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# Mechanics of Materials



journal homepage: www.elsevier.com/locate/mechmat

# An original force-displacement relationship for spherical inclusions in multilayered viscoelastic finite media

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#### ARTICLE INFO

Article history: Received 4 February 2009 Received in revised form 3 November 2009

Keywords: Living cells Magnetic tweezers Multilayer Biorheology Elasticity Viscoelasticity

# ABSTRACT

This paper presents original solutions of the force-displacement relationships for a rigid spherical bead embedded in a composite medium made of *n*-isotropic linearly viscoelastic finite layers. Analytical solutions were provided for both compressible and incompressible elastic and viscoelastic solids, assuming no-slip conditions between the rigid spherical inclusion and its adjacent medium as well as between each layer of the composite medium. Thanks to these general formulas, we investigated the effect of finite size media on the force-bead displacement response and derived the exact relationship linking apparent and intrinsic elastic moduli of the medium. Such theoretical solutions can be interestingly applied to identify layer's heterogeneities and to characterize accurately the mechanical properties of living material like cells when using translational microrheology assays. This point is especially illustrated by modeling animal cell cytoskeleton as a bilayer composite medium probed by magnetic tweezers. Interestingly, our results highlighted the influence of finite cell size effects, while allowing to distinguish viscoelastic properties of deep cell cytoskeleton from those of cellular cortex. Moreover, we established that translational microrheology experiments are well suited to characterize locally the viscoelasticity properties of the layer in contact with the probe as soon this layer thickness is larger than ten bead diameters.

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# 1. Introduction

Microrheology is now established as an important experimental tool for probing mechanical properties of living cells. Indeed it is now widely recognized that cell behavior heavily rely on their mechanical properties and intracellular stress distribution (Ingber, 2006; Wang et al., 2001). Several micromanipulation techniques have been developed during the past 10 years for probing biological materials (micropipette aspiration (Boudou et al., 2006; Sato et al., 1990), cell poking (Coughlin and Stamenovic, 2003; Goldmann et al., 1998), atomic force microscopy (Dimitriadis et al., 2002; Dulinska et al., 2006), microplates (Caille et al., 2002; Desprat et al., 2005), optical tweezers (Balland et al., 2005; Mills et al., 2004; Kamgoué et al., 2007), optical stretchers (Ananthakrishnan et al., 2006; Wottawah et al., 2005), magnetic tweezers (de Vries et al., 2005; Walter et al., 2006), magnetic twisting cytometry (Lenormand et al., 2004; Ohayon et al., 2004; Ohayon and Tracqui, 2005; Wang and Ingber, 1994) or particle tracking (Lau et al., 2003; Salamon et al., 2006; Tseng et al., 2002)), and appropriated mechanical models need to be designed and refined in order to quantify accurately the mechanical properties of living cells or tissues from the knowledge of their mechanical responses. Indeed, the cell cytoskeleton,

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<sup>0167-6636/\$ -</sup> see front matter @ 2009 Elsevier Ltd. All rights reserved. doi:10.1016/j.mechmat.2009.11.017

composed of actin filaments, intermediate filaments and microtubules, is a heterogeneous network that defines the overall cell stiffness. Different micromanipulations studies on cells, conducted in association with drugs inducing actin or microtubule disassembly, indicate that living cells behave mechanically as multilayered structures for which superficial and deep effects can be identified. This leads to the consideration of a cortical cytoskeleton and a deep cytoskeleton, with different mechanical properties that could be related to actin polymerisation/depolymerisation in the cortical layer and to microtubule polymerisation/depolymerisation in the deep cytoskeleton. Since the cortical layer is composed of a dense network of actin filaments, it is often modeled as a viscoelastic medium with a dominant solid behaviour. On the other hand, the viscous cytoplasm affects more strongly the mechanical properties of the deep cytoskeleton, which is then often modeled as a viscoelastic medium with a dominant fluid behaviour.

Magnetic cytometry experiments were first conducted to estimate adherent cell viscoelasticity from the cell response to the displacement of beads attached onto the cellular membrane (Crick and Hughes, 1950; Ziemann et al., 1994; Bausch et al., 1999; Hosu et al., 2003; Laurent et al., 2003). More recently, de Vries et al. (2005) designed and constructed an original multi-pole magnetic tweezers setup for investigating intracellular mechanical properties. While providing a real technical advance, such experiments may provide accurate estimates of cell mechanical properties only if experimental data analyses are based on reliable mechanical model of the cell response.

Considering the cell as a unique homogeneous isotropic medium, Lin et al. (2005) presented an elegant elastic solution for such translational microrheology experiments, taking into account cell compressibility and finite size. Nevertheless, this solution – used to extract the Young's modulus from the force–displacement measurements – is only valid when cell heterogeneity and viscoelastic effects are neglected. However, it has been reported that cell heterogeneity may be important in several experiments conducted on biomaterials or cells (Lim et al., 2006; Tracqui and Ohayon, 2007; Kamgoué et al., 2007).

In this context, we present original solutions of the force–displacement relationships for a rigid spherical bead embedded in a composite medium made of *n*-isotropic linearly viscoelastic finite layers. Thus, this study extends the approach of Lin et al. (2005) and provides exact expression of the force resulting from a given imposed translation as a function of the relative size, shear modulus and Poisson's ratio of each layer of the composite medium. We first derived an original solution for purely elastic compressible layers assuming no-slip boundary conditions at the bead-medium interface as well as between consecutive layers, up to a fixed external surface. Then, this analytical elastic solution was successfully extended to viscoelastic *n*-layer composite medium using the elastic-viscoelastic correspondence principle (Findley et al., 1989).

Interestingly, our results highlight the influence of finite cell size effects. We especially point out the existence of a critical relative thickness value of the first layer in contact with the microbead, above which the infinite monolayer solution of Phan-Thien (1993) remains valid. In addition, our theoretical solution allows to distinguish viscoelastic properties of the deep cell cytoskeleton from those of the cellular cortex.

