µEncapsulator Application Note for Encapsulation of Hydrogels

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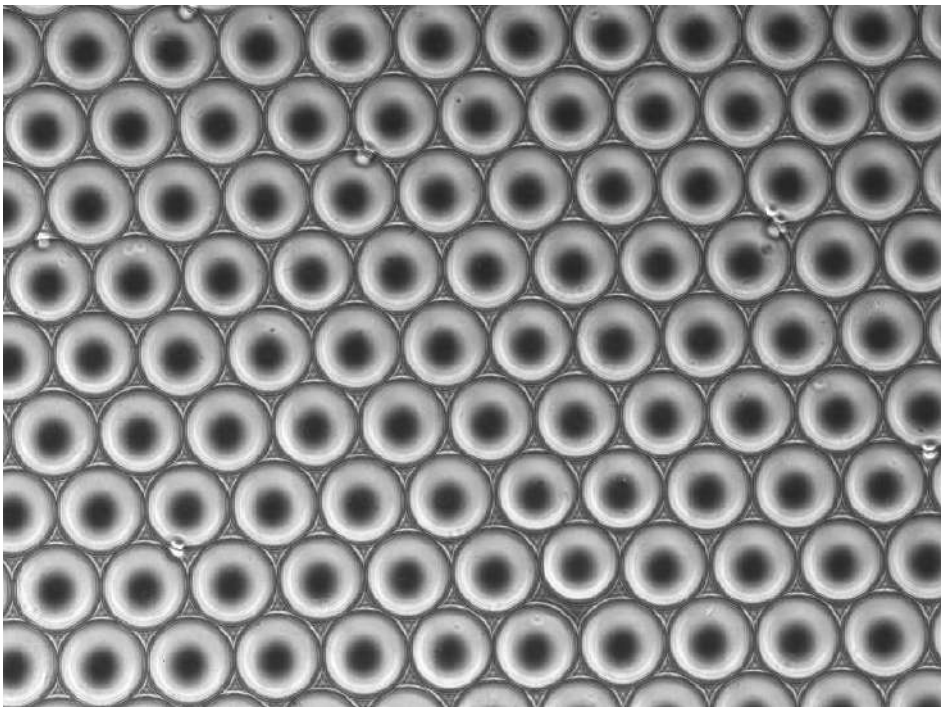

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Contents

1 Disclaimer 3

2 Summary 3

3 Introduction 4

4 Materials and Methods 5

5 Results 8

6 Conclusion 10

7 Product Information 11

7.1 µEncapsulator System 11

7.2 µEncapsulator Chips 11

1 Disclaimer

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2 Summary

This application note summarises the results of the encapsulation of Agarose and Matrigel on the µEncapsulator system using a 50 µm µEncapsulator 1- 2 Reagent Droplet Chip (Figure 1). It details parameters such as flow rates and temperature, recovery of the hydrogel beads as well as useful tips for cleaning the system after encapsulation of hydrogels.

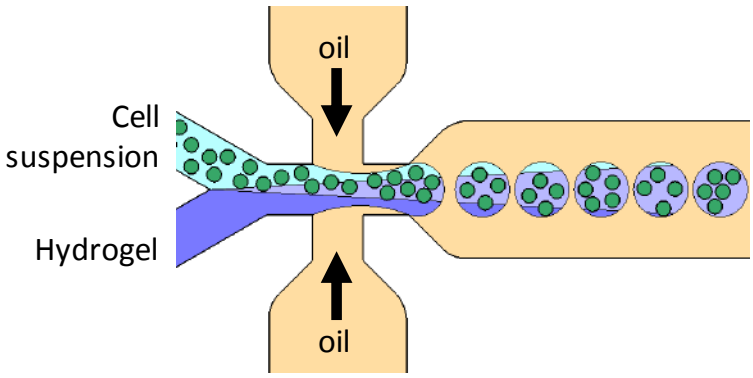

Figure 1 Schematic of hydrogel droplet generation. Tens of thousands of single cells are encapsulated with hydrogel inside droplets.

