High-Throughput Drop-Based Microfluidics To Prepare Stable Agarose Hydrogels

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Statement of Purpose:

Drop-based microfluidic systems are becoming valuable tools for various applications such as single cell analysis, complex multistep biological and chemical assays, diagnostics, DNA sequencing, and drug screening (1). Agarose can be used to produce spherical hydrogels, which can be used in drop-based assays (2, 3). This study was designed to establish the formation of chemically modified, Agarose based hydrogels in our laboratory.

Methods: (a) Microfluidic Device Fabrication: Soft lithography techniques were utilized to prepare microfluidic devices. AutoCAD software was used to create a UV photomask which contained micron-sized capillaries of desired structure and dimension. A silicon wafer was coated with a UV photoresist material, on which the photomask was placed. After UV exposure, the silicon wafer was developed with propylene glycol monomethyl ether acetate (PGMEA) to generate a positive resist with the desired exposed channels. Polydimethylsiloxane (PDMS) was poured on top of the positive resist and incubated at 65^{o}C overnight. After removing the PDMS from the silicon wafer, the inlets were punched and PDMS was bonded to glass by plasma-activated bonding. The devices were treated with hydrophobic Aquapel to prevent the wetting of channels during drop formation.

(b) Agarose Hydrogel Droplet Preparation: A 2% solution of low melting Agarose (Sigma Aldrich A2576) was prepared in water. The solution was heated to 60^{o}C and filled into a 1 ml syringe. A second syringe was filled with fluorocarbon oil (HFE-7500) with 1.5% Krytox-PEG-Krytox surfactant. These two syringes were attached to one end of PEEK tubing, and the other end of the tubing was attached to the microfluidic device. Figure 1 shows the preparation of the Agarose hydrogel droplets when two streams (oil and Agarose solution) were flowed to the drop making junction. The flow rates of Agarose solution and HFE 7500 oil were 25 µL/hr and 300 µL/hr, respectively.

(c) Agarose Hydrogel Droplet Characterization: The stability and compatibility of the Agarose hydrogel droplets was determined by re-suspending the droplets in different concentration of glycerol buffer solution. Agarose was covalently modified by Schiffs-base reaction. Various concentrations (0.13 µM to 1.3 µM) of sodium periodate ($NaIO_4$) were tested in 5 µL buffer solution (0.3 M sodium chloride, 0.2 M sodium bicarbonate, pH 8.5) which contained sodium cyanoborohydride (125.6 mg) and 6-aminofluorescein (14.9 mg). In some experiments, Biotin-PEO_3-amine was added to the buffer solution during the oxidation of the Agarose hydrogel droplets to study binding behavior between Biotin and Streptavidin.

Results: The suspended Agarose droplets in different concentration of glycerol solutions (5 to 75 vol %) did not shrink or break up to 3 weeks and were very stable. In order to conduct emulsion PCR or secretion assays, there is a need to conjugate proteins or primers to the Agarose hydrogel (2). For this reaction to occur, the hydrogel needs to be oxidized with NaIO4. Figure 2 shows stable droplets of Agarose oxidized with different amount of NaIO4. It shows that the oxidation of Agarose hydrogels does not interfere with gelation, and the fluorescein gets brighter as the concentration of NaIO4 increases. We used Streptavidin and Biotin as a model system in order to study the binding of proteins to enzyme in the hydrogel droplets. Figure 3 shows that the Streptavidin diffused into the hydrogel droplets and bound to biotinylated Agarose hydrogel.

Conclusions: We showed that biotinylated hydrogel droplets prepared from Agarose are stable and can thus be utilized for various applications such as droplet PCR (1) or droplet secretion assays (3).

References:

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3. Mazutis, L., Gilbert, J., Ung, W. L., Weitz, D.A., Griffiths, A. D., and Heyman, J.A., Nature Protocols, (2013), 8, 870-891.

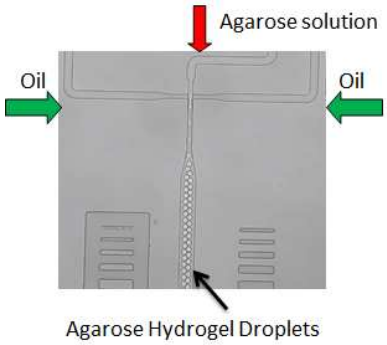

Figure 1. Microfluidic Device to Prepare Agarose Hydrogel Droplets

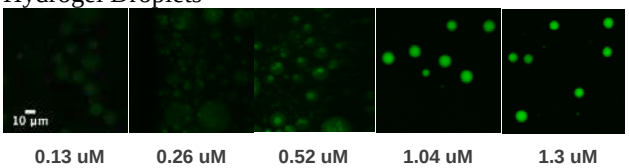

Figure 2. Oxidation of Agarose Hydrogel with Sodium Periodate

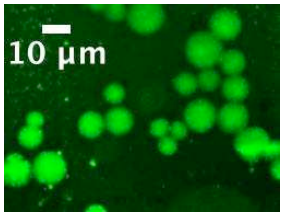

Figure 3. Binding of Streptavidin onto Biotinylated Agarose Hydrogels