# 2. Idealization of the cell-bead system and related mechanical problem formulation

Fig. 1 illustrates the experiment performed by de Vries et al. (2005) to characterize the mechanical properties of isolated cells. In their experiment, a translational magnetic force is applied on an intracellular spherical bead. Let us first notice that such micromanipulation does not provide directly a quantification of elastic Young's modulus of the probed cellular material (Ohayon et al., 2004; Ohayon and Tracqui, 2005). In the case of a rigid spherical bead, submitted to a known applied force (*F*) and fully embedded in an infinite isotropic incompressible linear elastic medium, one can estimate the apparent medium stiffness modulus ( $E_{app}$ ) from the resulting bead translation (*d*) as (Phan-Thien, 1993):

$$E_{\rm app} = \frac{2F}{S\delta} \tag{1}$$

where  $S = 4 \pi R_0^2$  is the spherical bead surface,  $R_0$  is the bead radius and  $\delta = d/R_0$  is the normalized rigid bead translation. This apparent stiffness modulus differs from the Young's modulus of the layer surrounding the micro-

**Fig. 1.** Schematic picture describing the micromanipulation setup used by de Vries et al. (2005) and supporting our idealized view of the isolated cell-bead system. (A–A) Side view illustrating the cell adhesion on the upper glass surface, which supports the spherical cell geometry we considered in our model. (B–B) Upper view indicating how translational magnetic forces are transmitted to the intracellular bead.



bead, since it may integrated geometrical effects such as medium finite thickness. In this study, we are first looking for the exact mathematical solution defining the relationship between the applied force and the resulting translation of a single spherical rigid bead of radius  $R_0$ embedded in a composite structure made of *n* isotropic, spherical, concentric and finite compressible elastic layers of radius  $R_i$ , Young's modulus  $E^{(i)}$  and Poisson's ratio  $v^{(i)}$ , where i = 1, n.

For this multilayer medium we will demonstrate that it exists a correcting function  $\Phi$  which relates the apparent stiffness modulus ( $E_{app}$ ) to the Young's modulus ( $E^{(1)}$ ) of the layer with thickness ( $R_1 - R_0$ ) surrounding the bead (Fig. 2) through a relationship of the form:

$$E_{\rm app} = \Phi \ E^{(1)} \tag{2}$$

The first original contribution of our work is to provide the analytical expression of  $\Phi$  as a function of the mechanical properties and geometries of the *n* elastic layers. In addition, this theoretical development has been extended to the case of composite medium made of *n* viscoelastic concentric layers.

#### 3. Analytical solution for purely elastic n-layer medium

The theory of linear elasticity was used to solve the problem defined above, assuming that each layer behaves as an isotropic linearly elastic medium following the constitutive Hooke's law. Thus, in each layer (i = 1, n), the condition for local equilibrium may be expressed in terms of the displacement vector  $\mathbf{u}^{(i)}$  as (Landau and Lifshitz, 1959; Green and Zerna, 1968)

$$\nabla \nabla \cdot \mathbf{u}^{(i)} + (1 - 2 \nu^{(i)}) \nabla^2 \mathbf{u}^{(i)} = \mathbf{0}.$$
 (3)

The following boundary conditions were considered in our study: (*i*) no-slip condition was assumed at the bead-cell interface (at  $r = R_0$ ), (*ii*) a small translation d(t) was imposed on the rigid bead, (*iii*) perfect adhesion was assumed at the interface between each pair of adjacent layers (at  $r = R_i, i = 1, n - 1$ ), and (*iv*) zero displacements were imposed on the external medium surface ( $r = R_n$ ). Such conditions are expressed respectively as

$$\mathbf{u}^{(1)}(R_0,t) = d(t) \, \left(\cos(\theta)\,\mathbf{e}_r - \sin(\theta)\,\mathbf{e}_\theta\right) \tag{4}$$



**Fig. 2.** Schematic representation of the *n*-layer viscoelastic medium made of successive concentric layers with radius  $R_i$ . For symmetry reason, only one fourth of the full geometry was presented.

$$\mathbf{u}^{(i)}(R_i, t) = \mathbf{u}^{(i+1)}(R_i, t), \quad i = 1, n-1$$
(5)

$$\sigma^{(i)}(R_i, t) \ \mathbf{e}_r = \sigma^{(i+1)}(R_i, t) \ \mathbf{e}_r, \quad i = 1, n-1$$
(6)

$$\mathbf{u}^{(n)}(R_n,t) = \mathbf{0} \tag{7}$$

where  $(\mathbf{e}_r, \mathbf{e}_{\theta}, \mathbf{e}_{\phi})$  and  $(r, \theta, \phi)$  respectively denote the spherical unit base vectors and the associated physical coordinates, while *t* is time. Interestingly, a displacement field solution for Eq. (3), which satisfies the boundary conditions Eqs. (4)–(7), can be obtained using the method of variables separation and looking for a displacement vector solution in each layer  $\mathbf{u}^{(i)}$  of the form

$$\mathbf{u}^{(i)}(\mathbf{r},\theta,\phi) = \mathbf{w}_r^{(i)}\cos(\theta)\,\mathbf{e}_r + \mathbf{w}_\theta^{(i)}\sin(\theta)\,\mathbf{e}_\theta \tag{8}$$

where  $w_r^{(i)}$  and  $w_{\theta}^{(i)}$  (i = 1, n) become the problem unknowns, with  $R_{i-1} \leq r \leq R_i$  and i = 1, n - 1. For having such solution fields  $\mathbf{u}^{(i)}$  satisfying the local equilibrium condition Eq. (3), the unknowns functions were found to be

$$w_r^{(i)}(r) = A_i + \frac{B_i}{r} + C_i r^2 + \frac{D_i}{r^3}$$
(9)

$$w_{\theta}^{(i)}(r) = -A_i - \frac{B_i}{4r} \left(\frac{3-4\nu^{(i)}}{1-\nu^{(i)}}\right) + C_i r^2 \left(\frac{3-2\nu^{(i)}}{1-4\nu^{(i)}}\right) + \frac{D_i}{2r^3}$$
(10)

where the 4*n* coefficients  $A_i$ ,  $B_i$ ,  $C_i$  and  $D_i$  (i = 1, n) have been determined using the boundary and continuity conditions Eqs. (4)–(7). Details of this resolution are given in Appendix A.

