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Viscoelastic modeling of the fusion of multicellular tumor spheroids in growth phase

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ABSTRACT

Background. Since several decades, the experiments have highlighted the analogy of fusing cell aggregates with liquid droplets. The physical macroscopic models have been derived under incompressible assumptions. The aim of this paper is to provide a 3D model of growing spheroids, which is more relevant regarding embryo cell aggregates or tumor cell spheroids.

Methods. We extend the past approach to a compressible 3D framework in order to account for the tumor spheroid growth. We exhibit the crucial importance of the effective surface tension, and of the inner pressure of the spheroid to describe precisely the fusion. The experimental data were obtained on spheroids of colon carcinoma human cells (HCT116 cell line). After 3 or 6 days of culture, two identical spheroids were transferred in one well and their fusion was monitored by live videomicroscopy acquisition each 2 h during 72 h. From these images the neck radius and the diameter of the assembly of the fusing spheroids are extracted.

Results. The numerical model is fitted with the experiments. It is worth noting that the time evolution of both neck radius and spheroid diameter are quantitatively obtained. The interesting feature lies in the fact that such measurements characterise the macroscopic rheological properties of the tumor spheroids.

Conclusions. The experimental determination of the kinetics of neck radius and overall diameter during spheroids fusion characterises the rheological properties of the spheroids. The consistency of the model is shown by fitting the model with two different experiments, enhancing the importance of both surface tension and cell proliferation.

General significance. The paper sheds new light on the macroscopic rheological properties of tumor spheroids. It emphasizes the role of the surface tension and the inner pressure in the fusion of growing spheroid. Under geometrical assumptions, the model reduces to a 2–parameter differential equation fit with experimental measurements. The 3–D partial differential system makes it possible to study the fusion of spheroids in non-symmetrical or more general frameworks.

1. Introduction

1.1. Motivations

For a decade, the fusion of cell aggregates has become a booming subject in bioengineering science. Biological self-assembly is the cornerstone of bioprinting techniques for tissue reconstruction (Fleming et al., 2010; Jakab et al., 2004, 2010; Mironov et al., 2009), which provide an alternative to classic solid scaffold-based approaches in tissue engineering. In cancer biology, tumor spheroids provide interesting *in vitro* meso-scale tools, which can help in a better understanding of the tumor organisation and of its viscoelastic properties, at least at the early stage of the tumor development (Hirschhaeuser et al., 2010). Indeed, tumor spheroids are interesting tools to reveal the macroscopic rheological properties of tumors. It is worth noting that such physical properties are crucial to accurately describe the tumor growth. Fusion of cell aggregates is also found in several development mechanisms, such as in early heart formation (Pérez-Pomares and Foty, 2006; Wessels

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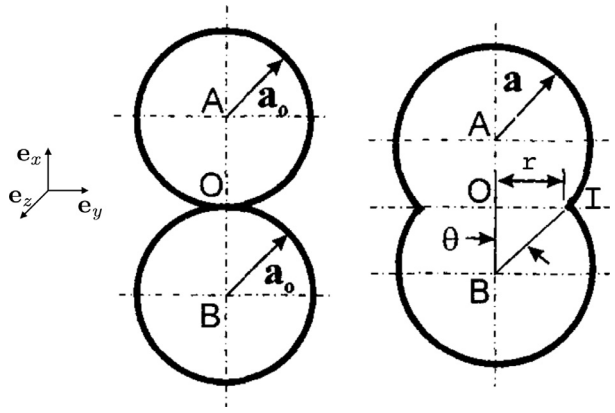

Fig. 1. Schematic descriptions of the fusion as defined by Frenkel (1945) and Pokluda et al. (1997).

and Sedmera, 2003). In this respect, cellular aggregates are commonly modeled as viscoelastic fluids. Actually, their fusion is highly reminiscent of the coalescence of liquid drops, driven by an effective surface tension, by analogy with the surface tension in liquid drops. Furthermore, other experiments exhibit cellular tendency to minimize interfacial area, which can be interpreted as a consequence of surface tension. For instance, a mixture consisting of two different kinds of cell tends to form an aggregate where the two cell populations are sorted, as presented in Sun and Wang (2013).

The analogy between the fusion of soft tissues and droplet liquids has been observed for several decades. One can cite for instance the cell sorting experiments on the early embryonic tissue behavior (Steinberg, 1963) or more recently the evolution of aggregate from irregular to spherical shape (Mombach et al., 2005). Moreover, specific devices have been developed to measure the viscoelastic properties of cell assembly (Forgacs et al., 1998; Foty et al., 1996, 1994). Regarding the fusion of cell assemblies, the experimental data consist in measuring the so-called neck diameter of the fusion, which is the length of the line contact between the 2 spheroids on a planar projection (it is twice the radius neck r of Fig. 1).

