Electronic Supplementary Material (ESI) for Lab on a Chip.

Controllable Generation and Encapsulation of Alginate Fibers Using Droplet-Based Microfluidics

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Electronic supplementary information (ESI)

1. Associated Movies

Movies of fiber generation using the conditions presented in Figure 2, Figure 3 and Figure 5 are provided in the following files

(a) A106_B90_140_06s_035_C110_O350.avi
(b) A116_B140_160_06s_035_C110_O350.avi
(c) A115_B120_160_06s_035_C110_O350.avi
(d) A102_B70_130_04s_035_C110_O350.avi
(e) A115_B120_160_09s_035_C110_O350.avi
(f) Encapsulation.avi

2. Segmentation of an alginate stream using a PDMS lateral valve

A PDMS lateral valve[26], with a membrane thickness of 33 µm, was tested at pressures ranging from 0.1 to 3.0 bar in order to control the flow along the alginate channel. A pressure in excess of 2.5 bar was needed to segment the alginate flow, however, inefficient production of fibers occurred due to the dead volume between the inflatable area and the upcoming junction and alginate shear thickening. Figure S1 shows fluorescence images of the device during an ON – OFF valve cycle (with a period of 0.5 s and a duty cycle of 25%).

Valve pressurization does not result in a separation of the alginate precursor solution but, on the contrary, leads to an alginate flooding of the channel (Figure S1c, arrow). Valve relaxation on the other hand causes the alginate to retract slightly by creating a negative pressure in the alginate channel, which leads to a cut of the alginate jet. Although segmentation can be achieved with the lateral valve, it comes at the cost of extensive alginate-sheathing buffer mixing and a very heterogeneous fiber geometry.