Taking benefit of our solution of the elasticity problem, the total force applied on the rigid bead *F* can then be determined by integrating the stresses over the surface of the rigid sphere.

$$\mathbf{F} = -\int_{0}^{\pi} \int_{0}^{2\pi} \sigma^{(1)}|_{R_{0}} \mathbf{e}_{\mathbf{r}} R_{0}^{2} \sin(\theta) \, d\theta \, d\phi$$
(11)

The apparent stiffness was found by carrying out this integration and using the boundary conditions (4)–(7). Then, one gets:

$$E_{\rm app} = \frac{2}{3} \left[ \frac{(M_{11} - M_{12})(2M_{23} + M_{24})}{M_{13}M_{24} - M_{14}M_{23}} + \frac{(2M_{13} + M_{14})(M_{22} - M_{21})}{M_{13}M_{24} - M_{14}M_{23}} \right]$$
(12)

where  $M_{ij}$  (i, j = 1, 4) are the components of a global transformation matrix  $\mathbf{M_n}$  which may be written as:

$$\mathbf{M}_{\mathbf{n}} = \boldsymbol{\Pi}_{\mathbf{n}} \ \boldsymbol{\Pi}_{\mathbf{n}-1}, \dots, \boldsymbol{\Pi}_{\mathbf{2}} \ \boldsymbol{\Pi}_{\mathbf{1}} \tag{13}$$

with

$$\Pi_{\mathbf{i}} = \boldsymbol{\Omega}_{\mathbf{i}} \ \boldsymbol{\beta}_{\mathbf{i}} \ \boldsymbol{\chi}_{\mathbf{i}} \ \boldsymbol{\chi}_{\mathbf{i}-1}^{-1} \ \boldsymbol{\beta}_{\mathbf{i}}^{-1} \ \boldsymbol{\Omega}_{\mathbf{i}}^{-1} \tag{14}$$

where  $\chi_i(R_i)$ ,  $\beta_i(\nu^{(i)})$  and  $\Omega_i(\mu^{(i)})$  are geometrical, compressibility and continuity matrices depending of the layer radius  $R_i$ . Poisson's ratio  $\nu^{(i)}$  and shear modulus  $\mu^{(i)}$  of the *i*th layer respectively. The analytic expressions of these matrices are detailed in Appendix A.

We checked the correctness of the theoretical solution we obtained for a bilayer composite medium by comparing the theoretical value  $E_{app}(th)$  and the numerically estimated value  $E_{app}(fea)$ , derived from the numerical solution of the elasticity problem performed by a 2D-axisymetric FE analysis (Ansys 11, Canonsburg, PA, USA). Taking a refined enough mesh to insure accuracy of the numerical computations, we found that for all different parameters sets we considered, the relative error  $100 \times (E_{app}(fea) - E_{app}(th))/E_{app}(th)$  is lower than 0.5%.

#### 4. Analytical solution for viscoelastic *n*-layer medium

# 4.1. Background: Solution procedure for the viscoelastic problem

In the general case, the constitutive law for a linear viscoelastic material is given through a convolution integral (Fung, 1981; Findley et al., 1989). For the *i*th layer and assuming a motion starting at time t = 0, the creep form of this integral is:

$$\boldsymbol{\epsilon}^{(i)}(t) = \int_0^t \mathbf{S}^{(i)}(t-\tau) : \frac{\partial \boldsymbol{\sigma}^{(i)}(\tau)}{\partial \tau} \ d\tau \tag{15}$$

where  $\mathbf{S}^{(i)}(t)$  is the fourth-order creep tensor (Georgievskii, 2007) and ":" denotes the double contracted product. Assuming that each layer is an isotropic medium, the stress-strain relationship may be split into deviatoric and volumetric components such as (Cheng et al., 2005; Xu et al., 2007):

$$\mathbf{S}^{(i)}(t) = \frac{\mathbf{J}}{2\,\mu^{(i)}(t)} + \frac{\mathbf{K}}{3\,\mathbf{K}^{(i)}(t)} \tag{16}$$

where  $\mu^{(i)}(t)$  and  $K^{(i)}(t)$  are two creep functions associated with the *i*th layer;  $\mathbf{K} = \mathbf{I} \otimes \mathbf{I}/3$  is the hydrostatic part of the symmetric fourth-order identity tensor  $\mathbf{I}$  (with Cartesian components  $I_{ijkl} = (\delta_{ik}\delta_{jl} + \delta_{jk}\delta_{il})/2$ , where  $\delta_{ij}$  is the Kronecker delta) and  $\mathbf{J} = \mathbf{I} - \mathbf{K}$  is its deviatoric part (Cheng et al., 2005; Xu et al., 2007).

In order to derive the viscoelastic solution, we used the elastic-viscoelastic correspondence principle (Findley et al., 1989; Georgievskii, 2007). Briefly, performing the Laplace transformation of the function  $\epsilon^{(i)}(t)$  given by Eq. (15), one can derive a complex form of the Hooke's law as

$$\bar{\boldsymbol{\epsilon}}^{(i)} = \mathbf{S}^{(i)} : \bar{\boldsymbol{\sigma}}^{(i)} \tag{17}$$

with

$$\widetilde{\mathbf{S}}^{(i)} = \frac{\mathbf{J}}{2\,\widetilde{\mu}^{(i)}} + \frac{\mathbf{K}}{3\,\widetilde{K}^{(i)}} \tag{18}$$

where  $\tilde{\mathbf{S}}^{(i)}(s) = s \mathscr{L}{\mathbf{S}^{(i)}(t)}$  is the Laplace–Carson transform of the fourth-order creep function of the *i*th layer;  $\tilde{\mu}^{(i)}$  and  $\tilde{K}^{(i)}$  are the Laplace–Carson operators of the viscoelastic material parameters (Findley et al., 1989; Cheng et al., 2005; Georgievskii, 2007; Xu et al., 2007). Then, based on the similarity between elastic and viscoelastic systems of equations to be solved in both cases, the correspondence principle allows – knowing the elastic solution of the associated system – to derive the viscoelastic one by replacing all the elastic material moduli by their corresponding Laplace–Carson operators. As a result, the viscoelastic solution is first given in the complex space and needs then to be expressed in the time space using the inverse Laplace transform (Findley et al., 1989; Georgievskii, 2007).

