Electronic Supporting Information 1: Gel growth kinetics at different fluid flow rates

Knowledge of the kinetics of the gel growth process allows to design timed switching protocols for producing layers with a predetermined width. We find that the alginate gel growth process is influenced by the fluid flow rate of the liquid alginate precursor solution along the gel. To characterize the gel growth process, we recorded the gel width as a function of time for different fluid flow rates in the alginate compartment (geometry of the alginate side of the reaction chamber: width 165 µm, length along the fluid flow direction 130 µm, and height 50 µm). The result is shown in Fig. SI-1. Only at the lowest flow rates does the gel growth follow what one predicts by considering the reaction stoichiometry and the diffusion of Ca^{2+} through the filters and the already gelled alginate (the theoretical line in Fig. SI-1). At higher flow rates, the gel growth process is slowed down substantially.

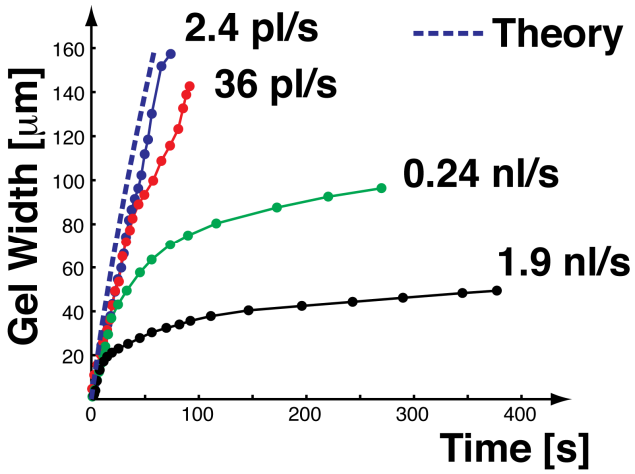

Fig.SI-1: Influence of the fluid flow rate on the gel growth process. With increasing fluid flow rate along the growing gel edge in the alginate compartment, the gel growth rate diminishes. The theoretical curve is obtained by considering diffusion of Ca^{2+} and the reaction stoichiometry only.

The decrease of gel growth rate at high fluid flow rates probably explains why we observe some deformation of the gel layers close to edges which speed up or slow the local flow field.

The use of fluorescently alginate for this experiment also allows to draw some conclusions concerning the mechanism leading to slower gel growth with increased precursor alginate flow rate along the gel edge. In principle, two distinct mechanisms can lead to a decrease of the gel growth rate: firstly, an increased alginate flow rate could lead to a densification of the gel by diffusion of additional alginate into the growing gel, thus requiring more Ca^{2+} per µm of gel and therefore decreasing the gel growth rate. And secondly, partially crosslinked alginate could be washed out from the reaction zone, thus decreasing the amount of Ca^{2+} without necessarily changing the gel density. By evaluating the fluorescence intensity, we find that both mechanisms play a role, but that quantitatively, the washout of Ca^{2+} from the reaction zone is much more important. Indeed, even at the highest flow rate, we find a maximum increase of gel density of about 50%, whereas the gel growth rate is slowed down by a maximum factor of about 50 under the same conditions. Thus, while the increased need for Ca^{2+} due to gel densification could explain a decrease of gel growth rate by a factor of 1.5, the remaining factor of about 30 must be due to a washout of partially crosslinked alginate and hence Ca^{2+} from the reaction zone.

Electronic Supporting Material 2: Theoretical Estimation of the Resolution of the Layer-by-Layer technique

General Expression for the fluorescent gel concentration

A general expression for the final concentration of fluorescent gel at $g_{\text{fluorescent}}$ at a fixed position x_0 is obtained by integrating of the reaction rate of fluorescent gel formation over time:

$$g_{\text{fluorescent}}(x_0) = \int_{-\infty}^{\infty} \frac{\partial g_{\text{fluorescent}}(x_0,t)}{\partial t} \cdot \mathrm{d}t \qquad (1)$$

Assuming that we can switch instantaneously between fluorescent and non-fluorescent alginate, the formation rate of fluorescent gel can be expressed as:

$$\frac{\partial g_{\text{fluorescent}}(x_0,t)}{\partial t} = \frac{\partial g(x_0,t)}{\partial t} \cdot A(t) = R(x_0,t) \cdot A(t) \qquad (2)$$

where $A(t)$ is 0 when non-fluorescent alginate is flowing along the gel boundary, and 1 when the fluorescent alginate is flowing along the gel, and $R(x,t)$ is the reaction rate. Assuming a relatively constant advancement rate v and internal structure of the gel formation front, we can express the reaction rate and concentration profile using a compound variable to $x'=x_0-vt$, indicating the position relative to the reaction front at a given time t. For the reaction rate and the local gel concentration we therefore have:

$$R(x_0,t) \equiv R(x') \tag{3}$$

and

$$g(x_0,t) \equiv g(x') \tag{4}$$

Also, using elementary calculus, we can establish a relation between the spatial and temporal derivates:
and

$$R(x_0,t) = \frac{\partial g(x_0,t)}{\partial t} = -v\frac{\mathrm{d}g(x')}{\mathrm{d}x'} = -v\frac{\partial g(x_0,t)}{\partial x_0} \tag{5}$$

