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Dynamic in situ chromosome immobilisation and DNA extraction using localized poly(N-isopropylacrylamide) phase transition

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A method of in situ chromosome immobilisation and DNA extraction in a microfluidic polymer chip was presented. Light-induced local heating was used to induce poly(N-isopropylacrylamide) phase transition in order to create a hydrogel and embed a single chromosome such that it was immobilised. This was achieved with the use of a near-infrared laser focused on an absorption layer integrated in the polymer chip in close proximity to the microchannel. It was possible to proceed to DNA extraction while holding on the chromosome at an arbitrary location by introducing protease K into the microchannel. © 2011 American Institute of Physics. [doi:10.1063/1.3637631]

In recent years, advances in fluorescence imaging, fabrication of micro- and nanofluidic lab-on-a-chip technology, and DNA manipulation have resulted in novel techniques for manipulation of individual biomolecules.1 Recent developments in DNA manipulation have included the use of nanopores2 and optical mapping of DNA.3 The latter is carried out by stretching fluorescently tagged DNA in combination with objectives with high numerical aperture and high quantum-yield CCDs. Due to loading processes and transport through the microchannels, analysis using microfluidic devices suffer from missing fragments of DNA.4 The ability to carry out in situ sample preparation, DNA extraction, and disentanglement are therefore of significant interest in order to carry out genome analysis. Transporting the DNA through a microfluidic system while it is packaged as a metaphase chromosome is an advantageous sample handling technique. Quake et al.5 used single chromosome handling by randomly dispersing individual chromosomes from a single cell into separate chambers. In order to avoid both homologous copies of a chromosome co-locating in the same chamber, the number of chambers was increased. An alternative approach is to actively capture single chromosomes and extract the DNA at the analysis location. Such methods include immobilising a chromosome using optical tweezers as carried out by Liang et al.6 However, immobilisation and manipulation were only carried out on chromosome fragments and the force was found to be highly dependent on the shape, size, and orientation of the chromosome. In a previous work, a stagnant flow device was used to isolate a chromosome and extract the DNA in a controlled manner.7 In this case, the immobilisation point was predefined by the design of the microfluidic system and could not be chosen arbitrarily.

Another technique for immobilisation is to embed the chromosome in a highly viscous thermosensitive polymer. Arai et al.8 presented a technique in which a single yeast cell was moved by optical tweezing to an area with a transparent microheater. The channel was filled...

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with an aqueous thermosensitive polymer and the heat transfer caused the polymer to undergo soluble-to-insoluble phase transition and immobilise the cell at a location predefined by the microfluidic design. Alternately, microchannel heating can be achieved using light-induced local heating (LILH). Thamdrup et al.\textsuperscript{9} developed a LILH technique using low-cost diodes at near-infrared wavelengths focused onto an absorption layer such that thermal conduction resulted in a large temperature gradient in a microchannel.

This paper presents the results of using a combination of LILH and reversible phase transition of a thermosensitive polymer as a method of metaphase chromosome immobilisation at a desired location in a microfluidic device. Furthermore, the paper also presents a method of carrying out proteolysis and DNA release while embedded in the poly(N-isopropylacrylamide (PNIPAAm) hydrogel.

The polymer based microfluidic chip, see Figure 1, was fabricated using the same process as reported by Thamdrup et al.\textsuperscript{9} However, the channel height was changed to 4.8 $\mu$m in order to accommodate metaphase chromosome. A light absorbing layer in close proximity to the microchannel enables LILH. The chip was mounted on an experimental setup described elsewhere\textsuperscript{9} that allowed for fluorescent microscopy, application of a pressure gradient in the microchannel, and LILH using a near-infrared laser. The positioning of the laser focus was controlled precisely using a motorized $x$, $y$, $z$ stage. The setup is sketched in Figure 1. Chromosome immobilisation was carried out by injecting a solution of chromosomes and aqueous PNIPAAm (Sigma-Aldrich) and carrying out LILH at the location of the chromosome, embedding it in hydrogel. Metaphase chromosomes were isolated from Jurkat cells (DSMZ Gmbh, Germany: ACC282) in a poly amine buffer as previously described\textsuperscript{7,10} and stained in 0.5$\times$ TBE (Tris/Borate/EDTA, Sigma-Aldrich) with 1 $\mu$M YOYO-1 at a concentration of $1.7 \times 10^6$ chromosomes/ml. Chromosomes were dispersed in a solution (10% (w/w) PNIPAAm in TBE, 0.1 mg/ml bovine serum albumin (BSA), 0.5% (v/v) Triton X-100 (octylphenol ethylene oxide condensate), 2.5% (v/v) 2-mercaptoethanol (BME), containing 10% (w/w) PNIPAAm in 0.5$\times$ TBE at a final concentration of $44 \times 10^3$ chromosomes/ml. The soluble to insoluble phase transition of this base mixture was characterized by measuring the temperature dependent viscosity using a cone-and-plate rheometer at a shear rate of 10 s$^{-1}$.\textsuperscript{10} At room temperature, a viscosity of 0.01 Pa s was measured and it increased sharply at 33 $\pm$ 0.1°C, above which viscosity could not be measured. This temperature is thus the lower critical solution temperature (LCST) of PNIPAAm. The final chromosome solution ensured a stable chemical environment for the DNA strands and reduced adhesion to the microchannel sidewalls.