# 4.2. Solution of the viscoelastic problem

The viscoelastic solution was obtained by assuming that the viscoelastic behaviour of each layer *i* may be described by a combination of two Kelvin models acting in series (Findley et al., 1989; Flügge, 1967).

According to this viscoelastic model (Fig. 3), the Laplace transform of the linear constitutive equation of the *i*th layer yields

$$\left(\sum_{m=0}^{1} \mathbf{P}_{m}^{(i)} s^{m}\right) : \bar{\boldsymbol{\sigma}}^{(i)} = \left(\sum_{n=0}^{2} \mathbf{Q}_{n}^{(i)} s^{n}\right) : \bar{\boldsymbol{\epsilon}}^{(i)}$$
(19)

where  $\mathbf{P}_m^{(i)}$  and  $\mathbf{Q}_n^{(i)}$  are the fourth-order tensors describing the viscoelastic behaviour of each layer. These tensors are defined by

$$\mathbf{P}_{0}^{(i)} = (\mu_{1}^{(i)} + \mu_{2}^{(i)}) \mathbf{J} + \mathbf{K}$$
(20)

$$\mathbf{P}_{1}^{(i)} = (\eta_{1}^{(i)} + \eta_{2}^{(i)}) \mathbf{J}$$
(21)

$$\mathbf{Q}_{0}^{(i)} = 2\left(\mu_{1}^{(i)}\mu_{2}^{(i)}\right) \mathbf{J} + 3K^{(i)} \mathbf{K}$$
(22)

$$\mathbf{Q}_{1}^{(i)} = 2\left(\mu_{1}^{(i)}\eta_{2}^{(i)} + \mu_{2}^{(i)}\eta_{1}^{(i)}\right) \mathbf{J}$$
(23)

$$\mathbf{Q}_{2}^{(i)} = 2\left(\eta_{1}^{(i)}\eta_{2}^{(i)}\right) \mathbf{J}$$
(24)

where  $\mu_j^{(i)}$  and  $\eta_j^{(i)}$  (j = 1, 2) are respectively the shear stress modulus and damping viscosity, while  $K^{(i)}$  stands for the bulk modulus of the *i*th layer. Then, by substituting in relation Eq. (19) all tensors by their expressions defined in Eqs. (20)–(24), and rewriting the equation in the form of Eq. (18), it becomes straightforward to identify, thanks to the elastic-viscoelastic correspondence principle, the Laplace–Carson transform of the shear modulus as

$$\tilde{\mu}^{(i)} = \frac{\mu_1^{(i)}\mu_2^{(i)} + \left(\mu_1^{(i)}\eta_2^{(i)} + \mu_2^{(i)}\eta_1^{(i)}\right)s + \left(\eta_1^{(i)}\eta_2^{(i)}\right)s^2}{\mu_1^{(i)} + \mu_2^{(i)} + \left(\eta_1^{(i)} + \eta_2^{(i)}\right)s}$$
(25)

Since we assumed that viscoelasticity only affects the deviatoric part of the tensors, i.e.  $\tilde{K}^{(i)} = K^{(i)}$ , one can directly obtain the Laplace–Carson transform of the Young's modulus and Poisson's ratio using the classical relations (Findley et al., 1989)



**Fig. 3.** Viscoelastic model template used in our study to describe mechanical properties of each layer. Notice that by setting  $\eta_1^{(i)} = 0$ , one obtains a three-parameter solid model, while  $\mu_1^{(i)} = 0$  corresponds to a three-parameter fluid model (Flügge, 1967).

$$\widetilde{E}^{(i)} = \frac{9K^{(i)}\widetilde{\mu}^{(i)}}{3K^{(i)} + \widetilde{\mu}^{(i)}}, \quad \widetilde{\nu}^{(i)} = \frac{3K^{(i)} - 2\widetilde{\mu}^{(i)}}{6K^{(i)} + 2\widetilde{\mu}^{(i)}}.$$
(26)

Then, Eq. (12) allows us to derive the apparent elastic complex modulus  $\widetilde{E_{app}}(s)$  from the Laplace–Carson transform  $\widetilde{M_n}(s)$  of the global transformation matrix  $M_n$  given in Eq. (13). Finally, with consideration of Eq. (1), the viscoelastic response for our problem was obtained by taking the inverse Laplace transform of the equation

$$\tilde{F} = S\tilde{E}_{app}\tilde{\delta}/2.$$
(27)

The normalized displacement history function  $\delta(t)$  resulting from the input force history function F(t) is then given by

$$\delta(t) = \frac{2}{S} \times \mathscr{L}^{-1}\{(\widetilde{E}_{app})^{-1} \times \mathscr{L}\{F(t)\}\}.$$
(28)

In the same way, using the inverse Laplace–Carson transform leads to the two time-dependent viscoelastic functions  $E^{(i)}(t)$  and  $v^{(i)}(t)$ . The inverse Laplace transform was computed using a numerical Laplace transform inversion (NLTI) algorithm (Gaver-Stehfest algorithm, Stehfest (1970)), which is described in Appendix B.

# 5. Application of our solutions to mono and bilayer composite media

In the following section, we will exemplify the use of our theoretical solution for the characterization of the mechanical properties of cells probed by intracellular microrheology experiments. Identification of cell mechanical properties will be conducted by assuming that the cell behaves, first as a monolayer and second, as a bilayer medium.

