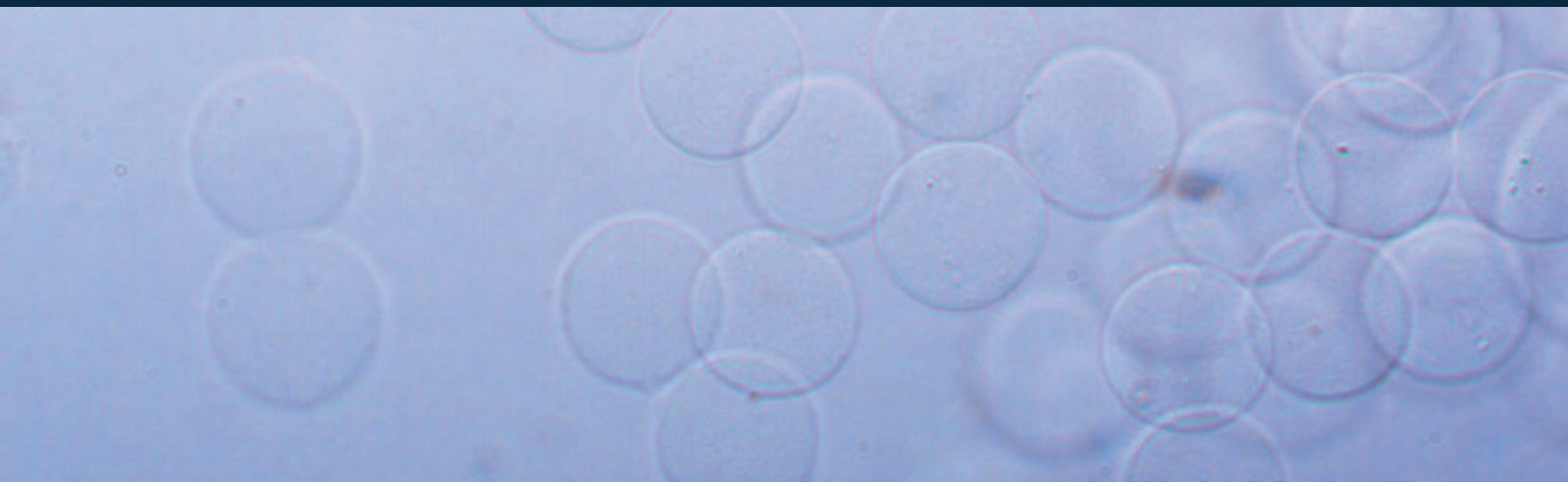
ELVE FLOW
PLUG & PLAY MICROFLUIDICS
an **ELVESYS** brand

User guide

Alginate particle production

EXPERIMENT: Alginate particle production

DOCUMENT REF: UG-ALG20-20-0604



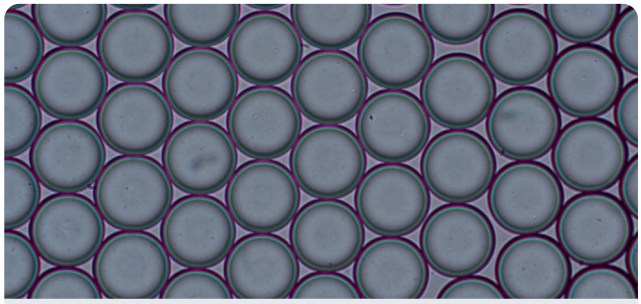
INRODUCTION

This user guide will show you how **highly monodispersed alginate beads** can be easily generated with a microfluidic droplet generation system:

We will first start by preparing an **alginate/ calcium-EDTA solution**.

Then microdroplets of this solution will be generated using a microfluidic system.

Finally, a calcium compound will be released in the droplet by a pH modifying agent (acetic acid), and will crosslink with alginate to generate **Ca-alginate beads**.



Several experimental conditions have been tested to achieve highly monodispersed droplet with size in the **50 µm range.** Other beads size are achievable with a similar setup.

Contact us at contact@elveflow.com to know more.