3 Introduction

There is an increasing amount of publications using hydrogels for single cell research. This is due to the unique capability of hydrogels to retain particles or cells of a certain size as well as being porous at the same time, allowing smaller molecules or buffers to diffuse in and out of the hydrogel. Furthermore, most of them can be kept liquid or harden at temperatures that are compatible when working with cells or bacteria, requiring temperatures most commonly between 4 and 37˚C. With Dolomite Microfluidics's µEncapsulator it is possible to generate hydrogel droplets from a liquid stream of hydrogel that can be hardened into solid hydrogel beads. These beads will retain cells or genomic DNA after breakage of the emulsion but allow for the exchange of smaller particles, such as enzymes, RNA, antibodies or cytokines between the bead and the medium surrounding it. These properties enable a plethora of different applications, one of which is the amplification of marker genes to detect somatic mutations in single cells ([1]). Hereby, cells are encapsulated in hydrogels and after recovery of solid hydrogel beads from the emulsion the cells are lysed inside the beads. The beads are then washed to remove cell debris, leaving clean genomic DNA inside the hydrogel beads. They can then be re-encapsulated with PCR mix to amplify the target marker gene of interest, unbiased from cell debris. Furthermore, hydrogel beads offer the opportunity to grow cells in a defines 3D environment for an extended period of time as hydrogels allow for the diffusion of nutrients and oxygen from the medium surrounding the bead to the cell ([2]). The following application note aims to introduce the user to the generation of agarose and Matrigel beads, two of the most commonly used hydrogels in research.

[1] Novak R., et al., "Single-Cell Multiplex Gene Detection and Sequencing with Microfluidically Generated Agarose Emulsions", Angew Chem Int Ed Engl. 2011 Jan 10; 50(2): 390–395.

[2] Dolega M. E., et al., "Controlled 3D culture in Matrigel microbeads to analyze clonal acinar development", Biomaterials. 2015 Jun;52:347-57

4 Materials and Methods

Droplet system. The µEncapsulator system (Figure 2 A) uses pulseless Dolomite Microfluidics P-Pumps and can be driven from a PC via Dolomite Microfluidics's Flow Control Centre (FCC) software. There are three highly precise pressure pumps driving the cell, hydrogel and oil lines (Figure 2 B). They are connected to a sample reservoir where cells and a liquid hydrogel is stored, which sits directly on top of the temperature control unit keeping the whole microfluidic path at a set temperature.

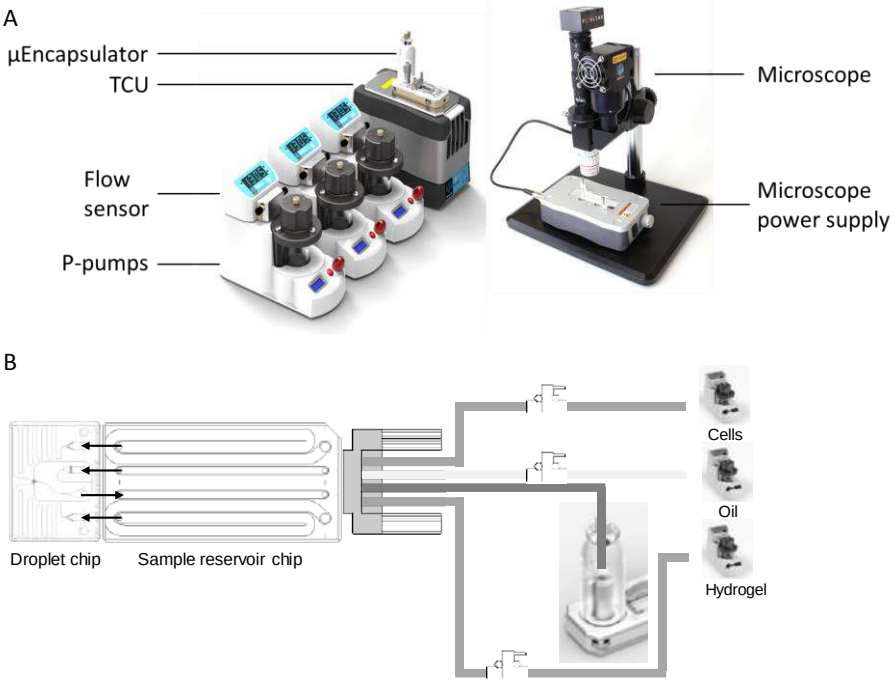

Figure 2 µEncapsulator system set up. (A) Image of the system set up depicting all the components of a µEncapsulator. (B) The schematic of the individual fluidic lines driving cells, hydrogel, and oil.

High Speed Microscope

The Dolomite High Speed Microscope (Figure 2 A) is a simple compact microscope, with a convenient long working distance lens. There are no eye-pieces, partly to protect users from the very bright light source. The microscope allows short exposure times (50 µs), which is useful in monitoring high speed droplet production.