As far as we know, the previous studies have been derived in an incompressible framework. In this paper we are interested in modeling the fusion of multicellular tumor spheroids, that are specific spheroids which grow during the experiments. Such a growth, which violates the incompressibility assumption, cannot be neglected to account precisely for the fusion. Experimental measurements of the diameter of the spheroid assembly shows the crucial importance of this growth: under volume conservation assumption, the increase of the neck radius implies necessarily the decrease of the diameter of the spheroids in fusion, while the experiments exhibit an increase or at least a stabilization of this diameter. We aim at providing a new viscoelastic modeling of the fusion of such growing cell aggregates, performing an analogy with droplet fusion, as proposed since several decades, but getting rid of the incompressible condition.

Growth and velocity field are tightly linked since the divergence of the velocity is the local growth rate of the tumor. In standard models of tumor growth, as proposed by Greenspan (1976), the choices of closure to determine the velocity are somehow arbitrary. The simplest choice consists of the standard Darcy's law, but Stokes equation or more complex rheological laws have been studied (Bresch et al., 2009; Sciumè et al., 2014). In this paper, we show that studying the fusion of tumor spheroids makes it possible to choose the viscoelastic law which is relevant with the experiments. We show that Stokes equation with a surface tension enables to recover the experimental observations. This approach exhibits the crucial role of surface tension and of the inner pressure on the growth and the fusion of spheroids. Our approach extends to the compressible framework the previous studies of cell aggregates fusion. An interesting feature of our approach lies in the fact that from experimental measurements, one can discriminate the rheological (viscoelastic) properties of the spheroid assembly from its growth parameters, which highly depend on the nutrient supply and the experimental set-up. In particular, we show that under simple geometrical symmetry, the measurements of the neck radius and the diameter of the fusing spheroids assembly determine entirely the growth of the fusing spheroids. In addition, the macroscopic rheological parameters of the spheroid, which consist of the visco-capillary velocity and the pressure-viscosity ratio are entirely characterized by these measurements.

Remark 1. Throughout the paper, we refer to the effective surface tension of spheroids, by analogy with the surface tension in fluid mechanics. However it is worth noting that multicellular spheroids are much more complex than liquid drops. The observed surface tension results probably from more complex biological phenomena, which are still unclear.

1.2. The standard modeling of spheroids fusion

Theoretical models for the coalescence of two identical spherical droplets of high viscous fluid under the action of surface tension have been proposed within the framework of sintering (Frenkel, 1945; Pokluda et al., 1997). In these papers, two spheres of radius a and center A and B respectively that have one contact point O are considered (see Fig. 1). During the fusion, both centers move towards the point O and the angle $\theta = \widehat{\mathrm{OBI}}$, where I is an intersection point between the spheres, goes from 0 to $\frac{\pi}{2}$, as reported in Fig. 1.

Equaling the works of the effective surface tension and of the viscous dissipation, the authors found a relation between the angle θ, the radius a and their time derivatives. Adding the volume conservation hypothesis leads to the following ordinary equation on θ as presented by Pokluda et al. (see Eq. (14), pp 3254 Pokluda et al., 1997):

$$\dot{\theta} = \frac{1}{2}\frac{\Gamma}{\eta a_0 \theta},$$

where η is the viscosity of the spheroid, Γ is the effective surface tension and a_0 the initial radius of the spheroid, before the fusion. The ratio Γ/η is referred to as the visco-capillary velocity. Several studies have adapted this approach in its simplified version to model the radius neck during the fusion of spheroids (Fleming et al., 2010; Flenner et al., 2008; Jakab et al., 2008) by setting

$$r^2 = 2^{\frac{2}{3}} a_0^2 \left(1 - e^{-t/\tau}\right), \tag{1}$$

where $\tau = 2^{\frac{2}{3}} a_0 \eta/\Gamma$. The above approach has been barely validated in the case where the fusion is much faster than the cell division. However, regarding growing spheroids, and in particular tumor spheroids, the time scale of fusion is much larger than the time of cell division. Moreover the exponential term has been set to fit with the experiments but it is not justified by physical considerations. For all these reasons, it seems necessary to provide a new model for the fusion of growing spheroids.

In addition, in this paper we are interested in studying the fusion of tumor spheroids, during several tens of hours (up to 72 h). Such cell spheroids are proliferating and the volume conservation is not satisfied: The previous approach of Shaler, Frenkel et al. fails to provide relevant results. One of the most clear justification of such inconsistency is provided by the measurement of the spheroid diameter (the largest diameter of the spheroid), which