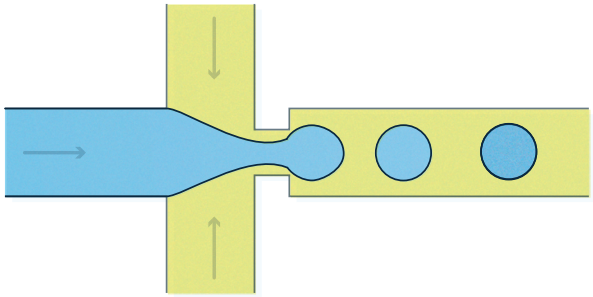
Step 1: **Solutions preparation**

Tank 1
Alginate & calcium-EDTA

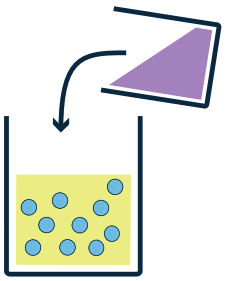
Tank 2
Oil reservoir

Vial 1
Acetic acid solution

Step 2: **Generation of alginate/calcium-EDTA droplets**

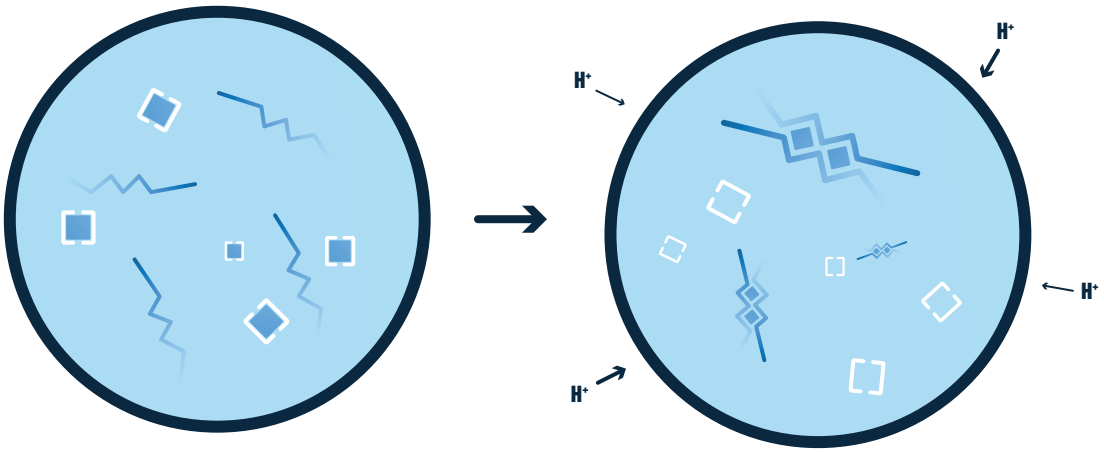


Step 3: **Adding acetic acid for Ca2+ ions release leadind to Ca-alginate crosslinking and beads formation**



Alginate **droplet** (pH 7)

