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Complex and biofluids / Fluides complexes et biologiques

Review: Rheological properties of biological materials

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Abstract

Eukaryotic cells and biological materials are described from a rheological point of view. Single cells possess typical microrheological properties which can affect cell behaviour, in close connection with their adhesion properties. Single cell properties are also important in the context of multicellular systems, i.e. in biological tissues. Results from experiments are analyzed and models proposed both at the cellular scale and the macroscopic scale. ***To cite this article: C. Verdier et al., C. R. Physique 10 (2009).***

Résumé

Revue : Propriétés rhéologiques des matériaux biologiques. Les cellules eucaryotes et les matériaux biologiques sont présentés du point de vue de leur rhéologie. Les cellules individuelles possèdent des propriétés rhéologiques typiques, qui peuvent affecter le comportement cellulaire, en étroite relation avec leurs propriétés adhésives. Les caractéristiques individuelles des cellules sont aussi utiles pour décrire des systèmes multicellulaires, tels que des tissus biologiques. Les résultats d'expériences sont analysés et des modèles proposés tant à l'échelle cellulaire qu'à l'échelle macroscopique. ***Pour citer cet article : C. Verdier et al., C. R. Physique 10 (2009).***

Keywords: Rheology; Tissues; Cell mechanics; Viscoelastic

Mots-clés : Rhéologie ; Tissus ; Mécanique cellulaire ; Viscoélastique

1. Introduction

Cells and biological tissues are complex materials. They are made of multiple elements or sub-elements [1] whose complexity is still far from being understood. Most of our knowledge concerning the rheological properties of cells and tissues comes from previous studies on soft matter, i.e. materials whose structure gives rise to intriguing properties [2–4] such as the ones of polymeric systems [5], suspensions, gels, emulsions, foams, pastes, etc. Such materials possess uncommon rheological properties (rheology is the science of flowing matter and deals with the study of the forces

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needed to achieve particular deformations or velocities) because their components contain both solid particles (or other types of inclusions like polymers, etc.) and fluids. Therefore one may say that they are viscoelastic but they may also be viscoplastic or elasto-visco-plastic. By plasticity, we generally mean that a given material can undergo very large deformations once the stress becomes higher than a certain yield limit. Cellular materials are different from usual soft materials due to the fact that they can develop an active response when submitted to stresses. This response is due to mechanotransduction, which is the ability of cells to transform mechanical external stresses into biochemical signals (and vice versa) in order to transfer information to and from the nucleus in order to achieve a particular cell function. This can lead to particular behaviours such as migration, adhesion, reaction to mechanical stress, etc.

Let us first consider that the materials or fluids of interest are viscoelastic, capable of possessing both viscous and elastic properties just like a Maxwell element (a spring of stiffness G in series with a dashpot of coefficient η). Such a model develops a stress τ related to the deformation γ by a simple 1D-constitutive law given by $\lambda\dot{\tau} + \tau = \eta\dot{\gamma}$, where $\lambda = \eta/G$ is the relaxation time, and $\dot{\tau}$ is the time-derivative of the stress. This characteristic time is fundamental to define the range of behaviours of the material:

- $t \ll \lambda$, the material behaves as an elastic solid of stiffness G, with a stress $\tau = G\gamma$;
- $t \gg \lambda$, the material behaves as a fluid of viscosity η and $\tau = \eta\dot{\gamma}$.

Apart from this simple fact, the material stress properties can be integrated to yield the actual exact stress $\tau(t) = \int_{-\infty}^{t} G(t-s)\dot{\gamma}(s)\,ds$, where it is found that $G(t) = Ge^{-t/\lambda}$. This relationship has two meanings: first the material has a memory and the state of stress will depend on previous deformations or past applied rates of deformations. Then this memory is fading, related to $G(t)$, and recent events will be remembered better than old ones, because $G(t)$ decreases in time. Usually, most complex materials possess more than just one relaxation time and therefore $G(t)$ becomes $G(t) = \sum_{i=1}^{n} G_i e^{-t/\lambda_i}$ where the pairs (G_i, λ_i) define the relaxation spectrum.

Generally the spectrum can be measured through oscillatory measurements where the imposed strain $\gamma(t) = \gamma_0 \sin(\omega t)$ varies with the same angular frequency $\omega = 2\pi f$ (where f is frequency) as the stress $\tau(t) = \tau_0 \sin(\omega t + \phi)$. In the case where the deformation γ_0 is small enough, one can deduce the elastic and viscous moduli (respectively $G' = \tau_0 \cos\phi/\gamma_0$ and $G'' = \tau_0 \sin\phi/\gamma_0$) which are a signature of the complex material. Recent developments of rheometry allow to study larger strains, leading to nonlinear responses including multiple frequency responses, not necessarily the prescribed one (ω). In the linear case, a typical curve giving the moduli $G'(\omega)$ and $G''(\omega)$ is represented in Fig. 1 for a polymeric biomaterial, such as an actin solution, or a network of actin polymers with crosslinks. Several features arise and can be explained using a simple multimode Maxwell model:

1. At low frequencies the system can flow or not. (a) If it flows, i.e. when no interactions between structural components exist that can prevent flow, then typical slopes of 2 and 1 respectively are obtained as $G' = G\frac{\omega^2\lambda^2}{1+\omega^2\lambda^2}$ and $G'' = G\frac{\omega\lambda}{1+\omega^2\lambda^2}$ with λ the longuest relaxation time. (b) If interactions are present and strong enough (e.g. crosslinks), then the material will exhibit a yield stress and no flow will be possible at low rates of deformation. This gives rise to constant moduli G' and G''. These two behaviours are indicated in Fig. 1.
2. At intermediate frequencies, the system is viscoelastic. Both moduli are of the same order but G' exhibits a plateau, also called the elastic plateau.
3. At larger frequencies, a transition occurs, a result of the presence of multiple closely spaced relaxation times and both moduli increase with a slow slope (exponent between 0.1 and 0.6).
4. Finally at very high frequencies the material reaches a glass behaviour, characterized by a constant elastic modulus G', and a decreasing loss modulus G''.

To be able to predict complex behaviours such as the one depicted in Fig. 1, multimode Maxwell models can be used, but they might require the use of a large numbers of modes. To improve such models, several authors have found ways to obtain a relaxation spectrum either in terms of a minimum number of modes [7] or using more sophisticated techniques like fractal derivatives [8]. Such methods are usually successful. Another means to interpret relaxation data is the reptation theory [9]. It assumes that a long polymer is constrained within a network of neighboring chains or tube [10] and that relaxation is limited. Therefore only special relaxation times and moduli are selected. This theory is successful for predicting the Newtonian region as well as the plateau domain (Fig. 1), but fails at high frequencies.

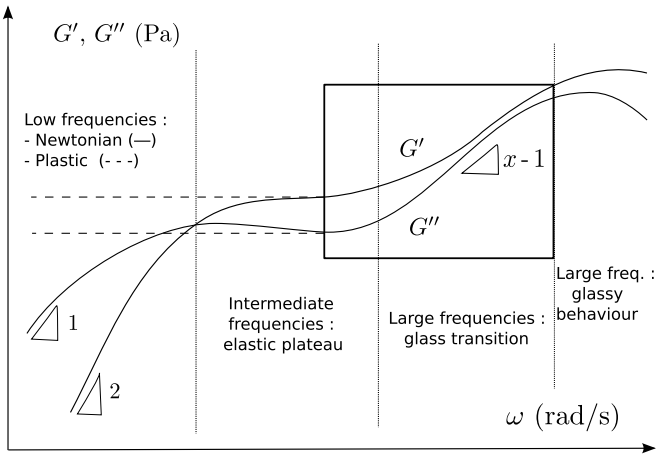

Fig. 1. Dynamic viscoelastic spectrum (G', G'') for a soft polymeric biomaterial (log–log scale). Full lines correspond to an uncrosslinked system whereas dotted lines indicate the presence of crosslinks. x is the exponent defined by Sollich [6]. The box indicates the typical range of microrheology techniques. Redrawn and modified from [3].

Going back to single cell behaviour, typical results are usually found in a limited range of frequencies, due to experimental limitations, like the range of the box shown in Fig. 1. The range of frequencies indicated corresponds to the plateau-transition zone discussed before. Sollich and coauthors [6,11] introduced the concept of disorder and metastability ("Soft Glassy Rheology" or SGR) to explain similarities in complex materials. They derive a mesoscopic model with a parameter x, the "effective noise temperature", and predict different behaviours for G' and G'' as power laws of ω, with an exponent function of x. In particular when x is above 1, the data in the typical intermediate frequency range behaves like $G' \sim \omega^{x-1}$, $G'' \sim \omega^{x-1}$, a behaviour close to the one described in Fig. 1. Note that it is also possible to predict a similar behaviour using a continuous distribution of relaxation times and associated moduli by replacing the summation for $G(t)$ by an integral, and again replacing the moduli by a power-law function of time [12]. Another possibility is to use Kelvin–Voigt models in series, whose distribution spacing increases in the transition zone [13]. We recall here that a Kelvin–Voigt model consists of a dashpot η in parallel with a spring G obeying to the equation $\tau = \eta\dot{\epsilon} + G\epsilon$. This model predicts solid-like behaviour with a constant $G' = G$ and $G'' = \eta\omega$.