## 5.1. The cell as a monolayer medium

#### 5.1.1. Elastic solution

The global transformation matrix for an elastic monolayer media ( $n = 1, E^{(1)} = E$  and  $v^{(1)} = v$ ) is, according to Eq. (13),  $\mathbf{M_1} = \mathbf{\Pi_1}$ . Using Eq. (12), we recover in this case the mathematical solution previously derived by Lin et al. (2005), namely

$$E_{\rm app} = \Phi(q, v) \ E \tag{29}$$

where  $\Phi(q, v)$  is the correcting function to apply to the real Young's modulus *E* in order to take into account the influence of medium finite size and compressibility. This correcting function, which depends on a geometrical factor through the normalized radius  $q = R_1/R_0$ , may be written as

$$\Phi = \frac{24(1-\nu)(2-3\nu)(1+\nu)^{-1}(q^5+q^4+q^3+q^2+q)}{5q(1+q-q^2-q^3)+4(2-3\nu)(5-6\nu)(q^5-1)}$$
(30)

from which several particular solutions can be derived:

# (i) for infinite compressible medium, $\Phi$ tends to

$$\Phi(q \to \infty) = \frac{6(1-\nu)}{(1+\nu)(5-6\,\nu)} \tag{31}$$

(ii) for finite incompressible medium,  $\Phi$  is equal to

$$\Phi(\nu \to 1/2) = \frac{4(q^5 + q^4 + q^3 + q^2 + q)}{(4q^2 + 7q + 4)(q - 1)^3}$$
(32)

(iii) for infinite incompressible medium,  $\Phi$  tends, as expected, to

$$\Phi(q \to \infty, \nu \to 1/2) = 1 \tag{33}$$

Taking benefit of this analytical solution, we originally investigated the influence of compressibility and finite size layer on the medium elastic response.

#### 5.1.2. Influence of compressibility and finite size

To analyse the compressibility and finite size effects on the Young's modulus estimation, we computed the amplitude of the correcting function  $\Phi(q, v)$  as a function of Poisson's ratio v and normalized radius  $q = R_1/R_0$ .

We found that the relative error  $\Delta E = (E_{app} - E)/E = (\Phi - 1) * 100\%$  made on the real Young's modulus *E* becomes lower than approximately 10% as soon as the monolayer external radius is approximately twenty times higher than the rigid bead radius (Fig. 4). In order words, the compressibility and finite size effects could be neglected when the relative thickness *q* is larger than 20.

#### 5.1.3. Viscoelastic solution

To exemplify the cellular viscoelastic response, we considered the data of de Vries et al. (2005). In their experimental work, they performed original three-pole magnetic tweezers experiments on cells, using magnetic bead of 1.05  $\mu$ m diameter submitted to force step of magnitude  $F_0 = 60$  pN. Among possible models of cell medium (Lim et al., 2006), we considered an incompressible three-parameter fluid monolayer model which corresponds to  $\mu_1 = 0$  in Fig. 3. Then, using an optimization procedure (nonlinear Levenberg–Marquardt algorithm, Levenberg



**Fig. 4.** Influence of monolayer thickness and compressibility on the estimation error made when approximating the intrinsic Young's modulus *E* by the apparent Young's modulus  $E_{app}$ . Relative error is computed as  $\Delta E = (E_{app} - E)/E = (\Phi - 1) * 100\%$ .

(1944) and Marquardt (1963)), we identified the optimum solution set of viscoelastic constants that fits accurately de Vries et al. (2005) experimental data (Fig. 5). Such identification was performed for increasing values of normalized inner radii  $q_1$ , taken in the range [3–100].

We successfully validated our transformation matrix method by considering the simple case of an incompressible infinite monolayer. Indeed, the numerical solution obtained for infinite medium (i.e. q > 30) agrees (relative error less than 1%) with the viscoelastic response ( $q \rightarrow \infty$ , marked  $\Diamond$  material parameters in Fig. 5). Formally, we have

$$d(t) = \frac{F_0}{6\pi R_0} \left[ \frac{t}{\eta_1} + \frac{1}{\mu_2} \left( 1 - \exp(-\frac{\mu_2 t}{\eta_2}) \right) \right] H(t)$$
(34)

where we assumed the input force signal to be a step force distribution of magnitude  $F_0$  ( $F(t) = F_0$  H(t) with H(t) the Heaviside step function).

Originally, our results highlight the influence of the finite cell size on the quantification of the viscoelastic parameters (q = 5 corresponding to a cellular radius of  $R_1 = 2.62 \mu$ m, Fig. 5). In such a case, considering the cell as an infinite medium may bias the identified values of viscoelastic constants up to a factor 1.5.



**Fig. 5.** (a) Cell viscoelastic parameters found when fitting the experimental measurements of the translational magnetic tweezers on living cells of de Vries et al. (2005) by an incompressible three-parameter fluid monolayer model. The marked  $\Diamond$  material parameters values correspond to the exact viscoelastic solution obtained for the limit  $q \rightarrow \infty$ . (b) Comparison between the experimental data of de Vries et al. (2005) and the exact solution we obtained when assuming a normalized cellular radius of q = 5 and when considering the material parameters values indicated by label ( $\blacklozenge$ ) in Fig. 5a.

# 5.2. The cell as a bilayer medium

#### 5.2.1. Elastic solution

For an elastic bilayer medium (n = 2), the global transformation matrix, according to Eq. (13), takes the form  $\mathbf{M_2} = \mathbf{\Pi_2} \ \mathbf{\Pi_1}$ . As presented for the monolayer case studied previously (Eq. (29)), we express the analytical elastic solution in terms of the correcting function to be applied to the Young's modulus of the first layer  $E^{(1)}$ ,

$$E_{\rm app} = \Phi(q_i, v^{(i)}, \beta_2) \ E^{(1)} \tag{35}$$

where the correcting function  $\Phi$  depends on the two normalized radii  $q_i = R_i/R_0$  (i = 1, 2), the Poisson's ratio  $v^{(i)}$  (i = 1, 2) and the shear moduli ratio  $\beta_2 = \mu^{(2)}/\mu^{(1)}$ . Interestingly, a simple analytical force–displacement relationship was obtained when the two layers are incompressible  $(v^{(1)} = v^{(2)} = 0.5)$  and when the external radius  $R_2$  tends to infinity  $(q_2 \rightarrow \infty)$ , i.e.