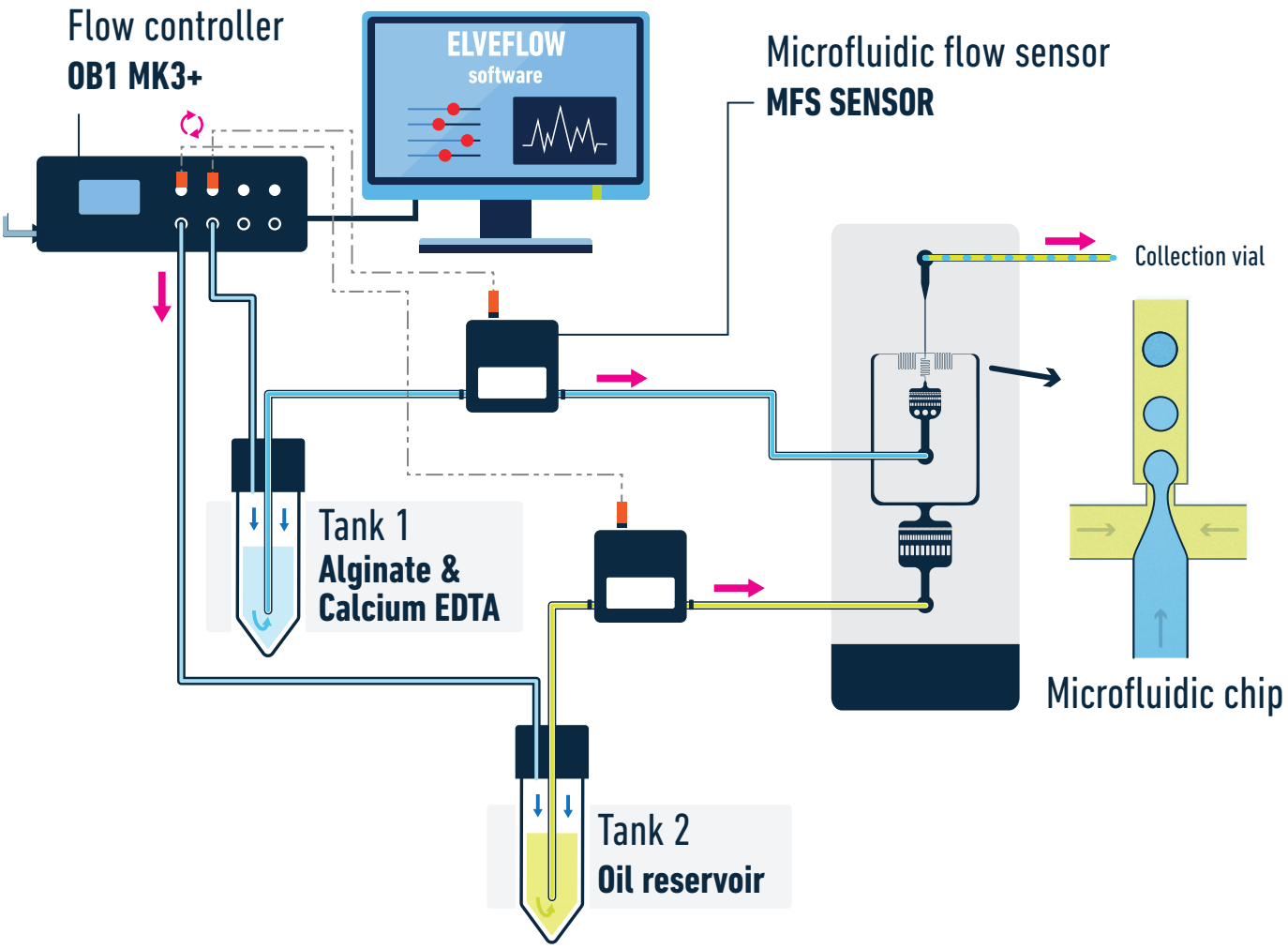
Alginate **bead** (< pH 5)



Calcium
EDTA
Calcium-EDTA
Alginate chain
Calcium crosslinked alginate chains

Step 4: **Adding the emulsion breaker to release beads from droplets.**

SETUP



MATERIALS

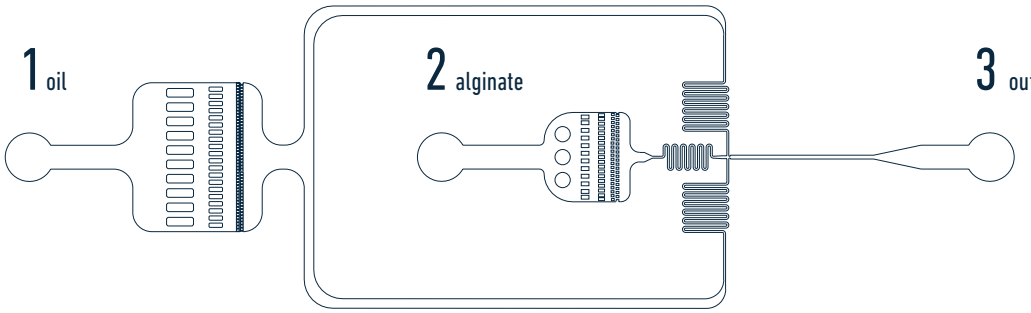
Hardware:

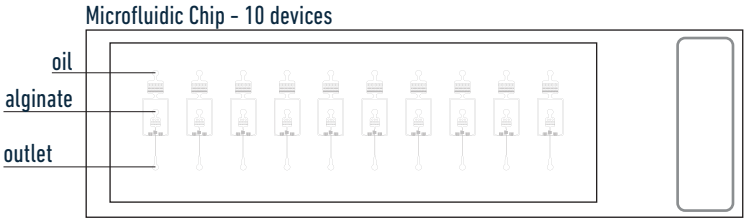
1. OB1 flow controller with at least two channels 0/2000 mbar
2. 2x flow sensors MFS2 0/7 µL/min
3. Kit starter pack Luer Lock + 1/32 tubings + 1/32 sleeves + 23G needles
4. 2x 15 mL Falcon reservoirs
5. Microfluidic chip (hydrophobic channels)
6. Microscope for observation (optional)
7. Fast camera to register droplets (optional)