This closes the discussion of simple 1D models, which have the advantage of bringing enough information on moduli and time scales. Nevertheless, cells and biological tissues undergo complex 3D-motions, thus more sophisticated models are required to predict experiments in shear, elongation or combined deformations. This is the principal motivation of this review.

In what follows, 3D constitutive laws will be used and the stress tensor $\boldsymbol{\Sigma}$ will be usually decomposed into two parts: an isotropic term $-p\mathbf{I}$ where p is the pressure, plus an extra stress tensor $\boldsymbol{\tau}$ whose form determines the constitutive equation. As in the 1D case, $\boldsymbol{\tau}$ will be a function of the linearized strain tensor (similar to γ) $\boldsymbol{\epsilon} = \frac{1}{2}[\mathbf{grad}\,\boldsymbol{u} + (\mathbf{grad}\,\boldsymbol{u})^T]$ (where $\boldsymbol{u} = \boldsymbol{x} - \boldsymbol{X}$ is the displacement between initial ($\boldsymbol{X}$) and present ($\boldsymbol{x}$) positions in Lagrangian coordinates), or any another tensor corresponding to large strains, as long as the tensor is 'objective'. 'Objectivity' or 'material frame-indifference' is introduced in classical mechanics [14] and means that constitutive equations should remain the same under a change of reference frame. In other words, this implies that tensors appearing in constitutive equations must verify certain properties under a change of frame. $\boldsymbol{\tau}$ will also depend on the objective tensor $\mathbf{D} = \frac{1}{2}[\mathbf{grad}\,\boldsymbol{v} + (\mathbf{grad}\,\boldsymbol{v})^T]$, the symmetric part of the velocity gradient (where $\boldsymbol{v}$ is the Eulerian velocity). In what follows, $\boldsymbol{\tau}$, $\boldsymbol{\epsilon}$, $\mathbf{D}$ will be respectively the 3D versions of the 1D stress (τ), strain (γ) and rate of strain ($\dot{\gamma}$) defined above.

The organization of this review is as follows: in the next part (Section 2), cell constituents and their properties are presented, and rheological models that can match these properties are described. These features are then put in the perspective of complex cell behaviours [15]. In the next part (Section 3), tissue properties and models will be presented. Special emphasis will be given first to biofluids, elastic and viscoelastic biomaterials [3,16]. Then, more complex models such as multiphase ones [17] and active tissues will be described, from both experimental and

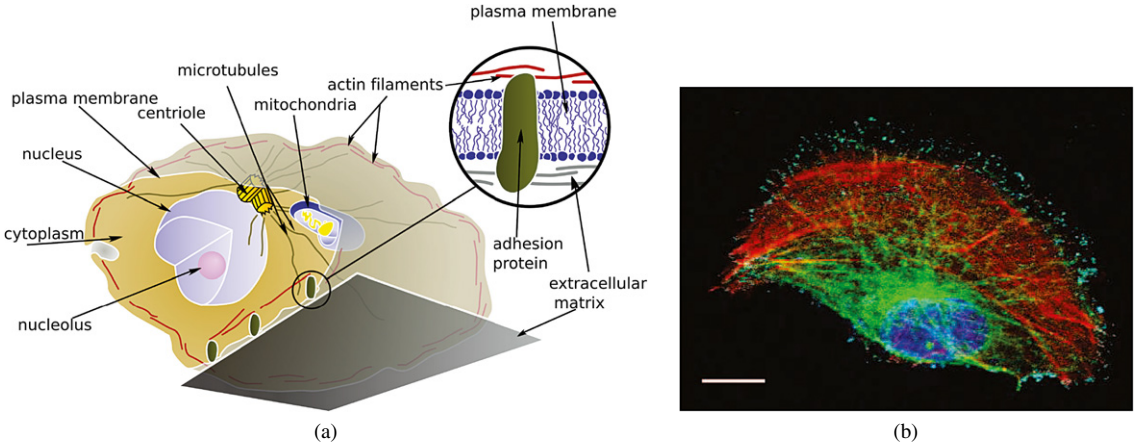

Fig. 2. (a) Model of an animal cell. Only a few organelles are shown. In reality, the organelles are smaller and more numerous. Inset: diagram of the cell membrane, with an adhesion protein interacting with the extracellular matrix and with the intracellular cytoskeleton. (b) Fluorescence image of a human cell (bladder carcinoma cell line T24). Microtubules are in green, actin in red, paxillin (a protein expressed in focal adhesions) in cyan, and the nucleus in blue. Scale bar: 10 μm.

theoretical points of view. In the last part, future directions, in particular multiscale modelling [18], will be discussed in order to deal with new concepts of cell and tissue mechanical properties.

2. Single cell properties and models

2.1. Cell constituents [19]

The cell is the basic unit of all living organisms. It contains organelles, cellular structures that have well-defined functions (see Fig. 2). Each eukaryotic cell consists of two parts, the cell body (with the cytoplasm) and the nucleus. The average size of a cell is 12 to 20 μm in radius, with a nucleus of 10–12 μm, with the exception of red blood cells, which have a diameter of 8 μm and no nucleus. Each cell is surrounded by a very thin membrane (6–10 nm) consisting of a bilayer of phospholipids (the plasma membrane). This flexible membrane maintains the integrity of the cell, but is also used for cellular functions, such as the selective passage of substances, or the attachment of the cell on its substrate. For these functions, proteins are inserted into the membrane, such as adhesion molecules which interact outside the cell with components of the extracellular matrix (ECM, composed of proteins such as fibronectin, collagen and proteoglycans), and inside the cell with the cytoskeleton and signalling proteins. In this part, we will focus on some cellular constituents, with a special emphasis on the cell structures that give the cell its rigidity and allow the cell to resist or exert forces.

2.1.1. The nucleus

The nucleus, which contains the genetic information, is the largest structure of the cell and the center of command for cell metabolism. Its shape is generally spherical or ovoid. The content of the nucleus is separated from the rest of the cell by the nuclear membrane. Genetic information is stored in the nucleus as DNA, which is linked with proteins to form chromatin. The nucleus contains one or more nucleoli, visible in optical microscopy, which are sites of production of ribosomal RNA.

2.1.2. The cell body

The cell body is composed of the cytoplasm, a structure bounded by the plasma membrane, which contains the cytosol and numerous organelles. The cytosol contains soluble proteins, sugars, salts, etc. The cytosol can switch from a gel (colloid) to a liquid structure and vice versa depending on cellular activity.

- The endoplasmic reticulum (ER) is an extension of the nuclear membrane. It is involved in the synthesis and glycosylation of proteins, and organizes the transport of substances in the cell.

- The Golgi apparatus is a set of membrane composed of flattened sacs that works in close relation with the endoplasmic reticulum. Vesicles delimited by membrane buds on the ER and merge with the Golgi. Protein maturation and protein sorting take place in the Golgi apparatus.
- Mitochondria are organelles that play an important role in the metabolism of the cell, converting energy from organic molecules derived from digestion (glucose) into energy used directly by the cell (ATP). Derived from bacteria, they contain their own DNA. Some mitochondrial proteins are synthesized by the mitochondria, but most of them are encoded by the nuclear genome and imported into mitochondria.
- The cytoskeleton is a complex network of polymers crossing through the cytosol that maintains cell shape and enables cell movement; it also plays important roles in both intracellular transport (the movement of vesicles and organelles, for example) and cellular division. The skeleton of the cell is composed of microtubules and microfilaments, protein structures consisting of globular subunits (microtubules and actin filaments), or fibrous elements (intermediate filaments).
  - **Microtubules** are polymers of tubulin. Microtubules are polarized and have the ability to polymerize and depolymerize rapidly to adapt to cell activity. In cells, microtubules radiate from the centrioles, located near the nucleus. These microtubules interact with motor proteins such as dynein and kinesin, which can bind intracellular organelles such as mitochondria or secretory vesicles. This allows these organelles to move quickly inside the cell. To understand the movement of these organelles, we can imagine microtubules as rails where organelles are pulled by these proteins.
  - **Actin microfilaments** are polarized, as microtubules, and can polymerize and depolymerize after interacting with other proteins (e.g. profilin retards the polymerisation). In each cell the microfilaments are concentrated beneath the cell membrane, to which they are connected to reinforce the cell surface for resisting tension and maintaining cellular shape. The actin microfilaments interact with myosin, a motor protein, that is able to generate contractile forces in actin networks. Finally, during mitosis (division of a cell into two daughter cells) the microfilaments form a contractile ring that allows invagination of the cytoplasmic membrane to the inside of the cell and thus its separation into two parts which will be the origin of two new cells.
  - **Intermediate filaments** are protein fibers with a twisted rope structure. They play a structural role in the cell. Different intermediate filaments are expressed in different cell types, vimentin in mesenchymal cells, cytokeratin in epithelial cells, desmin in muscle cells, and neurofilaments in the axons of neurons. Like actin filaments, these structures are important in maintaining cell shape, by bearing tension.
  - **Centrosomes and centrioles**. Many microtubules seem to be anchored by one end in the neighbouring region of the nucleus called the centrosome. The centrosome is the organization center of microtubules and contains a pair of organelles: the centrioles. These are small cylindrical structures perpendicular to one another composed of tubulin. Centrioles allow the establishment of the mitotic spindle during cell division.

