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## Modeling spheroids rheology

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### 1. Introduction

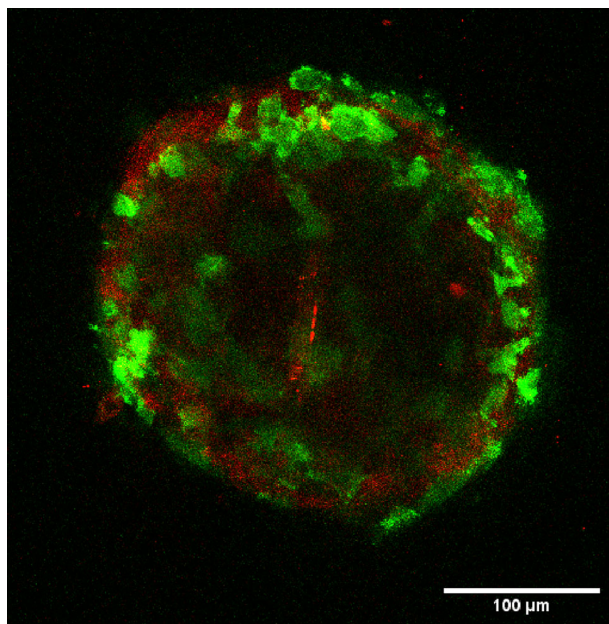
Cancer tumours are synonyms of poor vital prognostic and vary in size and aspect, depending on the location, cell invasiveness and neighboring tissues. They can also contain extracellular matrix (ECM) in different amounts, while cells can possibly synthesize their own matrix. Recent research has focused on tumour models, known as spheroids, obtained using cell culture in controlled conditions. They are good models since they are simple to obtain, have a round geometry and allow to test various drugs. Previously several authors tried to understand the effect of internal stresses within the spheroid (Ambrosi et al. 2017; Guillaume et al. 2019) and others studied the effect of an osmotic pressure (Dolega et al. 2021) applied to the spheroid. An important aspect of such spheroids is to determine their macroscopic behaviour, i.e. whether they can be considered as simple elastic media or poro-visco-elastic systems. Recently data on the viscoelastic properties of spheroids containing various collagen content was obtained (Tsvirkun et al. 2022) and reinforcement of the visco-elastic properties was observed. Here we analyze this data further and present an emulsion model (Palierne 1990) capable of predicting the global rheology of the spheroid based on the individual components, e.g. cells and the collagen matrix.

### 2. Methods

#### 2.1. Cells, collagen and spheroids

Cells were epithelial bladder cancer cells (invasive type, ATCC, HTB-4, Manassas, VA). They were cultured in T25 flasks using RPMI 1640 medium (Gibco, St Aubin, France) in an incubator at 37 °C, in a 5% CO<sub>2</sub> humid atmosphere. Note that they had been transfected for actin-GFP using a plasmid, therefore they can be visualized when using fluorescence.

Collagen I gels (2 mg/mL) were prepared from rat tail solutions (BD Biosciences) and mixed with culture medium. NaOH (0.1 M) was added to adjust the pH at 7.4 at 4 °C. Then polymerization was achieved at 37 °C during 30 min.

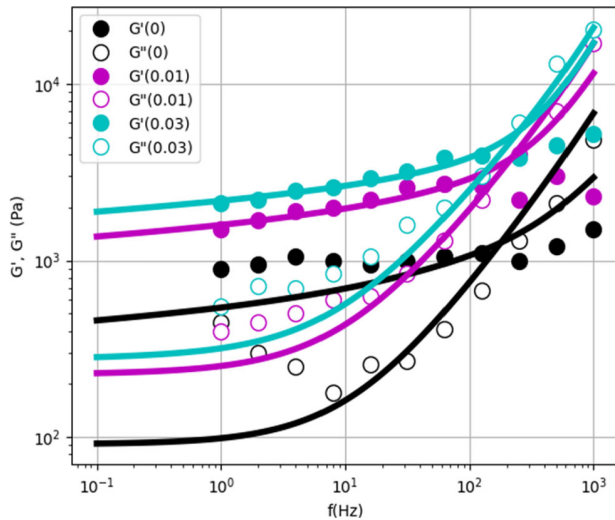


**Figure 1.** Confocal microscopy image of a spheroid prepared in culture medium containing 0.01 mg/mL collagen. T24 cells are in green, collagen in red.