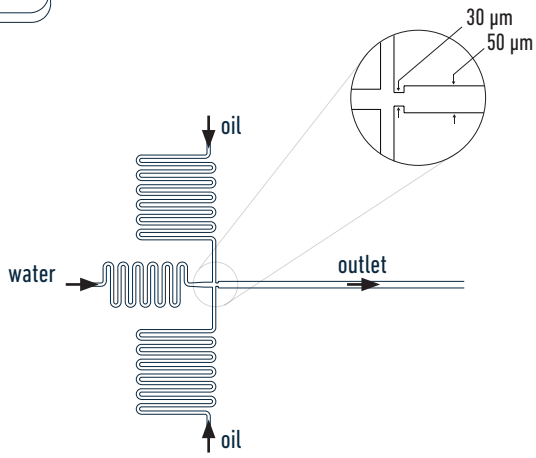
Chemicals:

1. Alginate (low viscosity) - Merck, SKU: A1112-100G
2. Calcium-EDTA - Merck, SKU: ED2SC-100G
3. Acetic acid - Merck, SKU: 33209-1L-M
4. HFE7500 oil with 2% surfactant - Droplet Genomics
5. Droplet breaking solution - Droplet Genomics or Merck, SKU: 370533-5G

DESIGN OF THE CHIP







QUICK START GUIDE

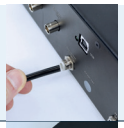
SOLUTIONS PREPARATION

1. Mix 2% w/w alginate/water solution and add 0.5M Calcium-EDTA into the mix.

2. Prepare the acetic acid solution. Depending on needed gelation time, use up to 2% acetic acid for immediate gelation and below 0.2% for slow gelation (not recommended to use below 0.05%).

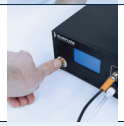
MICRODROPLET GENERATION

Standard droplet preparation procedure, here with alginate & calcium EDTA instead of water.

3.  **Connect your OB1 pressure controller to an external pressure supply** using pneumatic tubing, and to a computer using USB cable. For detailed instructions on OB1 pressure controller setup, please read the OB1 user guide.

4.  **Plug microfluidic reservoir to the OB1 pressure controller outlet.** The Elveflow reservoirs connection instructions are covered by a specific guide (see Elveflow microfluidic reservoirs assembly instructions).

5.  For the feedback loop, connect a flow sensor to the OB1.
Then, for flow measurement, connect flow sensors between the microfluidic reservoirs and the chip

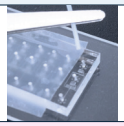
6.  Turn on the OB1 by pressing power switch.

7.  **Launch the Elveflow software.** The Elveflow Smart Interface's main features and options are covered in the Smart Interface guide. Please refer to those guides for a detailed description.

8. **Press Add instrument \ choose OB1 \ set as MK3+,** set pressure channels if needed, give a name to the instrument and press OK to save changes. Your OB1 now should be in the list of recognized devices.

9. **OB1 calibration is required** for the first use. Please refer to the OB1 user guide.

10. **Add flow sensor: press Add sensor \ select flow sensor \ analog or digital \ max flow rate for the sensor, give the name** for the sensor, select to which device and channel the sensor is connected and press OK to save the changes. For details refer to Microfluidic flow sensor user guide.

11. 

Use the supplied 1/32" OD tubing to connect microfluidic reservoirs with the chip.

Tips from the expert

Tubing connection to the chip:

For easier insertion of tubing into the PDMS chip, it is recommended to cut the tube at a slight angle, then use a flat (with ridges) tweezer to insert the tubing into PDMS. However, at high pressures, it is better to cut the tubing flat to make it hold better. At insertion, push the tubing until reaching the glass, then slightly pull back so as not to clog the channels with the tubing.

12. **Set pressures** (and other parameters if needed) and start pumping liquids into the chip.
Wait until all air bubbles escape from the chip and both liquids are flowing.

Change the pressure of water and oil channels to start generating droplets. Their size and frequency will depend on the pressure, flow rate and viscosity of the liquids used. **See droplet size/pressure diagram on page 6.**

Tips from the expert

Chip priming:

To begin with, run liquids into tubes until the liquid starts to drip. Only then, connect them to the chip, starting from the continuous phase (in this case - oil).

Once the oil tubing is connected, apply a pressure of around 100 mbar and wait until the chip is filled with oil. Only then plug the alginate channel (500 mbar could be used to flow in the tube, then after connecting, go back to 100 mbar).