2.1.3. Adhesion proteins

Cell adhesion is essential for many cell physiological functions (survival, proliferation, differentiation, migration) and pathological conditions (inflammation, metastasis, atherosclerosis, interaction of cell with biomaterials). Cell adhesion is regulated by proteins expressed at the cell surface, called cell-adhesion molecules (CAMs). These CAMs are transmembrane proteins containing an extracellular domain that interacts with specific ligands and an intracellular domain interacting with cytoplasmic proteins. These CAMs make it possible for the cells to adhere either on a substrate, such as an extracellular matrix, or with other cells. The adhesive molecules intervening in cell adhesion belong to four main families: selectins, molecules of the immunoglobulins superfamily, integrins, and the cadherins. Integrins and cadherins are the most ubiquitous.

The cadherins are transmembrane proteins involved in adhesion between cells. Their binding is usually homophilic, with a cadherin associated with another cadherin of the same type on an adjacent cell. Cadherins are involved in two types of cell–cell junctions, desmosomes and adherens junctions. Desmosomes are associated with the intermediate filament network, linking the cytoskeleton of adjacent cells. These junctions are responsible for maintaining the structural integrity of tissues.

Integrins form a large family of several dozen proteins. Each integrin consists of two transmembrane different polypeptides, the α and β subunits. The integrins have intracellular domains that link molecules of the cytoskeleton through intermediate cytoplasmic proteins such as α-actinin, vinculin, paxillin or talin. The main function of integrins is to mediate the adhesion of cells to the extracellular matrix. However, integrins of the β_2 family directly mediate

adhesion of leukocytes to endothelial cells. The $\alpha_5\beta_1$ integrin is the receptor for fibronectin at the surface of many cells. The extracellular part of this integrin binds to a specific sequence of fibronectin, and its intracellular domain is a binding site for proteins that make a link with actin microfilaments. By binding to extracellular molecules and intracellular microfilaments, integrin regulates the interaction between the cytoskeleton and the extracellular matrix.

2.2. Cell properties

As explained above, two important properties relevant to the cellular scale are its microrheological properties, but also its adhesion properties with respect to the extracellular matrix (ECM) or to other cells. Usually, investigating such properties requires to explore a wide range of forces, starting from single molecule interaction forces [20] in the range of a few piconewtons (pN) and going to quite high cell stretching forces (100 nN or more, see Refs. [21,22]) when the contact area is large. Covering such a large range of forces [1 pN–1 μN] demands the use of complementary tools [23].

2.2.1. Measuring cell properties

There are two kinds of techniques, active ones (perturbing cells), and passive ones, when no force is applied to the cells. Most of the techniques available today are active ones.

- Optical tweezers (OT) – The principle of optical tweezers [24] is now well known and consists in trapping a bead within a laser beam focalized by a microscope objective. By doing so, one can calibrate the trap using well-defined probes such as microbeads and apply controlled forces, while measuring deformations at the same time. Due to the limited power of the laser, forces range between [1–100 pN] usually. This method has now been used for many biological applications, in particular the determination of the mechanical modulus of Red Blood Cell (RBC) membranes [25] or the viscoelastic properties of actin-coated membranes [26]. It has also been used to extract tethers from neuronal growth cones. The instrument has been later modified to become the "optical stretcher", used with two opposed, nonfocused laser beams and allowing to stretch cells [27]. A review on optical tweezers [28] presents advantages and drawbacks of the method. Basically the main advantage of the method is to exert small forces on cell sub-elements, which is the major application.
- Atomic Force Microscopy (AFM) – It was invented twenty years ago as an extension of the tunnelling microscope [29] and is now considered as a classical tool for measuring forces between materials and a chosen probe. The AFM possesses a tip (usually conical or pyramidal) located at the end of a flexible cantilever which senses forces and therefore bends to a certain height called deflection. A laser reflected against the cantilever onto a photodiode is used to provide the deflection angle and therefore the force applied to the tip. Different modes have been developed: contact, tapping, and noncontact modes. Usually when it comes to measuring cell properties, very flexible cantilevers are used with typical constants [0.01–0.1 N/m]. The range of forces measured goes from 10 pN to 100 nN generally. Typical force curves are obtained by cell indentation, thus providing the cell's elastic Young modulus E (between 100 Pa and 10 kPa usually) using Hertz theory for elastic deformable bodies. Other researchers have used oscillatory movements [30,31] to obtain G' and G'' as defined in Section 1. Finally, topography maps (or elastic maps) are commonly obtained with spread cells. Finally, AFM is also a powerful method for measuring dynamic forces between receptors and ligands [32], similarly to the Surface Force Apparatus [23]. Such forces are essential because they control cell–cell interactions [33] as also shown in recent experiments [34,35]. AFM can also allow to achieve large cell deformations [22] which may become common thanks to recent improvements.
- Magnetocytometry (MTC) – This technique consists in coating ferromagnetic beads with a protein (usually RGD) which binds with the intracellular proteins such as integrins. These beads can therefore bind to the surface of cells. A high magnetic field is applied first to magnetize the beads and create parallel magnetic moments. Then a torque is applied, while following the bead motion using microscopy. Its rotation gives an information of the elasticity of the surrounding medium. Magnetic sinusoidal torque [36] can be used so that the beads rotate also sinusoidally. This allows to determine the real and imaginary parts of the complex modulus $G^*(\omega) = G'(\omega) + iG''(\omega)$, as defined in Section 1. Other types of measurements rely on the rapid application of a torque [37] and the follow-up of the time-dependent bead motion. This method allows to follow the elastic modulus (or stiffness) vs. the

mechanical stress to determine whether stress hardening occurs [38]. This can also permit to study the frequency dependence of the elastic and loss moduli G' and G'' to analyze cell regime changes [39].

- Micropipettes – Evans and coworkers developed micropipettes to study cell rheology [40,41]. By simple aspiration of a cell into a smaller micropipette, one measures its deformability while controlling the applied pressure (0.1–10^5 Pa). The results can provide the apparent cytoplasm viscosity (of the order $\sim$100 Pa s for granulocytes) as well as the cortical tension ($\sim$0.04 mN/m) of the cell, within the framework of an ad-hoc model [42]. Further use of micropipettes has led to studies in close relation with modelling aspects in particular to investigate cell membrane properties.
- Microplates – This is another alternative to AFM providing an interesting way to achieve stretching [43]. The method consists in binding a cell between two plates, one of them being deformable. By carefully following the cell in between the plates, one can measure its deformation and also monitor the plate's deflection, which gives the force (up to 100 nN, depending on the type of plate used). By designing this system, researchers [43] managed to obtain large cell deformations after binding cells on both sides (using concanavalin A). Force vs. time curves can be correlated to 1D or 3D viscoelastic models, allowing the determination of rheological parameters such as viscosities (η) and moduli (G), as mentioned in Section 1. The technique has been further improved to precisely monitor the plate's deflection and accurately determine the rheological response [44]. Furthermore, oscillatory deformations are also possible and have been recently used to propose new relaxation spectra [13].
- Cell poking – This is the last *active method* presented here. It was developed at the end of the eighties [45] and uses the round tip of a micropipette to indent cells from the side [46]. The tip is rapidly removed after this procedure. The knowledge of deformations combined with elastic theories permits to obtain the elastic modulus (E). Complementary microscopic observations (DIC or RICM) can reveal morphological changes like cell retraction. This method has been applied for detecting differences in viscoelastic properties using several cell types [46]. Note that a similar technique was also proposed to measure focal contact growth following application of a mechanical stress [47].
- Particle tracking microrheology (PTM) – This is the only *passive method* discussed here since no force is applied on the cell. The idea is to follow the Brownian motion of beads or organelles embedded within the cell cytoplasm. Doing so, the cell is not perturbed and is characterized in its natural state. The method proceeds as follows [48]: by following the random displacement $r(t)$ of small objects (radius a, typically μm size or smaller) located in the cytoplasm, one has access to the 2D mean squared displacement $\langle \Delta r^2(t) \rangle$, called MSD. From this is taken the Laplace transform $\langle \Delta \tilde{r}^2(s) \rangle$ (s is frequency in the Laplace domain) related to $G(s)$ by the generalized Stokes–Einstein relationship $G(s) = \frac{k_B T}{\pi a s \Delta \tilde{r}^2(s)}$ (where k_B is Boltzmann's constant and T is temperature). Then going into the Fourier space provides the complex modulus $G^*(\omega)$. Note that the generalized Stokes–Einstein relationship only applies at equilibrium. Experiments carried out on active cytoskeleton networks [49] indeed show that the previous relationship does not apply and that the difference between PTM data and conventional one can be attributed to motor activity which results in stiffness increase.

