Supplements to: Isotropic stress reduces cell proliferation in tumor spheroids

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1. 3D Reconstruction of the spheroid shape

We use confocal microscopy to extract the detailed shape of the spheroids. A spheroid is placed in a solution of wheat germ agglutinin conjugated with FITC ( Invitrogen Ref. W11261) for 10 min and rinsed. This protocol leads to the fluorescent labeling of the lipids at surface of the spheroid. We then acquire a stack of optical slices \( I(i,j,k) \) separated by 1 \( \mu m \) in the \( z \) direction. These images are analyzed using a home-made algorithm. For each pixel \( (i,j) \) we choose which \( z \)-index \( k \) correspond to the maximum focus for this lateral position. As the fluorescent labeling is positioned on the surface of the spheroid we obtain a 3D surface (smoothened to the level of one cell) which correspond to the shape of the spheroid.

We then obtain the radial profile of the aggregate by calculating the average radius for every \( z \) position. This curve as shown on Fig. 1 can be readily fitted by a quarter of a circle. This validates our assumption that the cell aggregates can be considered as spheres. In addition it enables us to extract the contact angle between the surface and the support (here a PEG-PLL treated glass) which in this case is about 157°.

2. Surface growth model

We propose a surface growth model in the following way:

\[ \partial_t N = N_d - N_a \]  \hspace{1cm} (1)

where \( N_d \) is the number of dividing cells and \( N_a \) the number of dying cells. They can respectively be written, with \( R \) standing for the spheroid radius:

\[ N_d = \int_0^R k_d(r)\rho(r)4\pi r^2 \, dr \]
We propose, consistently with our experimental and numerical observations, that the division rate reads:

$$k_d(r) = k_{dB} + \delta k_{dS} e^{-(R-r)/\lambda(R,P)}$$

with $k_{dB}$ the bulk division rate and $\delta k_{dS}$ a surface division rate increment; $\lambda$ is the typical decay length of the division rate. The apoptosis rate is taken as a constant in the bulk $k_a(r) = k_{aB}$. We then write $k = k_{dB} - k_{aB}$ as the bulk growth rate of the cells. Consistent with the cryosections observations, we also take the density $\rho(r)$ as constant $\rho_0$. Inserting in eq. 1 we get:

$$\partial_t N = 4 \frac{\pi}{3} R^3 \rho_0 k + 4 \pi k_{dS} \rho_0 \int_0^R r^2 e^{-(R-r)/\lambda} dr.$$  \hspace{1cm} (2)

Constant density implies $\partial_t N = 4 \pi R^2 \rho_0 \partial_t R$ which yields

$$\partial_t R = \frac{k}{3} R + \frac{\lambda}{R} \delta k_{dS} \left( 1 - 2 \frac{\lambda}{R} + 2 \frac{\lambda^2}{R^2} \left( 1 - e^{-R/\lambda} \right) \right).$$  \hspace{1cm} (3)

Expanding in $\frac{\lambda}{R}$

$$\partial_t R = \frac{k}{3} R + \lambda \delta k_{dS} + O \left( \frac{\lambda}{R} \right).$$  \hspace{1cm} (4)

neglecting higher order terms, this can be written in terms of volume:

$$\partial_t V = kV + (36 \pi)^{1/3} \lambda \delta k_{dS} V^{2/3}$$  \hspace{1cm} (5)
3. Analysis of the relative cell division density

The relative cell division density curves can be readily fitted by an exponential function of the form:

\[ \frac{\rho_d}{\rho_{cell}} = \rho_B + \delta \rho_S e^{-\left(\frac{R-r}{\lambda}\right)} \]  

(6)

The extracted parameters are gathered in the Table 1. We observe that the characteristic decay length of the proliferation \( \lambda \) is less affected by stress whereas the division density is reduced as much as a factor 300.

4. Osmotic effect of Dextran on the cellular proliferation/apoptosis

In order to test the effect of Dextran onto the cell proliferation and apoptosis rates, CT26 cells are cultured at 2D in conventional 25 cm\(^2\) flasks during 3 days. The cell culture medium is composed of DMEM, antibiotic-antimycotic, 10 % FBS and Dextran in a concentration sufficient to exert respectively P=0, 500, 2000, 5000 or 10 000 Pa. The total number of cells and the number of dead cells are then evaluated every day using a Beckman-Coulter cell counter. The results are presented in Table 2. Finally if we start with same initial number of cells and if we assume that the cell proliferation is exponential under these conditions it is possible to extract easily the ratio of two rates
Table 2. Table of the fitted parameters for the cell division density.

