a great improvement compared to static microfluidic structures, in which nanoparticles have to be constrained by an external force.

A major advantage of using thermally responsive polymers in microfluidics is the ability to create reconfigurable devices. The particular choice of triblock co-polymer used here offers a fast—on-the-spot—and reversible structural response to changes in temperature. Currently, a limitation of the system stems from the requirement to use Pluronic as the flow medium, which may not be compatible with all biological or chemical systems. However, a possible solution would be to create multilayer microfluidic structures, of which one could include Pluronic-based control elements such as valves and pumps.

Protein profiling with microgels

Sensitive, precise, and multiplexed high-throughput assays for the detection of proteins are of great importance in biological and medical studies. Currently, most protein detection platforms require antibodies for target capture and signaling. However, antibody selection is often expensive and time-consuming, and the production process begins *in vivo*.⁷ Hence, aptamers—short nucleic acid sequences that offer highly specific binding to protein targets—have been displacing antibodies in protein detection studies. Doyle and colleagues, for

example, have used microfluidics to develop microgel particles functionalized with aptamers for protein detection.

Srinivas *et al.*⁸ applied a simple microfluidic flow focusing device to co-flow an aptamer solution, a fluorescent code monomer solution and two blank monomer solution streams in mixtures of poly(ethylene glycol) diacrylate (PEG-DA 700), PEG 200 and buffer. When exposed to UV light under a transparency mask (stop-flow lithography), the co-flowing solutions crosslinked. Parts of the fluorescent solution that did not gel formed a graphical detection code. Thus, each gelled particle, $250 \mu\text{m} \times 70 \mu\text{m}$ in area, with a $38 \mu\text{m}$ height, contained a visual 5-digit code for multiplexing and one or more aptamer sections for protein detection. The aptamer containing region was framed by the two blank PEG-DA gel regions to prevent fluorescence bleed-over between the coding and detection sections. After binding to a protein, the microgel particles could be analyzed both in flow (25 particles per s) and statically using a fluorescent illuminating source.

For a proof-of-concept study the authors chose the medically relevant protease thrombin, which is involved in blood clotting by controlling the generation of fibrin from fibrinogen. Precise concentration measurements of thrombin are therefore important for monitoring of cardiovascular conditions. Two aptamers were selected to interact with the protease by binding to the fibrinogen and heparin exosites. Thus, one aptamer had a capture function, and the other was used as a reporter in this sandwich assay. The reporter aptamer was biotinylated to facilitate labelling with fluorescent markers.

The use of the porous hydrogels in this protein detection system allowed the aptamers to fold and modify their conformation upon attaching to the proteins and further bind to fluorescent labelling molecules. Furthermore, the 3D structure of the microgels provided sufficient distance between probe molecules and helped minimize steric interactions. Compared to surface-based detection systems, the microgels offered an increase of at least 1 order of magnitude in terms of effective surface probe density. Thus, the aptamers could reach sensitivities beyond those in surface-based detection, down to $\sim 65 \text{ pM}$.

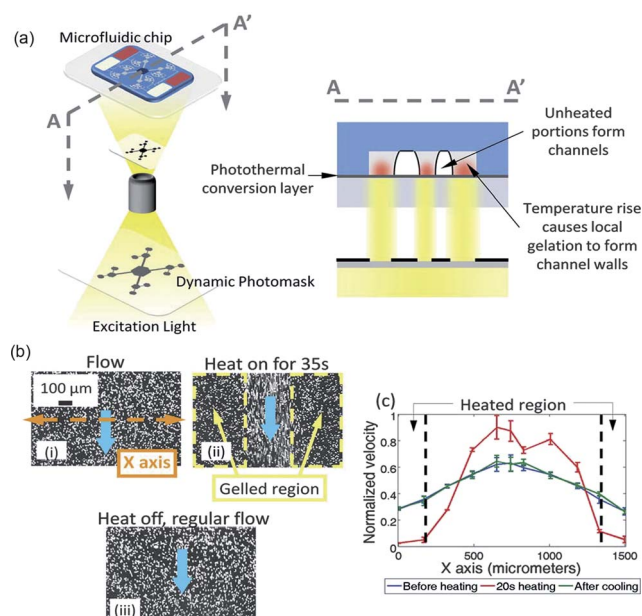


Fig. 2 (a) Schematic of the experimental setup: the microfluidic chip is filled with a heat sensitive polymer and exposed to light through a mask. The excitation light heats certain regions of the polymer, which then undergo a phase change from low viscosity liquid to gel. The gel forms channel walls, while the colder polymer regions continue to flow. (b) Formation of a channel in the microfluidic chamber. White tracer particles indicate the flow direction. (c) Average velocity of the tracer particles before and after heating and after return to the original temperature. Figure reprinted with permission from the Royal Society of Chemistry from Krishnan and Erickson.⁶

The detection sensitivity was shown to depend on the relative fractions of PEG-DA, PEG, and capture aptamer species in the monomer: the higher the concentration of PEG-DA was, the stronger the gel was and therefore the more probes were captured in the gel. This condition had to be balanced with the need for sufficiently large gel network pores that would allow diffusion of large biomolecules and labelling particles. At a 10% efficiency in aptamer trapping during crosslinking of the gel particles, the system showed

a proportional increase in detection with respect to the concentration of aptamers. The required amount of aptamer per assay was calculated to be between 1 and 7 pmol, which is comparable to alternative detection platforms.

The aptamer-containing microgel particles used here for protein detection have two major advantages over other methods: high sensitivity and high-throughput, multiplexed detection and analysis. As the fluorescence signal from the probes tends to decrease rapidly, often

by 40% in 10 min, the ability to combine coding with detection and scan many particles quickly helps vastly improve the accuracy of protein detection. In addition, the flexibility of the encoding and functionalization strategy provides high multiplexing capability with different aptamers and even other biomolecules. In the future, a potential research problem would involve combining this detection method with other lab-on-a-chip elements to track proteins from expression in cells, through collection and purification to detection.

References

- 1 P. Murray and D. Edgar, The topographical regulation of embryonic stem cell differentiation, *Philos. Trans. R. Soc. London, Ser. B*, 2004, **359**(1446), 1009–1020.
- 2 A. M. Ross, *et al.*, Physical Aspects of Cell Culture Substrates: Topography, Roughness, and Elasticity, *Small*, 2011, **8**(3), 336–355.
- 3 A. Chen, *et al.*, Shrink-Film Configurable Multiscale Wrinkles for Functional Alignment of Human Embryonic Stem Cells and their Cardiac Derivatives, *Adv Mater*, 2011, **23**(48), 5785–5791.
- 4 D. Psaltis, S. R. Quake and C. Yang, Developing optofluidic technology through the fusion of microfluidics and optics, *Nature*, 2006, **442**(7101), 381–386.
- 5 Y. Chen, *et al.*, Optofluidic microcavities: Dye-lasers and biosensors, *Biomicrofluidics*, 2010, **4**(4), 043002.
- 6 M. Krishnan and D. Erickson, Optically induced microfluidic reconfiguration, *Lab Chip*, 2012, **12**, 613–621.
- 7 R. K. Mosing and M. T. Bowser, Microfluidic Selection and Application of Aptamers, *J. Sep. Sci.*, 2007, **30**, 1420–1426.
- 8 R. L. Srinivas, *et al.*, Aptamer-Functionalized Microgel Particles for Protein Detection, *Anal. Chem.*, 2011, **83**, 9138–9145.