$$F = \frac{1}{2} S E^{(1)} \Phi \delta$$
(36)

where the correcting function  $\Phi$  reads:

$$\Phi = \frac{4(q_1^6 - q_1)\beta_2^2 + (6q_1^6 + 4q_1)\beta_2}{f_1(q_1)\ \beta_2^2 + f_2(q_1)\ \beta_2 + 6q_1^5 + 4}.$$
(37)

The functions  $f_i$ , describing the influence of the inner layer size effects onto the apparent Young's modulus, take the form

$$f_1(q_1) = 4q_1^6 - 9q_1^5 + 10q_1^3 - 9q_1 + 4$$
(38a)

$$f_2(q_1) = 6q_1^6 + 3q_1^5 - 10q_1^3 + 9q_1 - 8.$$
(38b)

Notice that, when the two layers have similar mechanical properties ( $\beta_2 = 1$ ), the function  $\Phi$  in Eq. (37) reduces to 1. This simplified solution was used to investigate the influence of the shear moduli ratio  $\beta_2$  and the normalized finite size  $q_1$  of the inner layer on the mechanical response of an incompressible bilayer elastic medium.

The correcting function appears to be very sensitive to both geometrical and rheological cellular parameters (Fig. 6). For an external cellular cortex much stiffer than the internal layer (e.g., if  $\beta_2 \ge 10$ ), and for small bead radii, the error made when considering the apparent stiffness as the intrinsic stiffness of the first layer is lower than 25% if  $10 < q_1 < 20$  and lower or equal to 10% if  $q_1 > 20$  (points A and B, Fig. 6). However, if the external cellular medium is much softer than the internal layer (e.g., if  $\beta_2 < 10^{-1}$ ), then beads with smaller radius are needed to reach similar acceptable error amplitude, but with enlarged sensitivity to the bead radius, i.e.  $30 < q_1 < 100$  for  $25\% > \Delta E > 10\%$ .

#### 5.2.2. Viscoelastic solution

In agreement with proposed rheological models of cells behaviour (Lim et al., 2006), we considered the solution obtained for a viscoelastic bilayer medium by assuming that the inner layer behaves as a three-parameter fluid model, while the external layer responds as a three-parameter solid model. We again gave special consideration to the experiment of de Vries et al. (2005) where bead diam-



**Fig. 6.** Influence of the normalized internal layer thickness  $q_1$  and of the relative shear modulus ratio  $\beta_2$  of the two layers on the relative error made when approximating the intrinsic Young's modulus  $E^{(1)}$  of the first layer by the apparent Young's modulus  $E_{app}$ . The indicated relative error was computed as  $\Delta E = (E_{app} - E)/E = (\Phi - 1) * 100\%$ .

eters were close to  $1.05 \,\mu\text{m}$ . The cell geometry was approximated by a spherical shape of external radius equal to 2.62  $\mu\text{m}$  and a cellular cortex thickness of 0.3  $\mu\text{m}$  (i.e.  $q_1 = 5$  and  $q_2 = 5.57$ ).

Notice that two sets of experimental results performed on the same biological sample but with distinct bead sizes – one with small bead radius  $(q_1 > 10)$  and the other with large beads  $(q_1 < 10)$  – are necessary to extract accurately the material moduli of the two cellular layers. Unfortunately, we did not find in the literature such sets of experimental results. Therefore, in addition to the experimental results of de Vries et al. (2005), we followed recent conclusions of Wei et al. (2008) reporting a time-dependent Young's modulus of the cellular cortex  $E^{(2)}(t)$  that is higher than that of the deep cytoskeleton  $E^{(1)}(t)$ . Thus, we imposed as viscoelastic moduli for the cellular cortex  $\mu_1^{(2)} = 360 \text{ Pa}, \mu_2^{(2)} = 420 \text{ Pa}$  and  $\eta_2^{(2)} = 48 \text{ Pa} \cdot \text{s}$ . We furthermore considered that the cellular cortex thickness is 0.3 µm. Then, an optimization procedure was used to identify the material moduli of the inner layer cytoskeleton layer, taking initial values  $q_1$  in the range [3-100]. Fig. 7 presents the best viscoelastic moduli we found when characterizing the deep cytoskeleton.

Interestingly, these results clearly emphasize the importance of distinguishing deep cytoskeleton from cellular cortex and highlight the influence of the finite cell size. Indeed, in such case, the results given in Fig. 7 show that the amplitude of the correcting factor is crucial, (up to a factor 2 particularly for the rheological constant  $\eta_2^{(1)}$ ). Notice that such results appear to be different from those obtained when the cell was modeled as a monolayer (compare q = 5 in Fig. 5 and  $q_1 = 5$  on Fig. 7). Considering the cell as a monolayer instead of a bilayer may bias the value of the inner layer viscoelastic constants up to 50%.

## 6. Conclusion

The main purpose of this study was to provide original solutions of the force–displacement problem arising when probing a composite medium made of *n*-isotropic linearly



**Fig. 7.** Viscoelastic parameters identified for the cell inner layer from the experimental measurements of de Vries et al. (2005). The cell was considered as a bilayer medium, with a cellular cortex of thickness 0.3 µm. The inner layer is modeled as an incompressible three-parameter fluid, while the cortical one is assumed to behave as an incompressible three-parameter solid. The marked values ( $\blacklozenge$ ) of the material parameters correspond to the exact solution we obtained for a normalized inner radius  $q_1 = 5$ .

elastic or viscoelastic finite layers with a rigid spherical bead in it. Our solutions may be computed rather easily and could notably improve the quantification of cell mechanical properties from experimental force-displacement measurements obtained in micromanipulation assays (de Vries et al., 2005; Wei et al., 2008). In this context, we also provided here an original method to characterize the viscoelastic properties of multilayered media from experimental data by using the elastic-viscoelastic correspondence principle (Findley et al., 1989). Our study was restricted here to the coupling of three-parameter solid and fluid models, which are often used to describe the viscoelastic response of adherent cells (Bausch et al., 1999; Hosu et al., 2003; Laurent et al., 2003; de Vries et al., 2005). However, the same approach could be extended to other viscoelastic models, thus providing a rather general framework which may help to quantify more accurately the mechanical properties of cells probed by translational magnetic tweezer technique. In addition, this study could help designing new experimental protocols when using intracellular translational magnetic tweezers experiments performed either under static or dynamic modes.

