Supplementary Material: Permeability and viscoelastic fracture of a model tumor under interstitial flow

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1 Measurement of aggregate length

To quantify aggregate rheology, we measure the evolution of the average aggregate length along the chamber, as an indication of aggregate deformation. We define the aggregate length as $L = S_a/W$, where S_a is the total projected area of the aggregate along the main channel, as measured in the experimental images, and W is the cross-sectional width of the main channel (see Fig. S1). Measurement of S_a was performed using ImageJ.



Figure S1: Measurement of aggregate length using ImageJ. (a) The area containing the shape of the cellular aggregate was cropped. (b) Using ImageJ, we convert the cropped image to binary, then conduct the area measurement to obtain $S_{\rm a}$, the total area of the aggregate along the main channel. (c) The average aggregate length L corresponds to the length of a virtual cellular aggregate with rectangular shape and the same $S_{\rm a}$. Scale bar: 300 µm.

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		$K_{ m a}/K_{ m s}$	
Run	$Q \; (\mu l \mathrm{min}^{-1})$	Average $(\times 10^{-3})$	Deviation $(\times 10^{-3})$
1	5	2.6	1.7
2	5	5.6	5.0
3	5	0.8	0.5
4	10	2.2	0.9
5	10	12.0	4.0
6	10	7.1	3.6
7	15	2.1	1.5
8	20	2.4	1.5
9	20	1.7	1.3
10	20	0.8	0.7
11	25	4.6	2.5
12	25	12.0	2.0
13	25	3.9	0.9
14	30	0.7	0.6

Table S1: Means and standard deviations of $K_{\rm a}/K_{\rm s}$ measured before aggregate fracture, under constant flow rates.

2 Determination of the hydrodynamic permeability of a cellular aggregate

Table S1 shows the average ratio $K_{\rm a}/K_{\rm s}$ measured for different experiments of constant flow rate applied through a cellular aggregate. The results show that before fracture $K_{\rm a}/K_{\rm s} < 0.012$ in all cases. Because $K_{\rm s} = 1.97 \cdot 10^{-10} \,\mathrm{m}^2$, we can estimate an upper bound of the aggregate permeability before fracture, $K_{\rm a} < 2.4 \cdot 10^{-12} \,\mathrm{m}^2$.

3 Fitting of the Zener's model parameters

The rheology of the cellular aggregate was described by the Zener model (Fig. 3(b) in the article). In the constant flow rate experiments, where Δp remains approximately constant, the relation between the pressure difference Δp and the aggregate strain ε is thus

$$\Delta p = E_2 \varepsilon + \eta \frac{E_1 + E_2}{E_1} \dot{\varepsilon},\tag{1}$$

where $\varepsilon = (L(t) - L_0)/L_0$, L_0 is the aggregate length at t = 0 and L(t) is the aggregate length at time t. As $t \to \infty$, the aggregate reaches a final length L_{max} .

This model is adjusted to each experimental realization at constant flow rate, by fitting the values of E_1 , E_2 , and η . An example of an experimental realization is shown in Fig. 3(a) in the article. From the experimental curve L(t), we measure L_0 (at t = 0) and L_{max} (at the maximum experimental time of t = 60 min). We then compute $E_2 = \Delta p L_0 / (L_{\text{max}} - L_0)$.

We next consider the time evolution of the deformation. For t > 0, Eq. (1) predicts:

$$\varepsilon - \varepsilon_1 = (\varepsilon_{\max} - \varepsilon_1) \left[1 - e^{-\frac{E_1 E_2}{\eta(E_1 + E_2)} t} \right], \tag{2}$$

where $\varepsilon_1 = (L_1 - L_0)/L_0$ is the rapid initial deformation of the cellular aggregate at very short time. In terms of L, Eq. (2) leads to:

$$\ln\left(\frac{L_{\max} - L}{L_0}\right) = \ln\left(\frac{L_{\max} - L_1}{L_0}\right) - \frac{E_1 E_2}{\eta(E_1 + E_2)}t.$$
 (3)

We replot our experiment data as suggested by Eq. (3) and fit the data by a linear regression of the form $\ln((L_{\max} - L)/L_0) = -at + b$. We then compute $L_1 = L_{\max} - L_0 e^b$, $E_1 = \Delta p L_0 / (L_1 - L_0) - E_2$ and $\eta_0 = E_1 E_2 / (a(E_1 + E_2))$.