In the case of a steadily advancing reaction front we can use the position of the reaction front $x=vt$ as a measure of the advancement of time, and define:

$$A(t) = B(x) \tag{6}$$

That is, B indicates whether fluorescent or non-fluorescent alginate is provided when the reaction front is at position x. Combining equations 2 and 4-6, as well as the appropriate definitions of x' and x with eq. 1, we get:

$$g_{\text{fluorescent}}(x_0) = \int_{-\infty}^{\infty} \frac{\mathrm{d}g(x_0-x)}{\mathrm{d}x} \cdot B(x) \cdot \mathrm{d}x \tag{7}$$

Finally, by defining

$$P(x') = -\frac{\mathrm{d}g(x')}{\mathrm{d}x'} = \frac{\mathrm{d}g(x_0-x)}{\mathrm{d}x} = \frac{1}{v}R(x') \tag{8}$$

we observe that the fluorescence intensity profile $g_{\text{fluorescent}}$ (x_0) can be obtained from the convolution integral of the stationary spatial profile of the reaction rate $R(x')$ and the original pulse function $B(x)$, indicating where fluorescent and non-fluorescent alginate should be deposited if the reaction front were infinitely sharp:

$$g_{\text{fluorescent}}(x_0) = \frac{1}{v}\int_{-\infty}^{\infty} R(x_0-x) \cdot B(x) \cdot \mathrm{d}x = \frac{1}{v} \cdot R * B \tag{9}$$

$R * B$ denoting the convolution of the functions R and B.

It possible to further develop this general expression for the case where $B(x)$ is a regularly alternating step function, i.e. if the nominal pattern to produce were regularly alternating stripes of fluorescent and non-fluorescent alginate, each stripe having a width Δx:

$$g_{\text{fluorescent}}(x_0) = \frac{1}{v}\sum_{i \in Z}\int_{(2i-1)\cdot\Delta x}^{2i\cdot\Delta x} R(x_0-x) =$$

$$g_{\text{fluorescent}}(x_0) = \sum_{i \in Z}\left(g[x_0+2i\cdot\Delta x] - g[x_0+(2i+1)\Delta x]\right) \tag{10}$$

Eq. 10 indicates that the knowledge of the gel concentration profile across the reaction zone is sufficient to determine the final concentration of fluorescent alginate in experiments where fluorescent and non-

fluorescent alginate is provided alternately. We shall now use a well-established model of the alginate gel formation, namely the Mikkelsen-Elgsaeter model[1, 2], to determine a theoretical estimation of the concentration profiles of the different chemical species across the reaction zone, allowing us to evaluate equation (10).

Evaluation using the Mikkelsen-Elgsaeter model under simplifying hypotheses

Two main processes contribute to the propagation of the reaction front in the alginate-Ca^{2+} system: the chemical reaction itself and diffusion.

The chemical reaction between Ca^{2+} ions and alginate can be written as[2]:

Alginate + N_c Ca^{2+} → Ca-Alginate

The value of the stoichiometric coefficient N_c depends on the guluronic acid content σ of the alginate. According to the half-eggbox model[3], the maximum binding capacity is 3/4 N_c Ca^{2+} per guluronic acid residue, such that the mean stoichiometric coefficient N_c per average residue is[2, 3]:

$$N_c = \frac{3\sigma}{4} \qquad (11)$$

One of the models successfully combining reaction and diffusion for the alginate reaction front is the Mikkelsen-Elgsaeter model[1]. This model is given by the following set of partial differential equations[2]:

$$\frac{\partial a}{\partial t} = D_a \frac{\partial^2 a}{\partial x^2} - \frac{\partial g}{\partial t} \qquad (12)$$

$$\frac{\partial c}{\partial t} = D_c \frac{\partial^2 c}{\partial x^2} - N_c \frac{\partial g}{\partial t} \qquad (13)$$

$$\frac{\partial a}{\partial t} = k \cdot a \cdot (a+g) \cdot c \qquad (14)$$

where t is time, and a, c, and g are the local concentrations of free alginate, free Ca^{2+} ions and Ca-Alginate gel respectively. D_a and D_c are the diffusion coefficients of free alginate molecules and the Ca^{2+} ions respectively, whereas k is the third order reaction rate constant.