  The method can be improved by tracking two objects and cross-correlating the thermal motion of this pair of tracers. The accuracy is enhanced [50] as shown for example in the case of F-actin solutions: the two-point microrheology method should therefore be preferred.

2.2.2. Cell microrheology

There have been many studies in the recent years focused on the study of cell microrheology, some of them consider *suspended cells*, others use *adherent cells*. As it was explained previously (Section 2.1), the properties may be quite different in the two cases, as the adherent cell expresses adhesion molecules (Section 2.2.3) which allows it to bind the substrate; therefore its cytoskeleton is modified. More generally, cell properties are *nonhomogeneous* and may depend on the precise locus of measurement. Differences in elastic moduli measured by AFM show that rheological properties are space-dependent. The cytoplasm and the membrane may possess different properties. Cytoplasm properties strongly depend on the amount of actin or tubulin (and other filaments), on the degree of polymerisation of these filaments, as well as the degree of reticulation of the network. High elastic moduli (if one may consider that the cellular material is elastic!) are associated with the nucleus or places where F-actin filaments are concentrated, such as the cell cortex or the leading edge. Softer places are found around the nucleus. Similarly, the membrane has a bending modulus which is dependent on proteins embedded into it.

Most interesting is the frequency dependence of the elastic and viscous moduli (G' and G'') as determined using OT, AFM [31], MTC [36], PTM [51] with attainable frequencies in the window shown in Fig. 1. This window covers the frequency domain available using such instruments from [0.1 Hz–10^3 Hz] generally and shows typical slopes for moduli of 0.1–0.5. It has been well known in rheology for years that such a frequency dependence reveals the existence of a continuous set of relaxation times [7,3], also described by the SGR model of Sollich [11]. This idea is still explored nowadays [52,53] when considering the above mentioned frequency range. But recently, new ideas were proposed [54,39] after measurements as slow as 10^{-3} Hz were carried out using MTC. Note that such measurements require a very good control of the experimental conditions since they are carried out over 15 minutes. This study indicates two slopes for the norm of G^*, one at intermediate frequencies $\sim$0.2 like in the SGR model, and a second one at lower frequencies of the order $\sim$0.33. This raises the question of whether or not new regimes (different power laws) can actually be obtained at low frequencies. From our knowledge of soft matter, it is usually accepted that when going to low frequencies, reorganization can always take place leading to flow behaviour as in the case of molten polymers for example. But it may happen that stronger links between molecules cannot allow such behaviour, this gives rise to a yield stress σ_S. This would be the case for cross-linked networks for example, as in the case of actin filaments connected by α-actinin molecules or other cross-links. Such a case gives a different picture for the G'–G'' diagram, with the two moduli reaching a plateau at low frequencies as shown in Fig. 1.

In the case of the cell cytoplasm, such processes can actually take place, and one may think that the results obtained recently reveal the presence of a new regime at lower frequencies, this one having a slope of roughly 0.33 [39]. It may also be true that a plateau regime is obtained in between. At the long times, it is difficult to understand what is really happening at the sub-cellular level, but one may suggest that other motions prevail such as those of myosin-activated entangled actin networks or the motion of molecular motors (which are ATP dependent [49]) or again, cytoskeletal remodelling.

To sum up, we may say that the cell cytoplasm acts like a soft biological material similar to a polymer solution with moduli in the range [1–100 Pa] and that the addition of crosslinkers can lead to elastic moduli in the range [100–1000 Pa], most of these moduli been frequency dependent. At the present time it is not clear how to define viscosities for such cellular materials because of processes occurring on longer time scales and not yet measured well in the literature. In any case, viscoelastic or viscoplastic (related to the existence of a yield stress) behaviours are commonly encountered for both *in vitro* reconstituted systems and with real cells.

2.2.3. Cellular adhesion

In this part we sum up the main results of the literature regarding the adhesion properties between cells or between a cell and a substrate. The first idea emphasized earlier [33] is to think about cell–cell area as the locus of competition between specific interactions and nonspecific ones (i.e. electrostatic, osmotic). In terms of modelling, there is a free energy whose minimization gives the contact area between two cells. It should be linked to:

1. The relevant chemical potentials of receptors and ligands.
2. The number of receptors and ligands.
3. The possible mobility of receptors/ligands.
4. The nonspecific repulsive potential.
5. The elastic deformation of cells.
6. The role of glycocalyx (a thin layer of sugars covering the cell surface).

In practice it is usually difficult to characterize cell–cell interfaces in 3D-real tissues, therefore biomimetic systems or cell–substrate interactions are more often considered, using functionalized substrates, or using receptors on the cell membrane [55]. The work on vesicles is helpful when combined with microscopy since it shows the cell area precisely using RICM (Reflection Interference Contrast Microscopy) [56]. Biomimetic systems representing cell–tissue interactions show typical values of the adhesion energy in the range of 10^{-5} J/m^2 [57]. There are fewer studies considering cell–cell or cell–substrate quantification of such energies but researchers have reported similar values [58]. As there are adhesion molecules expressed at the surface of cells, like in the case of cadherin–cadherin bonds, which play a fundamental role at cell–cell junctions (tissues, endothelium lining), reports give higher values of adhesion energies in the range of 1–5 mJ/m^2. These values depend strongly on cadherin density, and seem to increase linearly with molecule density [59]. This subject has motivated great interest, in particular the concept of surface tension

generalized from drops to cell aggregates is strictly based on cell–cell interactions: cell aggregates made of two cell types reorganize based on their respective surface tensions and mix preferably with their own type [60].

These situations assume equilibrium through a minimum in the free energy, but it is known that dynamic phenomena are present during cell–cell interactions and that they involve the formation and rupture of bonds [61,62] following kinetic reactions of the type $R + L \rightleftharpoons R - L$ (a receptor R reacts with a ligand L to form a bond $R - L$). These reactions have been studied theoretically and bonds can be modelled within the concept of free energy landscapes under the application of a force [63]. At the present time, few systems have been investigated but nevertheless interesting data is available for describing the rolling motion of leukocytes along the endothelium (mediated by PSGL-1 bounds to P- or E-selectins) [64,65].

To conclude this part, let us say that most of the actual physiological and pathological behaviours of cells are mediated by the complex interplay between adhesion and rheological properties of cells, this being illustrated in Section 2.4.

2.3. Models for the cell rheology

The extreme complexity of the cell, its obviously nonlinear response to mechanical assays (i.e. deformation no longer proportional to stress), and its ability to deform and generate forces in a programmed manner using chemical energy, are as many challenges for the modelling of its dynamics. Models have been proposed that successfully reproduce the behaviour of living cells in specific conditions, but because of this complexity they cannot pretend to generality, and a model that performs well in a given situation may not be appropriate in some other. An obvious example for this is the typical viscoelastic spectrum in Fig. 1: a model and set of parameters that faithfully reproduce the cell response in a given frequency range (say 1 to 100 Hz) cannot reliably predict response for a time scale of the order of 10 s, because of its nonlinearity. Though, most of the assays are performed within the former range, while much of the interesting cell dynamics happen at the latter timescale.

Two fundamentally different approaches have been explored: the up–bottom approach in which a global model is proposed that reproduces some of the mechanical features of the cell, and the bottom–up approach in which individual components of the cell, which are reckoned to be important for some mechanical feature, are modelled. The first approach usually leads to continuum models, the second to discrete models. For clarity we will only discuss the continuum approach, one can e.g. refer to [1] for a description of the other.

A basic feature of cells is that they form a closed object containing a fluid, the cytosol, and a meshwork of polymers, the cytoskeleton (see Fig. 3 for a sketch of these elements). The outer membrane allows only limited transfers of material, in a controlled fashion. Thus a general first assumption is that the material within the cell is incompressible, thus $\operatorname{div} \boldsymbol{v} = 0$, and that the outer membrane $\Gamma(t)$ is impermeable, thus its normal velocity is equal to $\boldsymbol{v} \cdot \boldsymbol{n}_{\Gamma(t)}$ ($\boldsymbol{n}_{\Gamma(t)}$ is the unit normal to $\Gamma(t)$).