| Pressure (Pa) | Viability (%) | Total cell number (10^5) | Cell diameter (µm) | Growth rate, k |
|--------------|--------------|--------------------------|-------------------|--------------|
| 0            | 94.4         | 6.6                      | 14.2              | k_0          |
| 500          | 93.5         | 7.5                      | 14.4              |              |
| 2000         | 94.1         | 5.7                      | 14.8              |              |
| 5000         | 90.9         | 5.4                      | 14.7              |              |
| 10000        | 91.7         | 4.4                      | 15.1              | 0.8 k_0      |

$k_2$ and $k_1$ for two different pressures $P_1$ and $P_2$ using:

$$\frac{k_2}{k_1} = 1 - \frac{1}{k_1 t} \ln\left(\frac{N_1}{N_2}\right)$$

(7)

In our case we have, $k_1 = 1/18$ hours$^{-1}$. We also measure the mean diameter of the cells.

We observe that the viability and cell mean diameter are not affected by the Dextran. This indicates that Dextran do not create any significant osmotic shock as reported for higher concentrations of salts or small sugar molecules. Moreover, the growth rate is weakly affected (20 % change) by Dextran compared with the 2 fold change observed in the spheroid experiments.

All together these results clearly indicate that the contact with Dextran only weakly modifies the proliferation and apoptosis of the cells that are placed in direct contact with it. It validates the idea that the growth rate change observed for the spheroid in the direct experiments is not due to an osmotic effect but a mechanical effect.

5. Osmotic effect of Dextran on the cellular concentration of other small molecules

To estimate the concentration changes due to osmotic effects, we view our setup as a set of two compartments separated by a semi permeable membrane. This membrane has pores sufficiently large to let the water (0) and small molecules (1), glucose in this example, diffuse freely but sufficiently small to block the molecule of Dextran (2). Dextran is confined to the compartment II at a concentration $c_2$. In the following calculation we derive analytically the concentration difference $\delta c_1 = c_1' - c_1$ for the small molecule (1) between the compartments II and I.

First the equilibrium of the chemical potentials for the different species that are exchanged through the membrane can be written as follow:

$$\mu_0(P, T) - v \Pi_{osm,I} = \mu_0(P + \Delta P) + v \Pi_{osm,II}$$

(8)

$$\mu_{1,I}(P, T, c_1) = \mu_{1,II}(P + \Delta P, T, c_1')$$

(9)
**Figure 3.** Schematic representation of the mechano-osmotic experiment. Our setup can be seen as a set of two compartments separated by a semi permeable membrane. This membrane has pores sufficiently large to let the water (0) and small molecules (1), glucose for example, diffuse freely but sufficiently small to block the molecule of Dextran (2). In this case the Dextran is block in the compartment II. at a concentration \(c_2\). In the following calculation we derive analytically the concentration difference \(\delta c_1 = c'_1 - c_1\) of the small molecule (1) between the compartments II and I.

If we consider that \(\mu_0(P,T) - \mu_0(P + \Delta P,T) = v\Delta P\), then for diluted solution of 1 we have from (7):

\[
\Delta P = \Pi_{osm,I} - \Pi_{osm,II} = kT(c'_1 - c_1) + \Pi_{osm,2} \tag{10}
\]

And from (8) we derive:

\[
\mu_{1,I}(P,T,c_1) = \mu_{1,I}(P,T,c_1) + v\Delta P + kT\ln(c'_1/c_1) \tag{11}
\]

And then,

\[
\frac{v\Delta P}{kT} + \frac{c'_1 - c_1}{c_1} = 0 \tag{12}
\]

Finally we have,

\[
\Delta P = \Pi_{osm,2}(1 - vc_1) \tag{13}
\]

and with \(\Theta_1 = \frac{V_1}{V}\):

\[
\frac{\delta c_1}{c_1} = \frac{v\Pi_{osm,2}}{kT}(1 - \Theta_1) \tag{14}
\]

In our case, the solute 1 can be chosen for example to be the glucose. Its concentration is in the 4 millimolar range and its diameter is 1 nm. The water molecule has a diameter of 0.29 nm and a concentration in the range of 50 molar. Thus \(v_1/v = 27\) and \(\Theta_1 = \frac{V_1}{V} = \frac{c_{1\text{glu}}}{c_{\text{water}}v_{\text{water}}} = 210^{-3}\) is small compared to 1. Finally, for a Dextran of \(10^5\) g/mol at 20 g/L we have \(\frac{\Delta c_1}{c_1} = 810^{-5}\).

Thus the effect of Dextran on the relative concentrations of the other soluble constituents of the system is negligible.