In order to obtain an analytical expression for $g(x)$ needed to evaluate eq. 10, we need to apply a series of simplifications to eq. 12-14.

Firstly, since alginate is a macromolecule, it should not diffuse significantly before reacting with the Ca^{2+} ions. Therefore, $a + g$ should remain approximately constant:

$$a + g \approx a_0 \qquad (15)$$

where a_0 is the initial alginate concentration. Since we are focusing on the reaction zone itself, where the reaction term $\partial g / \partial t$ is important, this also means that we can neglect the diffusion term $D_a \partial^2 a / \partial x^2$ in eq. 12.
Secondly, we expect diffusion to be the main transport mode for the highly mobile Ca^{2+} ions, such that we can neglect the temporal concentration change term $\partial c / \partial t$ in eq. 13 for the reaction zone.
Thirdly, and most importantly, we state a traveling-wave hypothesis: the reaction front in the Ca^{2+}-alginate system has been characterized as advancing at a relatively constant speed[2,4], such that the concentration profiles for a, g and c should be temporally relatively stable across the reaction front. In a reference frame fixed at the advancing reaction front (advancing with a speed v in the x direction), we can therefore write for the variables of eq. 12-14 :

$$\frac{\partial a}{\partial t} = -v\frac{\partial a}{\partial x} \quad , \quad \frac{\partial c}{\partial t} = -v\frac{\partial c}{\partial x} \quad , \quad \frac{\partial g}{\partial t} = -v\frac{\partial g}{\partial x} \qquad (16)$$

With the simplifications outlined, the Mikkelsen-Elgsaeter equations read:

$$v\frac{\mathrm{d}a}{\mathrm{d}x} = ka_0 \cdot a \cdot c \tag{17}$$

$$D_c\frac{\mathrm{d}^2c}{\mathrm{d}x^2} = N_c \cdot ka_0 \cdot a \cdot c \tag{18}$$

$$v\frac{\mathrm{d}g}{\mathrm{d}x} = -ka_0 \cdot a \cdot c \tag{19}$$

To solve eq. 17-19, we shall write them in a dimension-less form. To do so, we use a scaling argument. For a reaction zone of width W, the diffusion time of the Ca^{2+} ions is on the order of $t_D = W^2/D_c$. Further, by approximating $a \approx a_0$, a pseudo-first order reaction rate law for the Ca^{2+} is obtained from the right-hand side of eq. 18, the reaction rate constant being given by $N_c k a_0^2$. By setting the reaction time, given by the inverse of the first order reaction rate constant, equal to the diffusion time, we obtain a scaling expression for the width W of the reaction zone:

$$W = \sqrt{\frac{D_c}{ka_0^2 \cdot N_c}} \tag{17}$$

This allows us to define the dimension-less coordinate s:

$$s = \frac{x}{W} \tag{18}$$

Further, we define the dimension-less concentrations $\hat{a}$, $\hat{c}$ and $\hat{g}$:

$$\hat{a} = \frac{a}{a_0} \tag{19}$$

$$\hat{c} = \frac{c}{N_c a_0} \cdot \frac{D_c}{Wv} \tag{20}$$

$$\hat{g} = \frac{g}{a_0} \tag{21}$$

The dimension-less equations to be solved to obtain an analytical solution to the Mikkelsen- Elgsaeter equations are therefore:

$$\frac{\mathrm{d}\hat{a}}{\mathrm{d}s} = \hat{a} \cdot \hat{c} \tag{22}$$

$$\frac{\mathrm{d}^2\hat{c}}{\mathrm{d}s^2} = \hat{a} \cdot \hat{c} \tag{23}$$

$$\frac{\mathrm{d}\hat{g}}{\mathrm{d}s} = -\hat{a} \cdot \hat{c} \tag{24}$$

Since $\hat{g}$ does not figure explicitly in eq. 22 and 23, we can solve eq. 22 and 23 independently of eq. 24. $\hat{g}$ then follows from the knowledge of $\hat{a}$, according to eq. 15:

$$\hat{g} = 1 - \hat{a} \tag{25}$$

Supposing that the reaction front propagates into the free alginate located towards $s \rightarrow +\infty$, the boundary conditions for the solution of eq. 22-23 are as follows:

$$\hat{a}(s \to -\infty) = 0 \ , \ \hat{a}(s \to +\infty) = 1 \ , \ \hat{c}(s \to +\infty) = 0 \qquad (26)$$