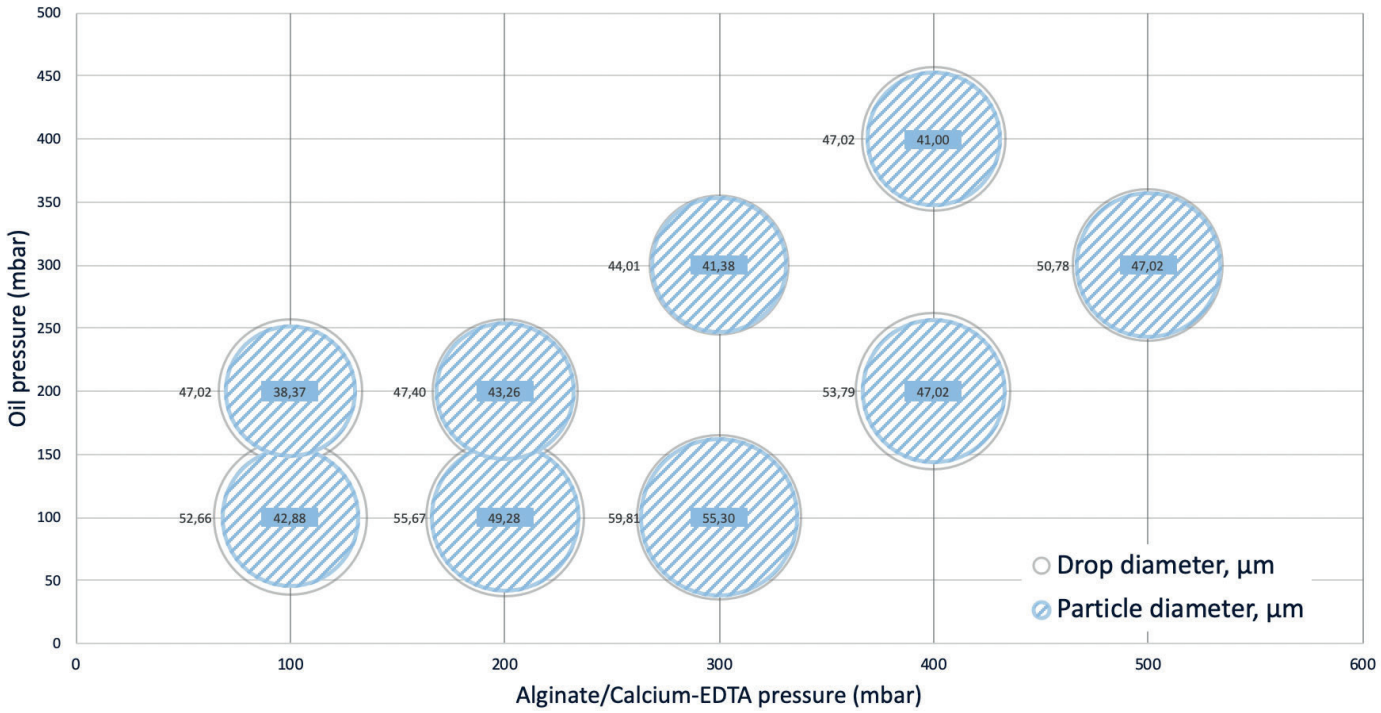
To begin with droplet generation, start by putting both channels at 100 mbar.

BEADS CROSSLINKING AND RELEASE

13. **Collect the droplets in the collection tube.** Then add the acetic acid solution, and mix for better acid access to the droplets.
Acetic acid will react with the calcium carbonate to release the calcium ions, and these calcium ions, through crosslinking reaction with the alginate, will produce Ca-alginate microspheres

14. **Once the gelation finished,** break the droplets using droplet/emulsion breaking solution.
The droplet breaking solution will remove surfactant from the droplets to release the alginate beads.

DROPLETS' SIZE/PRESSURE DIAGRAM



ALGINATE/ CALCIUM-EDTA PRESSURE mbar	OIL PRESSURE mbar	DROP DIAMETER µm	PARTICLE DIAMETER µm	FLOW ALG. µl/min	FLOW OIL µl/min
100	100	52,7	42,9	0,2	2,1
100	200	47,0	38,4	0,16	3,13
200	100	55,7	49,3	0,71	2,13
200	200	47,4	43,6	0,66	2,9
300	100	59,8	55,3	1,25	1,9
300	300	44,0	41,4	1,2	3,6
400	200	53,8	47,0	1,8	2,8
400	400	47,0	41,0	1,8	3,99
500	300	50,8	47,0	2,45	3,2

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