In general, the computational domain includes both the cell(s) and an outer medium (suspending fluid and ECM) which bears the outer boundary condition. Additional inner boundary conditions are needed to define the surface properties of the cell: these correspond to the stress jump at the boundary. Some authors continue with the analogy of drops and impose a surface tension force, $(p_{cell} - p_{medium})\boldsymbol{n}_{\Gamma(t)} + (\boldsymbol{\tau}_{cell} - \boldsymbol{\tau}_{medium})\boldsymbol{n}_{\Gamma(t)} = 2H\zeta\boldsymbol{n}_{\Gamma(t)} + \mathbf{grad}_s\,\zeta$, where p is the pressure, H is the local mean curvature of the membrane, ζ is the tension, usually constant on the membrane in this case, and $\mathbf{grad}_s$ the surface gradient. The membrane may also be considered elastic (e.g., [66]). Other authors have a completely inextensible membrane, the tension is thus the Lagrange multiplier imposing local inextensibility [67]. It is usually calculated by a penalty method, assuming an extensible membrane with a high extension modulus (e.g., [68]), a saddle-point approach is an alternative to calculate it exactly [69]. In the case of a membrane, an additional bending rigidity force $2\kappa(\Delta_s H + 2H^3 - 2HK)\boldsymbol{n}_{\Gamma(t)}$ applies [70,71], where Δ_s is the surface Laplace operator (also known as Laplace–Beltrami), κ is the bending rigidity modulus and K is the local Gaussian curvature. However, for cells other than red blood cells, a large excess area is available in surface wrinkles (see Fig. 3) or made available by membrane trafficking (e.g., [72]), and membrane forces are generally negligible compared to bulk forces. Surface tension forces and elasticity used in some models [73,74] thus do account for forces applied by the outer part of the cytoskeleton, close to the actual membrane and bound to it by transmembrane molecules. A second type of surface forces apply on cell membranes, they are interaction forces between the adhesion molecules that coat the membrane and the cell environment. Various levels of description of these forces can be chosen depending on the

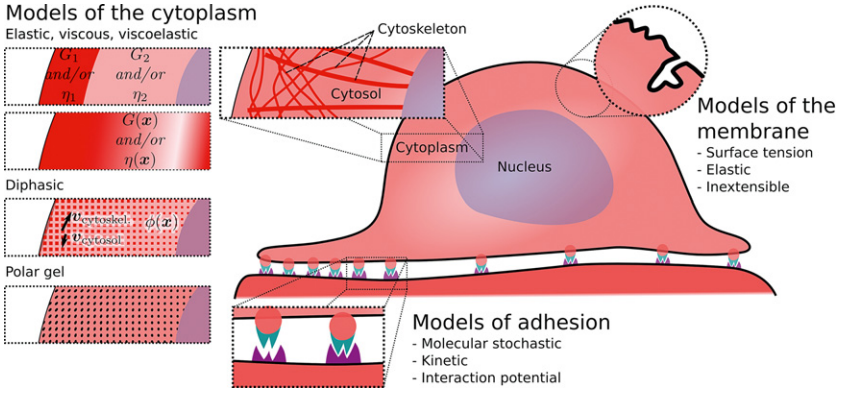

Fig. 3. Models for different components of an individual cell. The cytoplasm can be modelled as a continuum material, either as a monophasic or biphasic material. The phases can be considered elastic, viscous or viscoelastic, and the viscosity and shear modulus can vary abruptly or continuously as a function of position. Polar gel models track the local average polarity of the cytoskeleton and take into account forces associated to this polarization. Active processes (polymerisation, cross-linking and contractility) can be incorporated in these models. The membrane is mostly impermeable and its (weak) mechanical contribution approached by several models. Adhesion is due to molecular binding and can be modelled in more or less details.

case of interest, from stochastic binding equations (e.g., [75]) to kinetic equations (e.g., [76]) and averaged models, such as Van der Waals-type adhesion potentials (e.g., [77]).

Elastic and viscous behaviours are both exhibited by the cell. The elastic component originates from the cytoskeleton, which is strongly cross-linked and has a global organization. The dissipative component giving rise to viscous-like behaviours has a contribution from the flow of the cytosol along with and through the cytoskeleton meshwork. Another source of dissipation is the rearrangement of the cytoskeleton itself, whose cross-links have a lifetime of the order of 1 second [78].

Appropriate elementary models for describing these responses to strain will depend on its timescale. At very short times (milliseconds), cell response is dominated by the elastic response of the cytoskeleton. A linear model will thus have the following form $\boldsymbol{\tau} = 2G\boldsymbol{\epsilon}$. For times much longer than a second on the other hand, the response of the cytoplasm to sollicitations is essentially viscous, and one can take $\boldsymbol{\tau} = 2\eta\mathbf{D}$. Both these crude laws, which give the global picture of the cell response at the two ends of the frequency range in Fig. 1, can be refined to account for the spatial heterogeneity of the cell, introducing position-dependent viscosity or shear modulus (as shown in Fig. 3) to account for local higher density of cross-linked polymers in the cell nucleus and in the actomyosin cortex (e.g. the composite drops of [73]). The shear modulus may also be replaced by an elasticity tensor, however these refinements raise the issue of the measurement of the parameters involved. At intermediate times, both elastic and viscous responses are felt and combine in a complex, viscoelastic response. Again, different frequency ranges are best described with different models. For frequencies close to the longest relaxation time λ, one can use single relaxation time viscoelastic fluids described by the convected Maxwell model:

$$\lambda \overset{\triangledown}{\boldsymbol{\tau}} + \boldsymbol{\tau} = 2\eta\mathbf{D} \tag{1}$$

where the upper convected objective derivative is defined [2] as:

$$\overset{\triangledown}{\boldsymbol{\tau}} = \dot{\boldsymbol{\tau}} - (\mathbf{grad}\ \boldsymbol{v})\boldsymbol{\tau} - \boldsymbol{\tau}(\mathbf{grad}\ \boldsymbol{v})^T \tag{2}$$

Here $\dot{\boldsymbol{\tau}}$ denotes the material time-derivative of the stress. As compared to the 1D-Maxwell model, the simple time-derivative is now replaced by $\overset{\triangledown}{\boldsymbol{\tau}}$ in 3D. For higher frequencies, the power-law behaviour of the elastic and loss moduli can be reproduced for instance using the SGR constitutive law [79].

In addition to its timescale, the nature of the strain may also prompt for a more refined model: indeed, phase separation can occur, in which the cytosol flows relatively to the cytoskeleton. This was demonstrated for instance in the case of the formation of "blebs", membrane protrusions that only contain fluid in the first instance [80]. A biphasic model can then be introduced with a different velocity $\boldsymbol{v}$ and stress tensor $\boldsymbol{\tau}$ for each constituent (denoted by subscripts *csl* and *csk* for cytosol and cytoskeleton respectively), and a friction term k_F between them:

$$-\phi_{csl}\,\mathbf{grad}\,p + \mathbf{div}(\phi_{csl}\boldsymbol{\tau}_{csl}) + k_F\phi_{csk}\phi_{csl}(\boldsymbol{v}_{csk} - \boldsymbol{v}_{csl}) = \mathbf{0} \tag{3a}$$

$$-\phi_{csk}\,\mathbf{grad}\,p + \mathbf{div}(\phi_{csk}\boldsymbol{\tau}_{csk}) + k_F\phi_{csk}\phi_{csl}(\boldsymbol{v}_{csl} - \boldsymbol{v}_{csk}) = \mathbf{0} \tag{3b}$$

where ϕ_α is the volume fraction of each constituent, which is advected by the velocity of this constituent, and subject to a reaction term Γ_α that models mass exchanges of chemical origin (e.g., polymerisation) between the constituents (α denoting the generic constituent):

$$\frac{\partial\phi_\alpha}{\partial t} + \mathrm{div}(\phi_\alpha \boldsymbol{v}_\alpha) = \Gamma_\alpha \tag{4}$$

Dembo and collaborators (and subsequent papers [81]) consider both phases to be Newtonian fluids, while other authors (see e.g. [82]) consider one constituent (the cytoskeleton) to be an elastic solid, and the other (the cytosol) to be a Newtonian or a perfect fluid. In the limit when $\boldsymbol{v}_{csk} = \boldsymbol{v}_{csl}$, that is, when both phases flow together, such models yield respectively Newtonian fluid and linear viscoelastic solid models (whose parameters depend linearly on the volume fractions).

Finally, the most challenging aspect of cell dynamics is its active component, which allows for example thorough remodelling of its structure and motility. The basic ingredients for this are the dynamic formation of the cytoskeleton through polymerisation and cross-linking processes, and the contractile forces generated by molecular motors acting as active cross-linkers. Seminal work by Dembo and collaborators [81] has introduced phenomenological modelling of many aspects of the biological cell. Another approach by Kruse and collaborators ([83] and subsequent papers) gives this type of modelling in the framework of the hydrodynamic theory, within which phenomenological modelling of actin polymerisation, alignment and contractility can be developped.