Applying the substitution technique outlined in[5], we obtain a solution to eq. 22 and eq. 23; evaluating eq. 25, we also obtain $\hat{g}$:

$$\hat{a} = \beta(s) \qquad (27)$$

$$\hat{c} = \frac{\mathrm{d}\beta}{\mathrm{d}s} \cdot \frac{1}{\beta(s)} \qquad (28)$$

$$\hat{g} = 1 - \beta(s) \qquad (29)$$

where the β function is defined as to solution to:

$$\frac{\mathrm{d}^2[\ln(\beta(s))]}{\mathrm{d}^2 s} = 1 - \beta(s) \qquad (30)$$

with boundary conditions:

$$\beta(0) = \frac{1}{2} \qquad (31)$$

$$\beta(s \to +\infty) = 1 \qquad (32)$$

The boundary condition given by eq. 32 ensures proper convergence of the β function at $s \to +\infty$, whereas boundary condition eq. 31 centers the reaction zone on s=0. Note that since eq. 30 is an ordinary second order differential equation, indicating the two boundary conditions given by eq. 31 and 32 is sufficient to define the β uniquely. A numerical evaluation of the β function in the interval from s=-10 to s=10 is shown in Fig. SI-2, along with $\hat{c}$. The β function is a monotonically rising function, transiting from close to 0 towards 1 in an interval on the order of unity in terms of the dimension-less coordinate s. This confirms that indeed W is a valid order-of-magnitude estimation for the width of the reaction zone in the physical space.

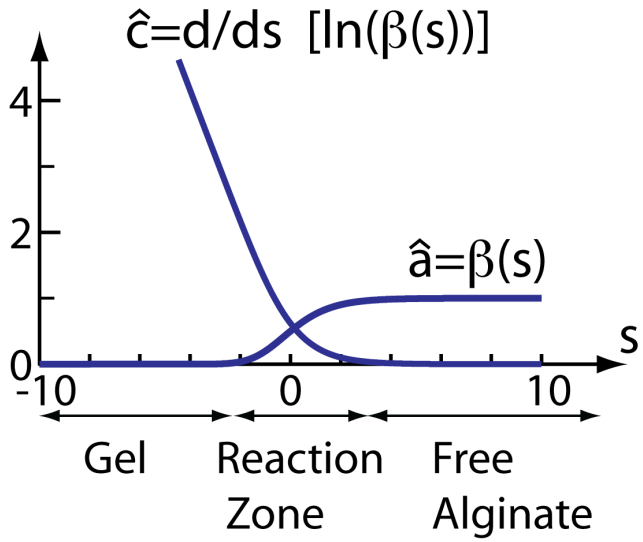

Fig.SI-2: Numerical evaluation of the expression for the dimension-less alginate concentration $\hat{a}$, given by the $\beta(s)$ function, along with the dimension-less Ca^{2+} concentration $\hat{c}$.

Using the definitions of s given by eq. 18 and the definition of $\hat{g}$ given by eq. 21, we finally obtain gel concentration profile $g(x)$ from eq. 29:

$$g(x) \approx a_0 \cdot \left[1 - \beta\left(\frac{x}{W}\right)\right] \qquad (33)$$

The final expression for the fluorescent gel concentration profile is obtained by combining eq. 10 and 33:

$$g_{\text{fluorescent}}(x_0) = a_0 \cdot \sum_{i \in Z} \left(\beta\left[\frac{x_0 + 2i \cdot \Delta x}{W} + \frac{\Delta x}{W} \right] - \beta\left[\frac{x_0 + 2i \cdot \Delta x}{W} \right] \right) \quad (34)$$

given as eq. (3) in the main text.

Electronic Supporting Material 3: Coupling alginate to aminofluorescein

1g of 3% w/w alginate solution in demineralized water is prepared. 300 µl of MES 100mM, pH 5 buffer solution are then added to the alginate, followed by 40µl of aminofluorescein 20mg/ml (aminofluorescein is soluble when ionized, so adding NaOH is necessary to dissolve it). The pH of the resulting solution should be around 5.0-5.5. EDC is dissolved at a concentration of 10mg/ml in cold 25mM, pH5 MES buffer. Then, 40µl of the freshly prepared EDC solution as added to the reaction mixture. After mixing and incubation for at least 15 minutes, the coupling reaction is finished, and removal of unreacted EDC and aminofluorescein follows, for instance by dialysis[6]. To suppress remaining residues activated by EDC, Tris buffer can be added prior to dialysis.