This procedure was applied to the different experiments at constant flow where aggregate fracture did not occur (Fig. 3(c) in the article). The resulting parameter estimates were $E_1 = 4320 \pm 700$ Pa, $E_2 = 1750 \pm 310$ Pa, and $\eta = 1.64 \pm 0.38 \cdot 10^6$ Pa s (mean \pm standard error, N = 11).

4 Control experiment without fibronectin coating

We carried out a control experiment where we did not coat the microchannels with fibronectin to emphasize the importance of cell-substrate adhesion on aggregate fracture. We performed the no-fibronectin assays following the same procedure as those with fibronectin coating. (1) Move a cellular aggregate into the cell chamber; (2) let the cellular aggregate relax for 2 hours; (3) then apply flow through it. Similar to the experiments with fibronectin, we apply either a constant flow rate or a loading rate. In both cases, the aggregate was observed to slide along the chamber in the same direction as the flow, until being pushed out of the chamber. No fracture inside the aggregate was observed in the absence of fibronectin. This is in contrast with the experiments with fibronectin coating, where a flow path through the aggregate is established at fracture.

In the first experimental series, we applied a constant input flow rate $Q = 5 \,\mu l \min^{-1}$. In this case, as the flow was rapidly increased from 0 to $5 \,\mu l \min^{-1}$ at the beginning, and the cellular aggregate was observed to slide along the PDMS walls, then be pushed out of the chamber after only 30 seconds (see Fig. S2(a)). There was no sign of fracture happening inside the aggregate.

Next, we studied aggregate behavior with no fibronectin coating and a gradually increasing loading rate of either $\dot{Q} = 0.5$ or $1.0 \,\mu l \,\mathrm{min}^{-2}$ (Figs. S2(b) and (c), respectively). In both cases, we observed that the aggregate was slowly dragged along the chamber wall towards the output channel, with the aggregate being eventually detached from the wall.

5 Supplementary Movie Legends

Supp. Movie 1. Method to introduce a cellular aggregate into the cell chamber. First, we inject the cellular aggregate into the inlet of the sample input channel. Then, we connect a syringe pump to this inlet and apply a flow rate of 50 to $100 \,\mu l \,\mathrm{min^{-1}}$ until the aggregate is partially inside the microchannel. We then reduce the flow rate to $10 \,\mu l \,\mathrm{min^{-1}}$, which is periodically alternated with periods of no flow, to push the aggregate slowly towards the cell chamber. When the aggregate reaches the cell chamber, the flow is stopped and the aggregate is let to relax at 37 °C for 2 hours.

Supp. Movie 2. Typical flow experiment showing cellular aggregate deformation and fracture under flow. After 2 hours of relaxation, the aggregate was subjected to an increasing input flow rate. The pressure difference (Δp) increased accordingly, inducing first deformation and eventually fracture of the aggregate.

6 Open data publication

The experimental dataset is openly accessible at this address: https://doi.org/10.5281/zenodo.1302801.



Figure S2: Flow experiments through cellular aggregates without fibronectin coating on microchannels. Experimental snapshots (left) and evolution of the measured pressure difference Δp (right) for (a) a constant flow rate of $Q = 5 \,\mu l \,\mathrm{min}^{-1}$; (b) a loading rate $\dot{Q} = 0.5 \,\mu l \,\mathrm{min}^{-2}$; (c) a loading rate $\dot{Q} = 1.0 \,\mu l \,\mathrm{min}^{-2}$. Scale bar: 200 µm.