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A general approach for the microrheology of cancer cells by atomic force microscopy

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ABSTRACT

The determination of the viscoelastic properties of cells by atomic force microscopy (AFM) is mainly realized by looking at the relaxation of the force when a constant position of the AFM head is maintained or at the evolution of the indentation when a constant force is maintained. In both cases the analysis rests on the hypothesis that the motion of the probe before the relaxation step is realized in a time which is much smaller than the characteristic relaxation time of the material. In this paper we carry out a more general analysis of the probe motion which contains both the indentation and relaxation steps, allowing a better determination of the rheological parameters. This analysis contains a correction of the Hertz model for large indentation and also the correction due to the finite thickness of the biological material; it can be applied to determine the parameters representing any kind of linear viscoelastic model. This approach is then used to model the rheological behavior of one kind of cancer cell called Hep-G2. For this kind of cell, a power law model does not well describe the low and high frequency modulus contrary to a generalized Maxwell model.

1. Introduction

The rheological properties of single cells have been investigated in the past using a large variety of methods like micropipette aspiration (Sato et al., 1990), traction or compression between two microplates (Thoumine and Ott, 1997), optical tweezers (Mills et al., 2004; Hénon et al., 1999), magnetic twisting cytometry (Wang et al., 1993; Maksym et al., 2000), rotational microrheology (Wilhelm et al., 2003), atomic force microscopy (AFM) (Radmacher et al., 1996; Lim et al., 2006; Kuznetsova et al., 2007) and many other methods. All these methods (except micropipette aspiration) have in common the tracking of a micro or a nanoparticle either at the surface of the cell or even inside the cell in response to an applied force. Then a model is needed to relate the force-displacement curve to the viscoelastic properties of the cell. If the probe is larger than the characteristic mesh size, ξ, of the actin network (typically $\xi = 100$ nm) a continuum approach can be used to model this response. A possible approach rests on a two fluid description with a viscoelastic network coupled by a frictional force to *a* viscous fluid (Levine and Lubensky, 2001). The approximation of low Reynolds number can be written as

$$Re = \frac{\omega^2 a l \rho}{G(\omega)} << 1 \tag{1}$$

with a the radius of the probe, l the amplitude of motion of the probe and ρ the density of the fluid. The order of magnitude of a and l being the micron and the order of magnitude of $G(\omega)$ being between 0.1 and 10 kPa it is readily seen that this condition holds even for frequencies as high as 100 kHz. The other condition needed for the use of a frequency generalized Stokes' law is that a compressive wave in the elastic network would be overdamped on a characteristic length equal to the dimension of the probe; this is realized for frequencies above (Levine and Lubensky, 2000, 2001):

$$\omega_b = \frac{(2\mu + \lambda)}{a^2 \Gamma} \tag{2}$$

where λ and μ are Lamé coefficients and $\Gamma \sim \eta/\xi^2$ is the friction coefficient between the fluid phase and the elastic network. Introducing $\tau = \eta/\Gamma$ with G the static shear modulus, the crossover frequency becomes

$$\omega_b = \left(\frac{1}{\tau}\right)\left(\frac{\xi}{a}\right)^2 \tag{3}$$

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We shall see in the following that the order of magnitude of the relaxation time is the second, so we get $\omega_b \sim 10^{-2}$; it means that the deviation from generalized Stokes' Einstein equation is only expected at very low frequencies, that is to say on a long time scale. In AFM experiments, as well as with optical tweezers the bead is not completely immersed in the viscoelastic medium, so this continuum approach must be modified to take into account different boundary conditions. When the motion of the probe is perpendicular to the surface as in AFM or indentation experiments, the basic relation which can be generalized in the frequency or time domain is no longer the Stokes law, but the Hertz law which applies to a spherical probe which settles in an elastic medium. Due to the change of the contact surface with time, the time dependent generalization of the Hertz law, which rests on the hypothesis of a linear superposition of delayed response to increments of force or displacement, must be properly written. Such a generalization was described, for instance in Johnson (Johnson, 2004) and applied to nanoindentation of cells (Srinivasa and Eswara, 2008). As the cell thickness is often not very large compared to the indentation depth, a correction of the Hertz formula has been proposed (Dimitriadis, 2002) for elastic media which introduces a function of the indentation depth over the cell thickness. This correction was used to model the time dependent response to an approximate step displacement, with the approximation that during the relaxation of the force, the indentation depth could be considered as constant (Darling et al., 2007). Besides the time dependent generalization of the Hertz model including the cell thickness correction, a description of the viscoelastic response function of the material is needed. In principle a deconvolution should allow to extract the creep response function $G(t)$ or the compliance function $J(t)$ but, due the limited range of time – or frequency – in the experiment, it is more direct to fit the experimental curve with a model response function. Furthermore the viscoelastic models can give some insight in the physical process underlying their representation. One of the most common models to describe a viscoelastic solid is the Zener model consisting of a Maxwell element in parallel with a spring representing the zero frequency elastic modulus G_0, the Maxwell element introduces a relaxation time:

$$\tau_i = \frac{\eta_i}{G_i} \tag{4}$$

This simple model of viscoelastic solid containing three parameters can describe quite well the mechanical response of different kinds of cells (Darling et al., 2006; Vadillo-Rodriguez and Dutcher, 2009), nevertheless some other experiments show a more complex response which can be described by a set of relaxation times or by a power law. In this last case an interpretation of the power law behavior was proposed in terms of a distribution of relaxation times associated with a power law for the distribution of the lengths of the elementary units of the actin network which was supposed to present a fractal structure (Balland et al., 2006). The mechanical response of a cell to different stimuli is important, because several biological functions are regulated by their contact with the neighboring cells or the extracellular matrix (Wang and Ingber, 1994). On the other hand it has recently been demonstrated that a small mechanical stimulus induced by the periodic motion of magnetic nanoparticles at the surface of the membrane can cause the death of the cell (Kim et al., 2010; Hu and Gao, 2010). Since a magnetic field can easily be applied in vivo it could be an attractive way to destroy tumors if the magnetic nanoparticles specifically bind to the cancer cells. In this paper our objective is to obtain the rheological characterization of a given type of cancer cells in order to use it in a future work to model the motion of different kinds of magnetic nanoparticles deposited on the surface of the cells. The knowledge of the viscoelastic response of the cell to the AFM probe will allow to determine the deepness at which magnetic particles can penetrate when submitted to an oscillating magnetic field of different frequencies. An atomic force microscope was used and in a first section we describe briefly the operating conditions and the biological material. As explained above, different approximated methods are used to identify the viscoelastic parameters from the experiments, so the second section will be devoted to a presentation of the equation used to deduce the material properties from the indentation depth versus time of a spherical indenter, and the prediction of this equation will be tested against the trajectory of the bead deduced from a finite element simulation which mimics the operating condition of the AFM. In the third section the results are analyzed through different rheological models and it is shown that the generalized Maxwell model gives a better agreement with the experimental data than a power law model.

2. Materials and methods

Besides imaging, another major application of AFM is force spectroscopy through the direct measurement of tip-sample interaction forces as a function of the indentation depth of the tip into the sample. Compared to conventional rheometers the use of AFM to get rheological properties raises three problems. The first one is the determination of the position of the probe relatively to the surface, the second one is due to the fact that the contact surface between the probe and the biological material is changing during the experiment and at least we have no independent measurement of the position of the probe and of the applied force. As presented in Section 1 there are many papers dealing with these problems and we do not intend to make a review of them here, but just to present what we think to be the best way to overcome them.

2.1. Cancer cells

The tumor cell which has been studied is called Hep G2, a human liver carcinoma cell line. HEP-G2 cells were grown at a BD Falcon™ 35 mm Easy-Grip™ Cell Culture Dish, in Dulbelcco modified Eagle culture medium (Invitrogen, Carlsbad, CA) containing 10% fetal calf serum, glucose (4.5 g/L), glutamine (2 mM), penicillin (100 units/mL), 10 mg/mL gentamycin in a wet (37 °C) and 5% CO_2/95% air atmosphere.

For fluorescence microscopy, HepG2 cells were grown on glass cover slips for 48 h and fixed with 4% paraformaldehyde for 15 min. Cells were then permeabilized with PBS 0.1% triton. For microtubule staining, cells were incubated with anti beta-tubulin followed by secondary anti mouse antibody coupled to Alexa-488 (Molecular Probe). For actin staining, cells were incubated with phalloidin (Sigma Aldrich). Nuclei were stained with Hoescht. Cover slips were mounted on slides using Permafluor (Thermo Scientific) and observed with Zeiss Axioimager microscope.

The organization of actin and microtubules is shown separately, respectively, on the left and right hand side of Fig. 1. The nucleus is stained in blue. It appears that the microtubule network is homogeneous and dense right above the nucleus.

2.2. Force spectroscopy with AFM

Atomic force microscopic experiments were performed with an Agilent series 5100 AFM/STM (Agilent, Santa Clara, CA). The probe of AFM which have been used to obtain the rheology of cancer cells is a spherical borosilicate glass probe (Novascan, Ames, IA). The spring constant of the cantilever is obtained by using an AFM option called Thermal K. which calculates the cantilever spring constant through the use of the equipartition theorem who states that the kinetic energy stored in a system, here on a coordinate which is the deflection of a cantilever from its equilibrium position, is equal to one half of the thermal energy of the system (Cook et al., 2006). The