Electronic Supporting Material 4: Measurement of Elastic Modulus

This part of the electronic supplementary material addresses the question whether the transient Ca^{2+} gradient across the gel during the microfluidic alginate gel deposition leads to a permanent gradient of elastic modulus even if the gel is subsequently immersed in a bulk buffer solution with homogeneous Ca^{2+} concentration.

To answer this question, we fabricated alginate hydrogels (1% w/w, disks of 1mm height and 8mm diameter) using either 10mM or 50mM Ca^{2+} concentrations. Specifically, the 1% alginate solutions supplemented with 150mM NaCl were poured onto agars blocks of at least 10x the volume of the alginate solutions, containing 10mM $CaCl_2$ respectively 50mM $CaCl_2$ in addition to 150mM NaCl. After solidification of the alginate by diffusion of Ca^{2+} ions from the agarose blocks into the overlaying 1mm high alginate solution, the blocks were submersed in bulk solutions with the same $CaCl_2$ and NaCl concentrations for several hours to ensure full equilibration. The alginate sheets produced in this way were harvested by inclining the agarose blocks, causing the alginate sheets to slide freely into the submersion bath; prior to the elastic modulus measurement, disks of 8mm diameter disks were stamped from the alginate sheet.

The elastic modulus measurements were carried out on a Bohlin CVO 120 rheometer (measurement of G' at a frequency of 0.1Hz with strains below 1%, with a vertical compression to a height of 850µm). To avoid slip between the stainless steel parts and the alginate hydrogel, we precoated the stainless steel chucks with an extremely thin layer of chitosan-HCl. We did so by exposing the chucks briefly to a 1% chitosan-HCl solution, to wipe them dry immediately after with a clean piece of paper.

First, we established the elastic moduli of the gels made at 10mM Ca^{2+} respectively 50mM Ca^{2+} as described above. Subsequently, gels made at 10mM Ca^{2+} concentration were immersed in a solution containing 50mM $CaCl_2$ (+ 150mM NaCl) whereas gels made at 50mM Ca^{2+} were immersed in a solution containing 10mM $CaCl_2$ (+ 150mM NaCl). The evolution of the elastic modulus was then followed over time.

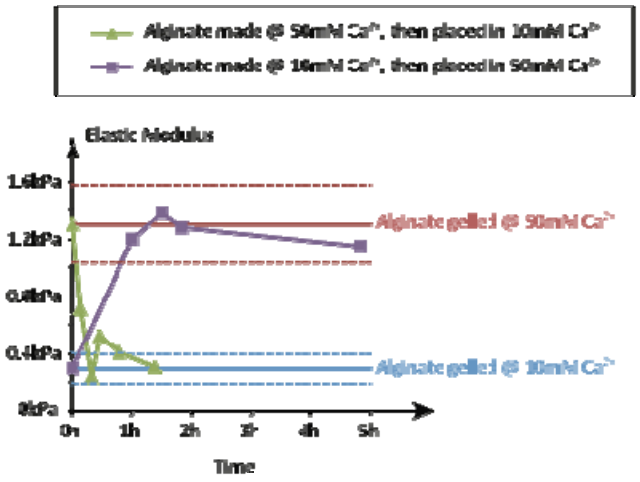

Fig.SI-3: Temporal evolution of the alginate gel modulus upon change of Ca^{2+} concentration

Fig. SI-3 shows the results. The horizontal lines indicate the native elastic moduli of the gels formed at 10mM Ca^{2+} and 50mM Ca^{2+} respectively, the dashed lines indicating plus minus one standard deviation. It can be seen that the gel formed at 10mM Ca^{2+} and immersed in the 50mM Ca^{2+} gains in strength and finally reaches the plateau level typical of a gel formed at 50mM Ca^{2+}. Gels formed at 50mM Ca^{2+}, and immersed in 10mM Ca^{2+} solutions gradually adopt the elastic modulus typical of a gel that had only been exposed to 10mM Ca^{2+}. This shows that on a time-scale of less than an hour for the 1mm high slabs used here, the elastic modulus reaches the value typical for the current immersion solution, irrespective of the past gel formation history. This is important for the microfluidic gel deposition technique presented here, because it indicates that whatever the Ca^{2+} gradients were during gel formation, when the gel is subsequently immersed in a homogeneous buffer, the gradients in elastic modulus will level out. This means that cells will experience a gradient in elastic modulus for the 5 to 10 minutes it takes to make the gel. Once the gel is perused with culture medium of homogeneous Ca^{2+} concentration, we expect the elastic modulus to level out rapidly, since diffusion time scales are short on the microscale, but certainly in less than the 1h needed for the 1mm high disks used for the mechanical testing.

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