Characterization of LILH in the microchannel was carried out by Thamdrup et al.\textsuperscript{9} using temperature dependent fluorescent dye imaging. Thamdrup et al. achieved temperatures above
LCST and heat generation was localized as room temperatures were measured within 50 μm of the laser focus. In our study, PNIPAAm phase transition was restricted to only part of the 50 μm wide channel by adjusting the laser power. The phase transition occurred within 50 ms. Typically, the diameter of the hydrogel was 25 μm representing a volume of approximately 2.3 pl at the onset of the infrared laser and slightly decreased over time due to the aging of the absorbing layer. The power of the infrared laser (the power was measured at the exit of the objective) was thus adjusted to 22 mW enabling flow through the microchannel and limiting the aging of the absorbing layer in the timelapse necessary to carry out proteolysis.

The chromosomes were transported through the microchannel by applying a pressure gradient of 50 mbar. Reduced fluorescence excitation light was used in order to minimize damage to the DNA strands due to photonicking. After identifying a chromosome (Figure 2(A)), the motorized x, y, z stage was used to place the laser focus downstream of the chromosome (Figure 2(B)). LILH generated a volume of PNIPAAm hydrogel, immediately embedding and immobilising the chromosome, as shown in Figure 2(C).

The PNIPAAm hydrogel was observed to be stable with applied pressures of 1.5 bar, allowing for introduction of a solution (10% (w/w) PNIPAAm in 0.5× TBE with 100 μg/μl protease K) containing protease K, in order to carry out proteolysis of the chromosome and unfolding of the DNA strands. This solution was injected into the opposite inlet/outlet and pressure was applied to fill the microchannel. PNIPAAm hydrogel forms a porous matrix through which small particles such as protease K can diffuse. As protease K is introduced to the microchannel, the fluorescent area expands with time, indicating proteolysis and the release of DNA from the protein scaffold of the metaphase chromosome as shown in Figures 3(A)–3(C).

After a proteolysis reaction running for 15 min, the laser was turned off and the DNA was released into the microchannel. As the DNA strand occupied a significantly larger volume after extraction, it was possible to embed only a part of the DNA in hydrogel. Immobilising the DNA and applying flow thus revealed that fluorescence was due to YOYO-1 bound to entangled DNA strands and the DNA was highly stretchable as observed in a previous study and shown in Figure 3(G). This observation showed that the DNA was indeed unfolded while embedded in the PNIPAAm hydrogel. This method of DNA extraction while immobilised was compared with proteolysis of a chromosome that was released into the protease K solution without being embedded in hydrogel, shown in Figures 3(D)–3(F). DNA was also extracted and stretched in a flow. In this case, the chromosome moved through the microchannel while expanding due to a slight pressure gradient still present. This drift is undesirable if one wishes...
to maintain the DNA at an analysis location. The fluorescent area was larger than in the immobilised case. Measuring the total fluorescence over the chromosomes in Figures 3(A)–3(C) and 3(D)–3(F) indicated that the chromosome in this case was five times larger. Carrying out proteolysis while immobilised provides a number of advantages: a higher local temperature resulted in a faster enzymatic reaction and therefore a faster proteolysis, the chromosome was maintained at the desired location, and the DNA strands did not adhere to the microchannel sidewalls as they were first released from the PNIPAAm hydrogel after all the proteins were digested.

In summary, a microfluidic chip capable of LILH was used to induce phase transition of PNIPAAm solution in order to embed and immobilise a single chromosome at an arbitrary location. Proteolysis was carried out while the single chromosome was immobilised, indicating diffusion of protease into the hydrogel. This method has a number of advantages over proteolysis carried out in free solution and demonstrates a controlled way to carry out DNA extraction from a single metaphase chromosome at an arbitrary location in a microfluidic system.

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FIG. 3. False color microscopy images of proteolysis of chromosomes. (A-C) A chromosome is encapsulated in the PNIPAAm insoluble phase and DNA unfolds within the hydrogel (Ref. 10). (D-F) A chromosome is released in the protease solution at $t = 0$ and the DNA unfolded in solution (Ref. 10). (G) The DNA strands extracted from a chromosome immobilised in hydrogel were stretched using the flow in a microchannel while a section was held in place using hydrogel (Ref. 10).