Glass µEncapsulator Chip

The current literature describes prototype PDMS Chips for droplet formation. For standard products, glass is preferred, as the glass chips are robust, achieve highly reproducible performance, and are chemically inert. Dolomite Microfluidics therefore produced a glass µEncapsulator droplet chip (Figure 3, see appendix for a full list of available chips) for the easy flow of reagents. The channels allow the use of robust, standard, leak-free connectors, while allowing the junction to be readily imaged. The chips used in this application note had a 50µm microfluidic junction and fluorophilic coating.

Agarose

Ultra-low Gelling Temperature agarose (A2576, Sigma-Aldrich) at 2 % concentration was mixed with water and molten in a microwave. The agarose was then filtered through a 0.2 µm syringe filter and either loaded immediately or stored at 50 ˚C in an

incubation oven. Equally a hot plate at 60 ˚C and constant stirring of the agarose might be used. The final concentration of agarose inside the droplets was 1 %.

Matrigel

Matrigel Matrix (Product #356231, Corning) was used undiluted and filtered using a 0.2 μm syringe filter. Matrigel was kept on ice until encapsulation. Final concentration of Matrigel inside the droplets was 50 %.

Hydrogel encapsulation

The TCU was set at a temperature that allowed the hydrogel to stay molten over duration of encapsulation. All the liquids used (oil, hydrogel, cell suspension buffer) were pre-filtered using 0.2 μm syringe filter prior to loading onto the system. Ultra-low gelling agarose was encapsulated at 37˚C and Matrigel at 4˚C, for each of the temperatures the μEncapsulator was allowed to cool down under the action of the TCU for 10 min before the hydrogel was loaded into the sample reservoir chip. The oil pump was loaded with 2 % FluoSurf, an oil commercialised by Dolomite Microfluidics and containing a proprietary surfactant, and the two pumps driving the aqueous lines with Novec 7500. Novec 7500 loaded into the 'aqueous' pumps functioned as a 'driving liquid', to push the cell buffer and hydrogel suspension out of the sample reservoir chip. The system was primed by running all three lines at 2 bar until liquid appeared at the linear connector (Protocol Figure 4, this will happen within seconds). The pumps were then stopped, and all three valves closed. The hydrogel and water were loaded into the sample reservoir chip (Protocol Figure 5) and the linear connector fixed to the μEncapsulator module. All three p-pumps were pressurised to 200 mbar, firstly the cell line valve was opened and switched to flow mode (flow rates listed in Table 1 for Matrigel and Table 2 for Agarose). Next the same was done by opening the valve for the hydrogel line and switching to flow mode to set the flow rate. Finally, the oil valve was opened and switched to flow mode. The flows were started in the order cells>hydrogel>oil, to avoid backflow of hydrogel solution into the cell line. Droplet production was stable and easily initiated at flow rates listed in the Table 1 for Matrigel and Table 2 for agarose. At these flow rates Matrigel flows at 2,500 droplets per second and agarose at 1,500 droplets per second. This means that a full run for encapsulating 2 x 100 μl will take about 20min for Matrigel and 30min for the agarose. The collected emulsion was transferred to room temperature/4 ˚C and 37˚C for agarose and Matrigel, respectively. The setting of liquid Matrigel into solid beads took about 30 min at 37 ˚C.

Table 1

Pump	**Flow rate [μl/ min]**
Oil	50
Matrigel	5
Cells	5
Temperature [˚C]	4

Table 2

Pump	**Flow rate [μl/ min]**
Oil	50
Ultra-low melting agarose	3
Cells	3
Temperature [˚C]	37

Cleaning of the µEncapsulator chip after hydrogel bead production

After using the system, it is essential to clean the system and the droplet chip, as otherwise the coating can be degraded, and the chip may then fail to make droplets. When working with hydrogels it is particularly important to keep the TCU running until all hydrogel has been removed from the droplet chip. Otherwise hydrogel might solidify inside the chip and clog the sample channel. For purging the chip of any remaining hydrogel, the easiest option is to run the µEncapsulator until the aqueous lines run out of aqueous sample (either cell suspension or hydrogel). If this is not applicable, the run can be stopped at any given time and the remaining liquid aspirated from the sample reservoir. From there, directly proceed to the cleaning procedure described below.

1. Remove all liquids from the P-Pumps and run them at 4 bar to empty tubing from any remaining liquid.
2. Rinse both sample reservoirs in the sample reservoir chip with 100 µL of detergent (e.g. 1% SDS).
3. Remove liquid from sample reservoirs.
4. Add 30 µL detergent to the reservoirs, connect the chip to the connector.
5. Run the pumps at 4 bar to flush detergent through the µEncapsulator chip for 1min.
6. Repeat steps 2 to 5 first with water, then 100 % isopropanol and finally air (remove any remaining liquid in sample reservoir chip).
7. Optional: dry the sample reservoir chip using a clean air supply (e.g. air gun), if available.
8. Put all chips back into zip lock bags to keep away from dust and particles.