Nevertheless, several limitations deserve to be pointed out at this stage in our work. First, we restricted our study to the small strain domain, i.e. to small bead displacements. Second, our analytical solutions are given under the assumption of perfect adhesion between the inner rigid bead and the first layer. Finally, the zero-displacement of the external layer is another assumption which may be only partly fulfilled for adherent cells.

#### 6.0.3. Biological implications

By a precise analysis of the influence of geometrical constraint on the mechanical response, our results could have several biological implications. Indeed, such accuracy is of special interest in experimental studies focusing on the role played by cell mechanical properties in the control of cellular processes. Notably, we found that finite size effects do not significantly influence the identification of the shear modulus of the layer in contact with the bead (inner layer) as soon as its thickness is larger than 10 bead diameters. More globally, the results presented here may provide a theoretical basis for probing, through controlled bead translation, the intracellular microrheology and particularly the cortex or other layers such as adhesion layers or extracellular matrix layer. Such guantification of cells viscoelastic properties could be used to investigate the cellular structural changes induced by stimulating or damaging agents (Trepat et al., 2004), as well as by endogenous cytoskeleton remodeling (An et al., 2006).

#### Acknowledgments

We acknowledge Dr. Simon Le Floc'h for useful discussions.

## Appendix A. Matrix formulation

### A.1. Solution of the elastic problem

In spherical coordinates, the elastic solution for the displacement is given by Eqs. (9) and (10). Using elastic properties, it comes for the stresses

$$\sigma_{rr}^{(i)} = \mu^{(i)} \left[ \frac{B_i}{r} \left( \frac{2 - \nu^{(i)}}{1 - \nu^{(i)}} \right) - 4C_i r^2 \left( \frac{1 + \nu^{(i)}}{1 - 4\nu^{(i)}} \right) - 6\frac{D_i}{r^3} \right] \frac{\cos(\theta)}{r}$$
(39a)

$$\sigma_{r\theta}^{(i)} = \mu^{(i)} \left[ \frac{B_i}{2r} \left( \frac{1 - 2\nu^{(i)}}{1 - \nu^{(i)}} \right) - 2C_i r^2 \left( \frac{1 + \nu^{(i)}}{1 - 4\nu^{(i)}} \right) - 3\frac{D_i}{r^3} \right] \frac{\sin(\theta)}{r}$$
(39b)

$$\sigma_{\theta\theta}^{(i)} = \mu^{(i)} \left[ \frac{B_i}{4r} \left( \frac{1 - 2\nu^{(i)}}{1 - \nu^{(i)}} \right) - 8C_i r^2 \left( \frac{1 + \nu^{(i)}}{1 - 4\nu^{(i)}} \right) + 3\frac{D_i}{r^3} \right] \frac{\cos(\theta)}{r}$$
(39c)

## A.2. Expression of the coefficients $A_i, B_i, C_i$ and $D_i$

While writing the elastic solution in the form

$$w_r^{(i)}(r,\theta) = \frac{u_r^{(i)}(r,\theta)}{\cos(\theta)} \text{ and } w_{\theta}^{(i)}(r,\theta) = \frac{u_{\theta}^{(i)}(r,\theta)}{\sin(\theta)}$$
$$s_{rr}^{(i)}(r,\theta) = \frac{r \sigma_{rr}^{(i)}(r,\theta)}{\cos(\theta)} \text{ and } s_{r\theta}^{(i)}(r,\theta) = \frac{r \sigma_{r\theta}^{(i)}(r,\theta)}{\sin(\theta)}$$

it comes for the boundary conditions at the bead-cell interface and on the external radius expressed in Eqs. (4) and (7), the form

$$w_r^{(1)}(R_0,\theta) = d \text{ and } w_r^{(n)}(R_n,\theta) = 0$$
 (41a)

$$w_{\theta}^{(1)}(R_0,\theta) = -d \text{ and } w_{\theta}^{(n)}(R_n,\theta) = 0$$
(41b)

$$s_{rr}^{(1)}(R_0,\theta) = k_1 \text{ and } s_{rr}^{(n)}(R_n,\theta) = k_3$$
 (41c)

$$s_{r\theta}^{(1)}(R_0,\theta) = k_2 \text{ and } s_{r\theta}^{(n)}(R_n,\theta) = k_4$$
 (41d)

Let us build the boundary conditions vectors  $\mathbf{c_0} = [d, -d, k_1, k_2]^T$  for boundary at the bead-cell interface and  $\mathbf{c_n} = [0, 0, k_3, k_4]^T$  for them on the external surface in which  $k_i$  are four unknown functions explicitly linked to stress values. Using the continuities conditions between each layer expressed in Eqs. (5) and (6), one can build the linear system

$$\mathbf{c_0} = \mathbf{\Omega}_1 \ \mathbf{\beta}_1 \ \mathbf{\chi}_0 \ \mathbf{a}_1 \tag{42a}$$

$$\boldsymbol{\Omega}_{i+1} \boldsymbol{\beta}_{i+1} \boldsymbol{\chi}_i \boldsymbol{a}_{i+1} = \boldsymbol{\Omega}_i \boldsymbol{\beta}_i \boldsymbol{\chi}_i \boldsymbol{a}_i \quad i = 1, \dots, n-1 \quad (42b)$$

$$\mathbf{c}_{\mathbf{n}} = \boldsymbol{\Omega}_{\mathbf{n}} \ \boldsymbol{\beta}_{\mathbf{n}} \ \boldsymbol{\chi}_{\mathbf{n}} \ \mathbf{a}_{\mathbf{n}} \tag{42c}$$

where  $\mathbf{a}_i = [A_i, B_i, C_i, D_i]^T$  is the coefficient vector of the *i*th layer,  $\Omega_i(\mu^{(i)})$  is a 4 × 4 matrix, function of the shear moduli of the *i*th layer,

$$\boldsymbol{\Omega}_{\mathbf{i}} = \operatorname{diag}(1, 1, \boldsymbol{\mu}^{(i)}, \boldsymbol{\mu}^{(i)}) \tag{43}$$