Spheroids were prepared as described previously (Tsvirkun et al. 2022) in hanging droplets of culture medium. Rat tail collagen (type I) was also included in the RPMI medium at lower concentrations (from 0 to 0.03 mg/mL) until 5000 cells were added to the solution. Spheroids formed after 3 days with a nice round shape, although it seemed that spheroids were more difficult to prepare without collagen. A characteristic confocal microscopy image is shown in Figure 1 where the spheroid showed a compact shape with fluorescent cells in green and collagen (0.01 mg/mL) could be observed in the red channel, using reflectance confocal microscopy. This showed in particular that cells could collect the collagen to make bonds like in a scaffold.

#### 2.2. Rheology characterization

Rheological data of collagen gels was gathered from previous works using low frequency (0.1 Hz–10 Hz) classical plate-plate rheometry (Jordan et al. 2010) and complementary AFM experiments in the higher frequency range (1 Hz–1 kHz). AFM force modulation mode (JPK, Nanowizard NWII, Berlin, Germany) was used as described previously (Abidine et al. 2021). Pyramidal tips (Bruker, MLCT, half angle 20°) were chosen to obtain pyramid-plane contact using Hertz model. Altogether, this allowed to cover several decades in frequency from 0.1 Hz to 1 kHz. The data is typical of a gel behavior with a plateau at low



**Figure 2.** Rheological data and model predictions for three initial concentrations (0–0.01–0.03 mg/mL).

frequencies and a slowly increasing behavior as a function of frequency. This rheology was predicted using power-law behaviours (data not shown).

Cell microrheology was also carried out using AFM as well and exhibited also power law behaviours as reported before (Abidine et al. 2018).

Finally spheroids at three initial collagen content were characterized (0–0.01 mg/mL–0.03 mg/mL). In this case, large flat cantilevers (TL-NCL, Nanosensors, 38 μm width) were put in contact with the spheroids (Tsvirkun et al. 2022). Here we only show the data for spheroids with these initial collagen contents in Figure 2. Full points represent the elastic shear modulus ( $G'$ ) whereas open symbols correspond to the loss shear modulus ( $G''$ ).

**2.3. Emulsion model for viscoelastic systems**

From the knowledge of viscoelastic data for collagen and cells, we can assume, based on the images obtained (Figure 1), that there are two phases, one is the collagen one (similar to a gel), and the other one consists of the cells with radius  $R$ . Previous work on viscoelastic emulsions are available and have been considered to predict accurately the behaviour of such systems. In particular, Paliarne (1990) proposed a model based on the rheology of both phases and interfacial tension was also included. The model reads as follows, in the large concentration case:

$$G^*(\omega) = (1 + 3\phi H^*(\omega)) / (1 - 2\phi H^*(\omega)) \text{ where}$$

$$H^* = \{4\alpha / R(2G_m^* + 5G_i^*) + (G_i^* - G_m^*)D^*\} / \{40\alpha / R(G_m^* + G_i^*) + (2G_i^* + 3G_m^*)D^*\}$$

$$\text{and } D^* = 16G_m^* + 19G_i^*.$$

$G_m^*(\omega)$  and  $G_i^*(\omega)$  are respectively the viscoelastic properties of the matrix and the cells, whereas  $\alpha$  is the adhesion energy per unit area, and  $R$  the cell radius.

**3. Results and discussion**

Based on this model, we tested our system using single cell viscoelastic properties, as well as collagen ones. Interfacial tension  $\alpha$  was found to have little effect therefore a typical value of the adhesion energy was used (30 mN/m) for the simulations. On the contrary,  $G_m^*(\omega)$  plays an important role and an optimal collagen concentration was found to be around 2 mg/mL, which could be related to the one in the extra-cellular space. Then the effect of cell content  $\phi$  was studied and showed to play an important role. The best fits are found below.

Cell concentration was found to be optimal at respective values  $\phi = 0.82, 0.95$  and  $0.97$ , and the initial collagen content was increased to  $\sim 2$  mg/mL. Thus it seemed that collagen addition reinforced the bonds between cells and reduced the intercellular spacing, which is an important finding of this work.

**4. Conclusions**

A viscoelastic emulsion model was used to predict the rheology of different spheroids. It was shown to be in good agreement with data obtained using AFM, after taking into account the properties of both phases measured separately. It could be a useful tool for further studies on the influence of the Extra-cellular matrix within tumours.

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**KEYWORDS** Spheroid; rheology; two-phase model

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