5 Results

The objectives of these tests were 1) to define hydrogel concentrations, flow rate conditions and temperatures that allow for stable encapsulation of hydrogels on a 50 µm glass µEncapsulator 1- 2 Reagent Droplet Chip, 2) test the recovery of hydrogel beads and 3) clean chips and system after hydrogel usage.

Testing flow rates and temperatures for the encapsulation of hydrogels. To achieve the successful encapsulation of hydrogels in droplets a series of flow rates and hydrogel types and concentrations were tested. For this experiment Matrigel was used undiluted in one channel and mixed with cell buffer loaded in the second channel. This resulted in a final concentration of 50% Matrigel in the hydrogel bead. At this concentration and 4˚C temperature the hydrogel was flowing without problems through the junction. Flow rates of 50 µl/min for the oil line and 7 µl/min for the aqueous lines were tested but these flow rates caused jetting. Reducing the aqueous lines to 5 µl/min led to continuous and monodisperse droplet formation. Ultra-low gelling (A2576, Sigma-Aldrich) agarose was tested at a 2% concentration, resulting in 1% final concentration in the droplets. A combination of flow rates was tested: 50 µl/min on the oil line and 7, 5 and 3 µl/min on the aqueous lines at 37 and 40 ˚C. Both 7 and 5 µl/min caused jetting at 37˚C but 3 µl/min lead to continuous and monodisperse droplet formation. When increasing the temperature to 40˚C also 5 µl/min produced a steady stream of droplets.

TIP:

When producing hydrogel beads for FACS, use a 50 µm droplet chip at 1 - 2 µl/min on the aqueous lines and 50 µl/min for oil. This produce hydrogel beads that are about 40 µm in size which is suitable for most FACS sorters.

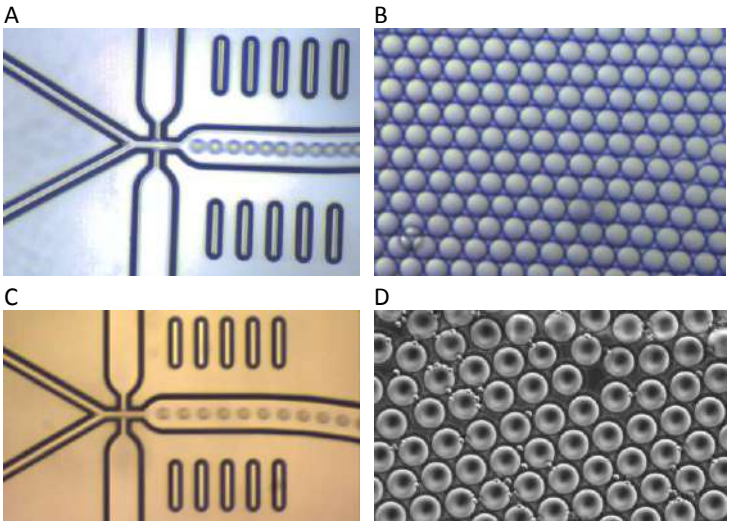

Figure 3 (A) Matrigel and (C) agarose emulsions were produced at the optimal flow rates, and droplet formation imaged at the chip junction.(B) Matrigel and (D) agarose emulsions imaged after formation show high monodispersity.

Recovery of Matrigel beads. Matrigel beads were recovered from the droplets via breaking of the oil emulsion with Fluoro-Stop. Before starting make sure to collect all the emulsion in a micro-centrifuge tube.

1. The emulsion was spun in a (micro) centrifuge tube for 30 seconds at 100 x g to ensure that the emulsion was floating on top of the oil.
2. Using a standard pipette, as much of the oil suspension as possible was removed from bottom layer. This minimised the amount of Fluoro-Stop necessary to break the emulsion.
3. The volume of emulsion was estimated, a typical µEncapsulator run should result in about 200 µl of emulsion
4. 2 to 3 volumes of Fluoro-Stop were added to the emulsion and the tube was inverted a few times by hand.
5. It was check that the emulsion has broken (a phase separation started appearing)
6. The plastic tube was spun in a micro-centrifuge for 1min at 1000 x g

Figure 4

7. Following phase separation, two layers appeared inside the tube (Figure 4)
8. A clear upper layer that contained the Matrigel beads
9. An orange coloured lower layer with the unwanted fluorous phase
10. The tube was tilted at 45° and the aqueous supernatant layer was removed and kept in a clean micro-centrifuge tube for further experimentation or analysis
11. The beads in the aqueous phase were observed under a light microscope. Figure 5 shows the recovered Matrigel beads, arrows are pointing toward one of the beads due to their low contrast their visibility is very low.