 $\beta_i(\boldsymbol{\nu}^{(i)})$  a  $4\times 4$  matrix, function of the ith layer Poisson's ratio

$$\boldsymbol{\beta}_{\mathbf{i}} = \begin{bmatrix} 1 & 1 & 1 & 1 \\ -1 & -\frac{3-4\,\nu^{(i)}}{4(1-\nu^{(i)})} & \frac{3-2\,\nu^{(i)}}{1-4\,\nu^{(i)}} & \frac{1}{2} \\ 0 & \frac{2-\nu^{(i)}}{\nu^{(i)}-1} & \frac{4(1+\nu^{(i)})}{1-4\,\nu^{(i)}} & -6 \\ 0 & \frac{2\,\nu^{(i)}-1}{2(\nu^{(i)}-1)} & \frac{2(1+\nu^{(i)})}{1-4\,\nu^{(i)}} & -3 \end{bmatrix}$$
(44)

and  $\chi_i(R_i)$  a  $4 \times 4$  matrix function of the *i*th layer external radius.

$$\chi_{\mathbf{i}} = \text{diag}(1, R_i^{-1}, R_i^2, R_i^{-3})$$
(45)

To simplify the continuity condition between each layer, we build the step matrix  $\Gamma_i$  linking the coefficient vector  $\mathbf{a}_i$  of the *i*th layer and  $\mathbf{a}_{i+1}$  of the (i + 1)th layer, such that

$$\mathbf{a}_{i+1} = \boldsymbol{\Gamma}_i \ \mathbf{a}_i \tag{46}$$

with  $\Gamma_i = \chi_i^{-1} \beta_{i+1}^{-1} \Omega_i \beta_i \chi_i$ . This formulation allows the determination of all coefficient vectors. Finally, we build the global transformation matrix  $\mathbf{M_n}$  so as to solve the linear system  $\mathbf{c_n} = \mathbf{M_n c_0}$ . This matrix takes the form

$$\mathbf{M}_{\mathbf{n}} = \boldsymbol{\Pi}_{\mathbf{n}} \ \boldsymbol{\Pi}_{\mathbf{n}-1}, \dots, \boldsymbol{\Pi}_{\mathbf{2}} \ \boldsymbol{\Pi}_{\mathbf{1}} \tag{47}$$

where  $\Pi_i$  describes the  $\mathit{i}\text{th}$  layer behavior and is defined as

$$\Pi_{\mathbf{i}} = \boldsymbol{\Omega}_{\mathbf{i}} \ \boldsymbol{\beta}_{\mathbf{i}} \ \boldsymbol{\chi}_{\mathbf{i}} \ \boldsymbol{\chi}_{\mathbf{i-1}}^{-1} \ \boldsymbol{\beta}_{\mathbf{i}}^{-1} \ \boldsymbol{\Omega}_{\mathbf{i}}^{-1}. \tag{48}$$

# A.3. Force-displacement relationship

The applied force F on the rigid bead is determined by integrating the stresses over the surface of the rigid sphere. Carrying out this integration yields to the following relationship between the applied external force F and the small bead translation d which is implicitly include in the expression of the constant  $B_1$ .

$$F = 4\pi \ \mu^{(1)} \ B_1 \tag{49}$$

Notice that we only need one coefficient  $B_1$  which could be determined from the previous linear system. It takes the form

$$B_1 = \frac{R_0}{3\,\mu^{(1)}} \,\,(2\,k_2 - k_1) \tag{50}$$

where the constants  $k_1$  and  $k_2$  are explicit functions of the global transformation matrix components  $M_{ij}$  (i, j = 1, 4)

$$k_1 = d \left[ \frac{M_{14} (M_{21} - M_{22}) - M_{24} (M_{11} - M_{12})}{M_{13} M_{24} - M_{14} M_{23}} \right]$$
(51a)

$$k_2 = d \left[ \frac{-M_{13} (M_{21} - M_{22}) + M_{23} (M_{11} - M_{12})}{M_{13} M_{24} - M_{14} M_{23}} \right]$$
(51b)

Finally, the substitution of constants  $k_1$  and  $k_2$  in the force– displacement relationship gives Eq. (12).

# Appendix B. The Gaver-Stehfest algorithm for NLTI

Given a Laplace transform  $\overline{f}(s)$  of an original time space function f(t) (t > 0), the function f(t) can be approximated by the product of the reciprocal of time by a finite linear combination of the transform values where the Laplace variable is replaced by  $\alpha_k/t$ :

$$f(t) = \frac{1}{t} \sum_{k=1}^{2n_g} \omega_k \,\bar{f}\left(\frac{\alpha_k}{t}\right) \tag{52}$$

We used 8 Gaver functionals ( $n_g = 8$ ) in this inversion formula with  $2n_g$  the number of terms used in the Salzer summation to accelerate convergence (Valko and Abate, 2004). The nodes  $\alpha_k = k \ln(2)$  and the weights  $\omega_k = \zeta_k \ln(2)$  are real numbers which only depend on  $n_g$ . The  $\zeta_k$  coefficients are given by Abate and Whitt (2006) in the form

$$\zeta_{k} = (-1)^{k+n_{g}} * \sum_{j=\left\lfloor \frac{k+1}{2} \right\rfloor}^{\min(k,2n_{g})} \frac{j^{n_{g}} (2j)!}{(j)!(n_{g}-j)!(k-j)!(2j-k)!}$$
(53)

with  $\lfloor z \rfloor$  being the greatest integer less than or equal to *z*. Notice that this numerical method remains robust event when considering purely incompressible layers (i.e.  $v^{(i)} = 1/2$ ).

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