2.4. Understanding cell behaviour: beyond cell rheological properties

The material properties of the cell described above are of course not sufficient to explain complex behaviour, such as cell migration, division or morphogenesis during the embryonic development of a living organism. These behaviours are obtained by programmed reaction to stimuli and tuning of processes such as adhesion and polymerisation of the cytoskeleton. The motile machinery of the cell is regulated by networks of signals: for example, association of the motor protein myosin with actin is regulated by fast phosphorylation/dephosphorylation of proteins, leading to contraction of the actin network, and activation of the small G proteins Rac, Rho and Cdc42 is crucial for coordinating actin polymerisation. Intracellular calcium concentration is also important for regulating cell contractility. In the case of cells cultured on surfaces coated with an extracellular matrix component such as fibronectin, it has been shown that cells form focal adhesions. A focal adhesion consists of a cluster of proteins (integrins and their cytoplasmic partners) which is a site of attachment of a bundle of actin filaments called a stress fiber. These structures are not only sites of attachment of the cell. They can send and receive signals.

Cell migration is a good example of its coordinated active behaviour. Adhesion is a prerequisite of cell movement on matrix proteins. We have already seen that cells can adhere to a substrate using specific receptors such as integrins. The integrins are linked to the cytoskeleton, allowing the cell to apply forces on its substrate (see Fig. 4). Migration requires an initial polarization of the cell, followed by an enhanced polymerisation of the actin cytoskeleton, extending a protrusion at the 'front', which in turn attaches by adhesive complexes to the substrate on which the cell is migrating. Microtubules and actin microfilaments have been shown to be important for establishing and maintaining cell polarity. Actin monomers polymerize at the front to form filaments that stabilize cell extensions. The cell then retracts its trailing end, where attachments are released. Although this global picture is now well established (e.g., [84–88]), the precise mechanisms that generate movement are still being explored, and some specialized models are able to capture its dynamics [89,90]. Note that 3D cell migration still needs to be explored more carefully, like cell migration in 3D-collagen matrices, where new techniques for stress characterization can be used [91].

Cell diapedesis, that occurs in the process of inflammation, is an even more complex mechanism [93]. This process takes place inside the blood vessels, when circulating white cells (leukocytes) must migrate to fight infections in the surrounding tissues. After activation by chemicals released by bacteria, leukocytes adhere and pass through the endothelium, a layer of cells that cover the inside of blood vessels. Diapedesis involves a precisely coordinated adhesion cascade and signalling events, first to slow down leukocytes in the blood flow, and then make them spread and adhere firmly to the endothelium (see e.g., [94] for experiments and a viscous drop and elastic membrane model). The final step when the leukocyte squeezes through interendothelial junctions demands an important reorganization of the cell shape [95].

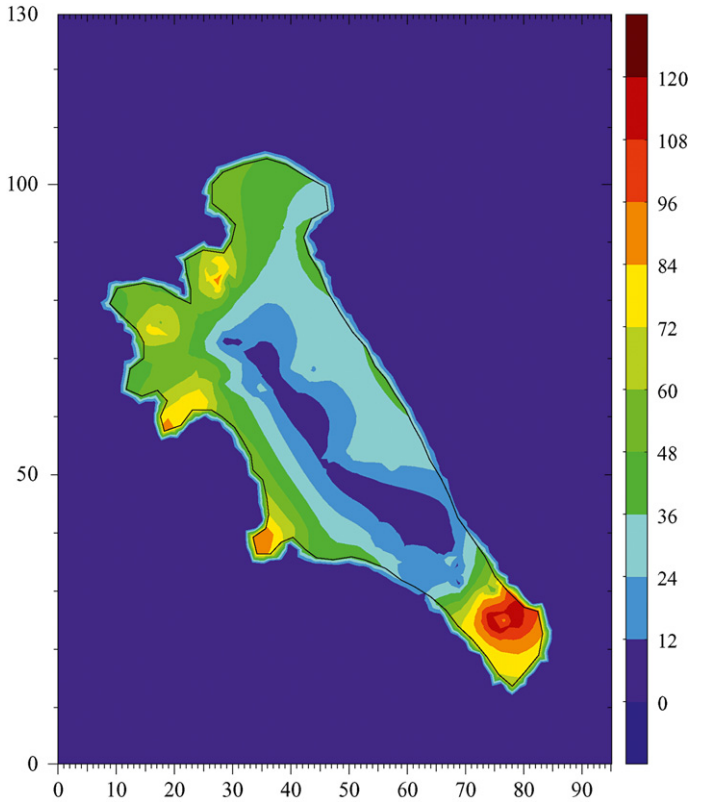

Fig. 4. Cell traction forces exerted by a migrating T24 cancer cell on a polyacrylamide substrate (Young's modulus $E = 10$ kPa). The colour code indicates the magnitude of the computed traction force in pN/μm^2, with a maximum around 100 pN/μm^2. The box size is given on vertical and lateral axes in μm. The cell is moving from bottom-right to top-left, is initially attached and develops a large lamellipodium to proceed [92].

Cell division, or mitosis, is an instance in which the cell incurs a global reorganization of its cytoskeleton, allowing to duplicate and to sort cellular elements so as to create two viable daughter-cells [96]. Mitosis is a highly regulated process, that involves numerous signalling events and control points. After the genetic material inside the nucleus has been duplicated, the nuclear envelope disappears and the DNA condenses into chromosomes. Chromosomes attach to microtubules forming the mitotic spindle, and motor proteins pull apart chromosomes to opposite ends. The division itself involves the formation of a contractile ring made of actin filaments and myosin at the 'equator' of the cell, which constricts the cell membrane and forms the cleavage furrow. The model by Dembo and collaborators could reproduce these steps [97].

Finally, individual cells are capable to sense the mechanical characteristics of their environment and react accordingly. For instance, endothelial cells organized in a monolayer and submitted to a shear flow slowly adopt an elongated shape aligned with the flow direction [98]. Also in morphogenesis, some of the shape generations have been shown to follow cell rearrangements dictated by stresses applied by neighbouring parts of the embryo [99]. Individual cells also exhibit sensitivity to the mechanical properties of their environment, such as its rigidity (e.g., [100]). This conversion by cells of mechanical stimuli into intracellular signalling events is called mechanotransduction, that can influence cell shape by modification of its cytoskeleton, gene expression, proliferation, and cell survival [101]. Adhesive proteins such as integrins which are connected to the cytoskeleton have been shown to play a role in this process, but to date, no real mechanoreceptor has been clearly identified.

3. Tissue properties and models

Biological tissues include both biofluids and tissues. The descriptions of their properties can start with biofluids and elastic materials. Then the problem of more complex materials requires the use of multiphase models, to finally include active tissues and remodelling.

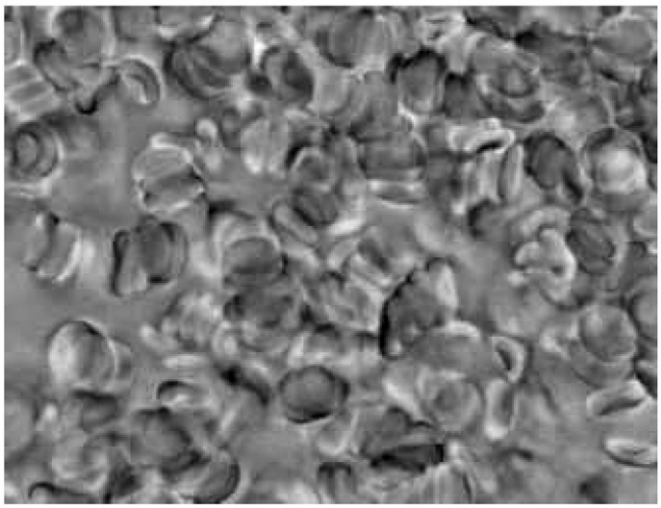

Fig. 5. Picture of full blood, including RBCs, white blood cells and plasma.

3.1. Biofluids

There are several types of fluids commonly encountered in the human body. To name a few of them, synovial fluids allow lubrication of knee joints [16,102] and are shear thinning fluids. Mucus also exhibits similar properties, due to the presence of polymer chains and behaves as a polymer solution. It has pronounced viscoelastic properties at concentrations above the gelation threshold, as shown by rheometry. Typically behaviour of mucus is a constant elastic modulus G' and slowly decreasing G'' of the order of a few tens of Pascals [103]. Saliva is another biofluid with properties close to water (viscosity below 0.01 Pa s) and also presents viscoelastic properties with some elasticity [104], appearing at higher frequencies in the range [10–100 rad/s].