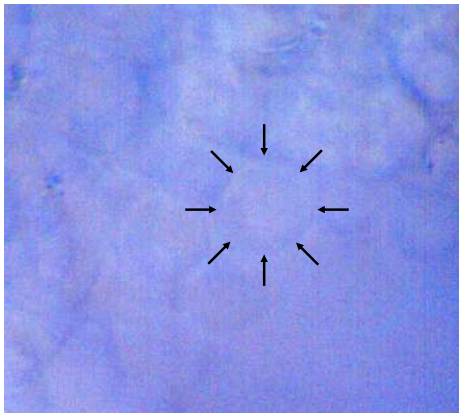

Figure 5 Recovered Matrigel beads

Cleaning system after usage. Cleaning the system after using hydrogels is very similar to the method suggested in the µEncapsulator module. The main difference when dealing with hydrogels is the potential danger of blocking channels of the droplet chip with hardened hydrogels. Therefore, two different methods were tested for removing hydrogel from the droplet chips. The most straightforward approach was to run the µEncapsulator until all liquid from the aqueous sample reservoir was used up, as this resulted in the droplet chip being flushed with Novec 7500. It was also possible to stop the run at any given time, aspirate the left-over liquid from the sample reservoirs and then proceed straight to the cleaning protocol. In any case it was found that it was important to leave the TCU at production temperature as long as hydrogels are still present in either the droplet or sample reservoir chip. Failure to do resulted in chip blockage.

TIP:

To unblock a droplet chip from hardened agarose, set a hot plate to 90 ˚C and lay down the droplet chip with the gasket facing away from the metal surface. After 5 min at 90 ˚C re-insert the chip into the µEncapsulator and continue with the cleaning procedure.

6 Conclusion

Hydrogels are a very useful tool when wanting to grow or image cells, perform step-wise reactions or even FACS sort cells. This application note showed that by using the µEncapsulator system hydrogels can be readily encapsulated inside droplets and solid Matrigel beads can be formed. The produced beads were extremely monodisperse and their size could be easily adjusted by altering flow rates or the droplet chips used.

7 Product Information

7.1 µEncapsulator System

Orders from	Instrument/Consumables	Order Number
US and Canada	µEncapsulator System with Enhanced Control (110v, 60Hz, US) - Excludes Applications Pack	3200554
	High Speed Digital Microscope and Camera	3200531
UK	µEncapsulator System with Enhanced Control (230V, 50Hz, UK) - Excludes Applications Pack	3200556
	High Speed Digital Microscope and Camera	3200531
Europe	µEncapsulator System with Enhanced Control (230V, 50Hz, EU) - Excludes Applications Pack	3200558
	High Speed Digital Microscope and Camera	3200531
Japan	µEncapsulator System with Enhanced Control (100V, 50-60Hz, JP) - Excludes Applications Pack	3200560
	High Speed Digital Microscope and Camera	3200531
Rest of the world	µEncapsulator System with Enhanced Control (230V, 50Hz, UK) - Excludes Applications Pack	3200556
	High Speed Digital Microscope and Camera	3200531
	Installation and Basic Training (supplement for 2 days, on site RoW)	3200571

7.2 µEncapsulator Chips

Instrument/Consumables	Order Number
µEncapsulator 1 Sample Reservoir Chip (2x 100µl) Pack of 3	3200562
µEncapsulator 1 - 2 Reagent Droplet Chip (50µm etch depth), fluorophilic, Pack of 3	3200563
µEncapsulator 1 - 2 Reagent Droplet Chip (50µm etch depth), hydrophilic, Pack of 3	3200564
µEncapsulator 1 - 2 Reagent Droplet Chip (30µm etch depth), fluorophilic, Pack of 3	3200567
µEncapsulator 1 - 2 Reagent Droplet Chip (30µm etch depth), hydrophilic, Pack of 3	3200568
µEncapsulator 1 - 2 Reagent Droplet Chip (15µm etch depth), hydrophilic, Pack of 3	3200565
µEncapsulator 1 - 2 Reagent Droplet Chip (15µm etch depth), fluorophilic, Pack of 3	3200566