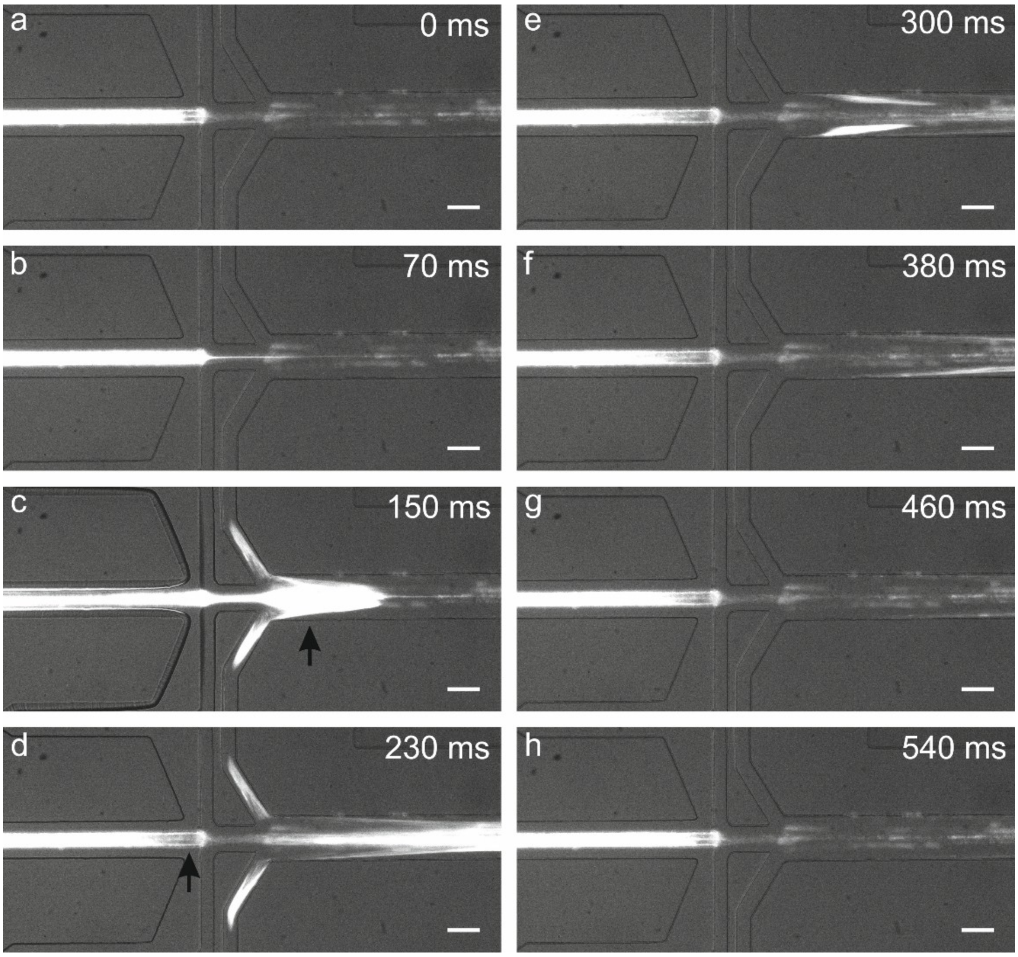

Figure S1 Alginate pulsation using a lateral valve. (a-h) Time sequence displaying an alginate solution seeded with fluorescent nanoparticles during an ON – OFF (0.1 – 0.4 s) cycle. The lateral valve (having a membrane thickness of 33 µm) was pre-filled with deionized water, interfaced with a solenoid valve and controlled by a Python script. Valve frequency and maximum pressure were kept constant at 2 Hz and 2.5 bar. Alginate solution, sheathing buffer, calcium buffer and the oil were pressurised using pressure controllers at 127 mbar, 160 mbar, 110 mbar and 350 mbar respectively. (c) When pressurized, the lateral valve provided a partial channel occlusion that did not break the alginate jet. The applied pressure propagated into the alginate stream, deformed its shape (denoted by the arrow) and flowed into the calcium buffer channel. (d) The alginate jet eventually segmented (shown by the arrow) and polymerised. Traces of its polymerization are visible all along the channel (d-h). Scale bars are equal to 50 µm.

3. Fiber length quantification

Automated quantification of fiber length was performed by applying a Gaussian blur filter (sigma = 6) and background subtraction, followed by auto local thresholding using the Phansalkar thresholding method (r = 170). The length of each object was measured only when the entire fiber was visible within a frame. In order to exclude debris from analysis, a general size cut-off of 4000 µm^2 was applied. Fibers with a length less than 300 µm required a cut-off of 2000 µm^2. Figure S2 shows two representative images extracted during fiber identification and quantification process. The code identifies fibers in the presence of debris and sets a threshold (yellow line) for length quantification.

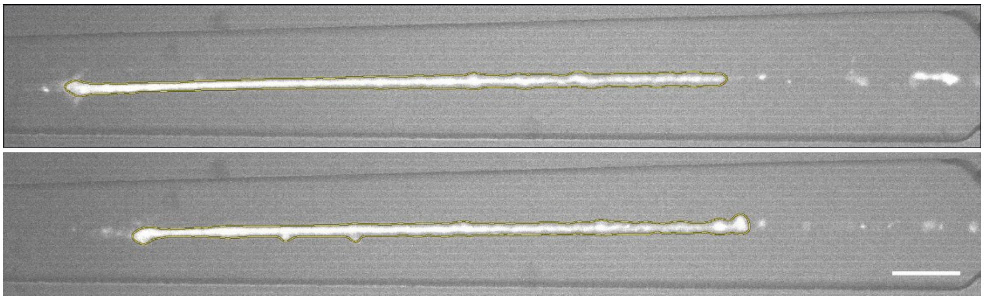

Figure S2 Fiber identification and length quantification. Frames extracted during the analysis. The threshold is set to distinguish fibers from debris, with the yellow line defining the contours of the entire fiber. Scale bar is equal to 50 µm.

3. Variation of alginate fiber length

Variation of parameters including P_A, P_B (P_{B_low}/P_{B_high}) and the period cycle lead to changes in the alginate fiber length. Figure S3 shows a box plot generated using all the conditions listed in Table S1.

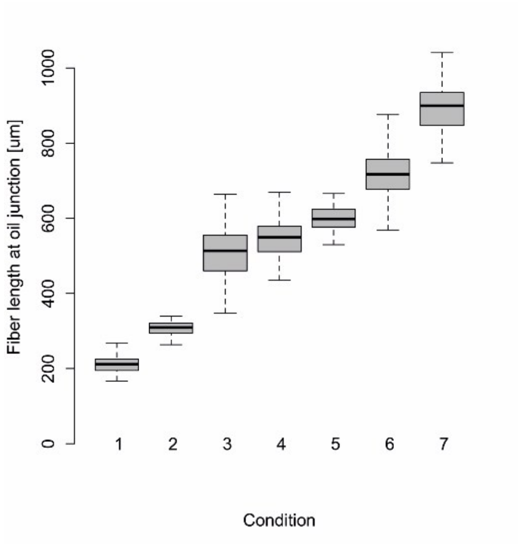

Figure S3 Box plot of fiber length obtained under the operating conditions listed in Table 1.

Condition	P_A (mbar)	P_B (mbar)	Period (s)	Duty Cycle (%)	P_C (mbar)	mean Fiber Length ± stdv (µm)
1	102	70/130	0.4	68	110	**211 ± 24**
2	106	90/140	0.6	68	110	**309 ± 17**
3	110	120/140	0.6	68	110	**514 ± 63**
4	106	100/140	0.4	68	110	**549 ± 49**
5	115	120/160	0.9	68	110	**598 ± 33**
6	116	140/160	0.6	68	110	**717 ± 72**
7	115	120/160	0.6	68	110	**900 ± 106**

Table S1 Operating conditions leading to different alginate fiber length.