The most widely studied biofluid is blood (Fig. 5). Blood contains mostly plasma (an aqueous solution of electrolytes and proteins such as fibrinogen and albumin) and red blood cells (RBCs or erythrocytes, volume concentration around 40%), but also a few other cells like white blood cells (WBCs or leukocytes, 0.06%) and finally platelets (around 0.25%). The rheological properties of blood have been studied by Cokelet [105], Chien [106–108]. The existence of a yield stress has been shown by Chien [106]. This yield stress is due to the ability of cells to bind which each others and form the so-called 'rouleaux'. Blood can be well described using Casson's model [109] $\sqrt{\tau} = \sqrt{\tau_s} + \sqrt{\eta\dot{\gamma}}$, where τ_s is the yield stress and $\dot{\gamma}$ is the shear rate.

More generally, the yield stress is a very important quantity in rheology, associated with the existence of interactions (weak or strong) causing the impossibility for a fluid to flow at small shear stresses. Therefore, below the yield stress, the material usually behaves as an elastic solid, and above it, can flow. The most commonly used model for describing such fluids exhibiting yield stresses τ_s is the Bingham model (equivalent to Casson's model at low and high shear rates), which can be generalized to three dimensions as follows:

$$\begin{aligned} &\text{if } II_\tau \leqslant \tau_s^2, \quad \mathbf{D} = \mathbf{0} \quad \text{or} \quad \boldsymbol{\tau} = G\mathbf{B} \\ &\text{if } II_\tau \geqslant \tau_s^2, \quad \boldsymbol{\tau} = 2\left(\eta + \frac{\tau_s}{\sqrt{II_{2\mathrm{D}}}}\right)\mathbf{D} \end{aligned} \tag{5}$$

where $II_\tau = \frac{1}{2}(\mathrm{tr}(\boldsymbol{\tau})^2 - \mathrm{tr}(\boldsymbol{\tau}^2))$, $\mathbf{B} = \mathbf{F}\mathbf{F}^T$ is the deformation tensor adapted for large strains ($\mathbf{F} = \frac{\partial \boldsymbol{x}}{\partial \boldsymbol{X}} = \mathbf{grad}\,\boldsymbol{x}$ is the deformation gradient tensor), G is the shear elastic modulus, and η is the viscosity, which is a constant for large shear rates $\dot{\gamma}$, like in Casson's model. The first inequality means that stresses are not large enough for the system to flow because of intrinsic interactions, then no flow exists but the material behaves as a (nonlinear) elastic solid. When stresses are high enough, the fluid can flow and presents a viscosity η.

There are other empirical models available for such fluids, also called viscoplastic fluids, like the Herschel–Bulkley model [110] predicting a shear thinning behaviour for the viscosity at large shear rates, together with a yield stress at small rates.

Another interesting idea when describing complex fluids containing particles or cells (radius a) is the concept of fractal aggregates [111,112]. The basic concept is to consider a system with particles (concentration ϕ) at rest. Particles organize in a fractal way and above a certain gel concentration $\phi \geqslant \phi_g$, the interactions between particles become effective and a yield stress (τ_s) can exist, due to the presence of aggregates of a certain size. When flow is applied at a typical shear rate $\dot{\gamma}$, aggregates are broken but may re-form due to possible encounters. For a given shear rate, equilibrium is obtained giving rise to a typical aggregate size, which is stress-dependent. Then using empirical relations for suspension viscosity [113], one obtains a 1D-constitutive equation which exhibits a yield stress $\tau_s = \frac{\Gamma}{a}(\frac{\phi}{\phi_0})^{\frac{1}{m(3-D)}}$, where $\Gamma \sim 10^{-5}$ N/m is the adhesion energy between cells [111,58], and ϕ_0 is the maximum packing volume fraction. D is the fractal dimension and m is the only adjustable parameter. Such models work well but still remain to be developed in three dimensions.

Finally, let us mention that in recent years, some authors have re-investigated the concept of blood and vesicle suspensions (as a model of blood), by carefully studying the motion of individual cells (vesicles) in a shear flow. This has led to interesting new behaviours [114] which can then be included in a more general model for *dilute suspensions* rheology [115]. These new models seem to be in better agreement with experiments [116] than earlier ones, which were modelling blood as a suspension of capsules [117].

3.2. Elastic materials – strain energy – viscoelastic materials

Tissues are divided into different classes [118] which include:

- Epithelium, i.e. layers of cells (skin, vascular wall).
- Connective tissues, containing cells embedded in the ECM: bones, cartilage, ligaments, tendons.
- Contractile tissues (cardiac, skeletal, smooth muscles).
- Nervous tissues.

Tissue behaviour can usually be described at first glance by classical elastic laws for the stress tensor $\boldsymbol{\tau} = \lambda\,\mathrm{tr}(\boldsymbol{\epsilon})\mathbf{I} + 2\mu\boldsymbol{\epsilon}$ where $\boldsymbol{\epsilon}$ has been defined in Section 1, and λ and $\mu = G$ are the Lamé coefficients. Such a simple constitutive equation is usually not enough to cover the large deformation range [16] where nonlinear elastic models are necessary, as sometimes shown by exponential behaviour of the stress [119] meaning that deformation is limited. The usual idea is to introduce a more general definition of the strain tensor, which will tend to the classical limit strain $\boldsymbol{\epsilon}$ corresponding to linear elasticity or small deformations. There are several possibilities for the generalized strain tensor such as $\mathbf{E} = \frac{1}{2}(\mathbf{F}^T\mathbf{F} - \mathbf{I})$ or $\mathbf{E_e} = \frac{1}{2}(\mathbf{I} - \mathbf{B}^{-1})$. We choose to use relationships making use of $\mathbf{B}$ (see definition in Section 3.1) rather than $\mathbf{C} = \mathbf{F}^T\mathbf{F}$ here, for the sake of simplicity. Then one can construct a nonlinear elasticity law by relating $\boldsymbol{\tau}$ to $\mathbf{B}$ or $\mathbf{B}^{-1}$. The Mooney–Rivlin model $\boldsymbol{\tau} = 2C_1\mathbf{B} - 2C_2\mathbf{B}^{-1}$ is well adapted for such biological materials. C_1 and C_2 are two adjustable moduli, linked to the shear elastic modulus G. Therefore, only one parameter can be adjusted. Most generally, nonlinear elasticity or hyper-elasticity can be treated through strain energy functions to describe the behaviour of a soft tissue. The extra stress tensor $\boldsymbol{\tau}$ is then a function of the large deformation tensors $\mathbf{B}$ and $\mathbf{B}^{-1}$ with the addition of a strain energy function $W(I_B, II_B)$ [120,121] and its derivative with respect to the first and second invariants of the tensor $\mathbf{B}$ (I_B and II_B respectively). This model has been applied successfully to the study of brain tissue for example [122]. A typical form of W is the one given by [123]: $W = \sum_{i+j=1}^{n} C_{ij}(I_B - 3)^i(II_B - 3)^j$, and has been applied to very soft tissues (brain, liver, kidney) [124].

Following early experimental measurements giving the stress as a function of the strain rate [16], it was shown that the above models are not sufficient to describe the rate dependence of most standard tests (elongation) and that there is a need to include viscoelasticity in the models. At first a simple 1D quasilinear model was used $\tau(t) = \int_{-\infty}^{t} G(t-s)\dot{\tau}_e(s)\,ds$ to introduce this rate dependence [125,126], in the case of collagen networks or aortic tissue. In this integral, $\tau_e(t)$ represents the elastic stress response corresponding to a step-stretch. A more sophisticated model is the K–BKZ model [5] which is commonly used for describing the behaviour of soft biological tissues [127] $\boldsymbol{\tau}(t) = \int_{-\infty}^{t} G(t-s)(\frac{\partial W}{\partial I_B}\mathbf{B}(t,s) - \frac{\partial W}{\partial II_B}\mathbf{B}^{-1}(t,s))\,ds$. This model contains the energy function W as defined above, as well as the memory function $G(t)$ of the biological material. Note that the viscoelastic Maxwell model earlier defined above in Eqs. (1)–(2) can be recovered in its integral form by simply taking $W = I_B$.

Finally, another important property of tissue is their anisotropy. This is often due to the presence of ECM fibers or cell alignment (like in epithelia). To consider these features, two options are possible: one can replace the elastic

moduli by an elastic tensor [128]. This can give rise to different elastic moduli and Poisson coefficients in different directions. The next possibility is to consider fibers or objects individually and assume that stresses are functions of the fiber direction $\boldsymbol{p}$. After considering the fiber dynamics [129], one can obtain a stress tensor depending on fiber direction through the averages $\langle \boldsymbol{p}\boldsymbol{p} \rangle$ and $\langle \boldsymbol{p}\boldsymbol{p}\boldsymbol{p}\boldsymbol{p} \rangle$, with viscous (solvent) and elastic parts [2]. These angle brackets $\langle \cdot \rangle$ are averages with respect to the probability density function (for the fiber orientation), which satisfies a Fokker–Planck equation. This idea is the one employed within the context of multiphase models which is presented in the next section.

