LOCALLY DEFINED THERMALLY REVERSIBLE HYDROGEL FORMATION IN MICROCHANNELS
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ABSTRACT
On the way to encapsulate/culture cells in a microscale three dimensional matrix we use a thermally reversible hydrogel. Poly(N-isopropylacrylamide) (pNiPAAm) and its derivatives have shown promising results for cell culturing. By means of resistive heaters embedded in the chip we can control gel formation. The formation of a gel slab and the generation of droplets and the ‘on the fly’ gelling has been demonstrated in this work. The temperature gradient inside the channel has been visualized using Rhodamine B as a fluorescent marker.

KEYWORDS: Temperature sensitive hydrogel, droplet formation, microfluidics, poly(N-isopropylacrylamide)

INTRODUCTION
Current trends aim to culture cells in a microscale three dimensional matrix that more closely mimics the natural extracellular environment [1]. Hydrogels provide a three-dimensional scaffold for cells and offer attachment points and mechanical support as well as the possibility for delivery of nutrients and removal of waste products from the cell through the porous gel matrix. By choosing polymers that reversibly undergo gel formation through changes in environmental conditions, such as temperature, cell trapping and release can be triggered externally.

Poly(N-isopropylacrylamide) (pNiPAAm) and its copolymers have shown promising results in the encapsulation of cells due to the mild conditions under which gelation occurs and due to its resemblance to the extra cellular matrix (ECM) [2,3]. PNiPAAm in pure water has a lower critical solution temperature (LCST) of 32-34°C (Fig.2a). A 10%w/w solution is used in this work.

EXPERIMENTAL
This work includes gel formation of pNiPAAm in two geometries: 1. Gel slabs are created through local heating using embedded electrodes in channels which are entirely filled with the polymer solution (fig.1a).

Figure 1. (a) The whole channel is flushed with the polymer solution. By means of resistive heating the temperature is locally brought above $T_{gel}$. (b) Droplets are formed using shear focusing.

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The interface gel/solution corresponds to the isotherm $T = T_{gel}$. 2. Building on previously reported methods of droplet formation [4], this work includes a temperature-controlled phase change of the droplets (fig.1b). Through viscous shearing droplets are created above a critical stress. Channel geometry and surface properties of the channel wall, flow rates, viscosity and hydrophilicity or hydrophobicity of the fluids play an important role in controlling droplet size and frequency of generation. The droplets are then gelled and collected using gold electrodes as resistive heaters to increase the temperature further downstream.

The channels are fabricated using PDMS soft lithography and coated with Parylene C. Due to the porous nature of PDMS water is evaporating through the wall and the concentration of the polymer solution is changed. This effect is amplified when temperature is increased. The Parylene coating minimizes the changes in concentrations.

Figure 2. (a) The viscosity of poly(N-isopropylacrylamide) (pNiPAAm) is plotted as a function of temperature. Gelling point is around 32-34°C. (b) The temperature gradient is visualized with a fluorescent dye (Rhodamine B). The grey zone is the electrode’s masking.

The temperature gradient can be assessed by measuring the fluorescent intensity of Rhodamine B (Fig.2b) [5]. The intensity decays exponentially with temperature due to increased efficiencies of non-radiative processes (decay -2.3%/°C). By calibrating the temperature/intensity relationship the intensity profiles can be correlated to an absolute temperature profile.

Figure 3. The application of a current induces visible (dark areas) gel formation over the heater electrodes due to resistive heating. Velocity profile measurement confirms that the dark regions with zero velocity are gelled and thus immobilizing the fluorescent beads (3µm).
RESULTS AND DISCUSSION

Figure 3 shows the local gel formation in a 10% w/w pNiPAAm solution. The three electrodes can be switched on individually. When no current is applied the temperature is below the LCST and no gel is formed over the heaters. A DC current (37 mA) in one of the gold electrodes (20 µm wide) brings the temperature locally above the LCST and gel formation is induced. The particle image velocimetry (PIV) measurement (Fig. 3) shows the velocity distribution along a line perpendicular to the channel. Zero velocity identifies gel regions (or dark areas). The size of the gel region can be controlled by the applied current. The pNiPAAm droplets (10% w/w) are dispersed in hexadecane using a flow focusing configuration (Fig. 4). By varying the flow rate ratio, droplet sizes in the range from 80-110 µm can be achieved with a nozzle width of 60 µm. The generation rate can be changed from 5-50 droplets per second. Further downstream the droplets are gelled and trapped.

CONCLUSIONS

This work uses Au electrodes as resistive heaters to locally change temperature in a microfluidic chip to induce gel formation. In this way gel slabs and gel droplets are created. Future work would include the replacement of the liquid phase by culture medium and the encapsulation of cells for viability studies.

REFERENCES