3.3. Multiphase models

Since the 1960s multiphase models have been applied with success to describe the behaviour of articular cartilages [130,131] and other biological tissues, such as arteries, heart, brain, subcutaneous layer, and so on [132]. As it is impossible to give a complete review of this whole field in a few pages, we will focus on cancer modelling and tissue engineering which have undergone the highest development in the past decade [133]. Actually, most of the concepts and models used in these applications were also applied to other biological tissues.

Regarding cancer modelling, the first multiphase models to appear were, of course, biphasic models mainly derived with the aim of describing the behaviour of (avascular) multicellular spheroids *in vitro*. The tumour mass was then treated as an ensemble of cells wetted by an extracellular liquid filled with nutrients and growth factors [134–137]. Since protein size dissolved in the extracellular liquid, absorbed or produced by the cells is very small as compared to cell size, the protein evolution is usually described through suitable reaction–diffusion equations.

The observation that tumours, as many other tissues, are made of at least three constituents, i.e., cells, extracellular matrix, and extracellular liquid, that can be easily identified in Fig. 6, led then to the development of tri-phasic models. Actually, more general multiphase models were also developed to describe the interaction of tumours with external tissues, to include different cell populations, e.g., macrophages or fibroblasts, to consider polyclonality of tumour masses, to distinguish the different constituents making up the extracellular matrix. Examples can be found in [138–146] dealing with tumours, and in [147] dealing with cell growth in an artificial scaffold showing how similar models can be applied in tissue engineering.

Referring to [148,149] for more details, in addition to the reaction–diffusion equations for the molecules considered essential for the description of the evolution of the system, the structure of the model consists in a set of mass and momentum balance equations for the constituents. Denoting by α the generic constituent, one can then write, similarly to Eq. (4)

$$\frac{\partial \phi_\alpha}{\partial t} + \nabla\cdot(\phi_\alpha \boldsymbol{v}_\alpha) = \Gamma_\alpha \tag{6}$$

(where ϕ_α is the volume ratio of the constituent, $\boldsymbol{v}_\alpha$ its velocity, and Γ_α its growth/death term) and, considering that inertia is negligible in the description of growth phenomena (similarly to Eqs. (3a)–(3b)),

$$-\phi_\alpha \nabla p + \nabla\cdot(\phi_\alpha \boldsymbol{\tau}_\alpha) + \mathbf{m}_\alpha = \mathbf{0} \tag{7}$$

where p is the interstitial pressure of the extracellular fluid, $\boldsymbol{\tau}_\alpha$ is the so-called excess stress tensor, and $\mathbf{m}_\alpha$ is the interaction force accounting for the forces acting on the constituent α due to the interactions with the other components of the mixture.

The model then simplifies according to the constituents considered. For instance, in many cases, the tissue can be modelled as a porous material, or some of the interactions among the constituents can be neglected.

To be specific as an example we here report the tri-phasic model that can be deduced by the more general model introduced in [146] that assumes that the interactions with the liquid are negligible and that the extracellular matrix is a deformable porous medium in which the cellular population moves and grows like a sticky granular material

$$\begin{aligned}
&\frac{\partial \phi_c}{\partial t} + \nabla\cdot(\phi_c \boldsymbol{v}_c) = \Gamma_c \\
&\frac{\partial \phi_m}{\partial t} + \nabla\cdot(\phi_m \boldsymbol{v}_m) = \Gamma_m \\
&\boldsymbol{v}_c - \boldsymbol{v}_m = K\left(1 - \frac{\sigma}{|\nabla\cdot(\phi_c \boldsymbol{\tau}_c)|}\right)_+ \nabla\cdot(\phi_c \boldsymbol{\tau}_c)
\end{aligned}$$

$$\nabla \cdot (\phi_c \boldsymbol{\tau}_c + \phi_m \boldsymbol{\tau}_m) = \mathbf{0} \tag{8}$$

where c refers to the cellular population, m to the extracellular matrix, $(\cdot)_+$ stands for the positive part of the parenthesis, K is the mobility coefficient and σ (energy per unit length) measures the adhesive properties between the cell and the extracellular matrix. The first two equations represent mass balance with mass source terms describing duplication and death of cells and matrix remodelling. The third equation is a generalization of Darcy's law for a deformable porous material with adhesion between cells and the ECM. It means that if the stress gradient within the cell population is lower than σ, then the parenthesis will be zero, and both velocities (cell and matrix) will be the same, otherwise the relative velocity between cell and matrix depends on the stress gradient and the amount of adhesion between cells. There is no reference to the third phase since its contribution is considered negligible. Of course, the equations above need to be accompanied by the constitutive equations for the excess stress tensors $\boldsymbol{\tau}_c$ and $\boldsymbol{\tau}_m$, describing the response of both cells and ECM to deformation.

In this respect, in most of the multiphase models of tumour growth appearing in the literature the tumour is modelled as a compressible fluid, either inviscid $\boldsymbol{\tau}_c = -p(\phi_c)\mathbf{I}$ (i.e. directly relating the stress to the volume ratio through an isotropic tensor), or viscous (e.g., [147,150]). The deformability of the extracellular matrix is often neglected. When this effect is taken into account, as in [17], a nonlinear elastic model is considered.

We need to mention that this multiphase framework allows to justify, on the basis of sound mechanical hypotheses, what is generally called Darcy's closure and named as potential flow assumption in [134], that is used with success in many papers, e.g., [151–157].

However, there are some theoretical difficulties that appear when one wants to describe tumours and tissues as solids, because they are growing, remodelling and re-organizing while deforming. We will briefly describe this issue in the next section.

Recently, Lowengrub and coworkers [158,159] paid attention to the adhesion properties of cellular populations introducing an adhesion energy. Still working with a multiphase framework, the final model consists of a fourth-order nonlinear advection–reaction–diffusion equation of Cahn–Hilliard type.

Another aspect that is attracting more and more attention is the effect of mechanical and environmental cues on the behaviour and growth of tumours and tissues, in general. In this respect, Chaplain et al. [143] developed a model accounting for contact inhibition of growth and showed on the one hand how cells physiologically stop duplicating upon reaching confluence due to the correct activation of the mechanotransduction pathway and, on the other hand, how a misperception of the compression state of the local tissue related to a misinterpretation of the mechanochemical signal can generate by itself a clonal advantage on the surrounding cells, leading to the replacement and the invasion of the healthy tissue. Also [160–162] looked at the same problem, using individual cell-based models (IBMs). The results obtained using IBMs and multiphase models were then compared in [163–165].

3.4. Active, growing, and remodelling tissues

As already mentioned, there is a conceptual difficulty in describing growing and remodelling biological tissues as solids, because the cells forming them duplicate and die, the extracellular matrix is continuously remodelled and even in the absence of growth the whole of the cells undergo an internal re-organization in response to strain. There is then a difficulty in defining a reference configuration and in using a Lagrangian coordinate system. In particular, the meaning of strain loses the immediate meaning it has in classical continuum mechanics when dealing with inert matter. In fact, when dealing with a living tissue, with respect to what should we measure strain, if the material is always changing? That is why the concept of evolving natural configuration introduced by Rajagopal and coworkers can be of help. In particular, Humphrey and Rajagopal applied this concept to describe the growth and remodelling of tissues [166], arteries [167,168], ligaments and tendons [169]. The basic idea of this formalism, also present in plasticity theory, started to be applied in a biomechanical context to describe growth [170–172].

The theory for materials with evolving natural configurations is an ideal setting to investigate the growth and remodelling of tissues because it allows to separate the contribution due to growth from the one due to deformation alone and to model each of them individually.

The aim is then to distinguish in the evolution of the tissue given through the deformation gradient $\mathbf{F}$ the contribution of pure growth from the stress-induced deformation. In particular, it is natural to work so that no growth occurs during stress-induced deformation. From the biological point of view, the two contributions could be easily tested in principle since growth occurs on a much longer time scale (several hours) than deformation.

The way one can conceptually identify the two contributions is to take a neighbourhood of a point in the present configuration $\mathcal{K}_t$ and allow it to relax to a stress-free configuration while keeping its mass constant. The atlas of such configurations obtained changing the point forms a natural configuration relative to $\mathcal{K}_t$ which is denoted $\mathcal{K}_n$. Of course, this natural configuration depends on time. The deformation without growth is identified by the tensor $\mathbf{F}_N$, which then describes how the body is deforming locally in going from the natural configuration $\mathcal{K}_n$ to the present configuration $\mathcal{K}_t$. It is then connected to the stress response of the tissue to strain. The tensor

$$\mathbf{G} \equiv \mathbf{F}_N^{-1}\mathbf{F} \tag{9}$$

tells how the body is growing locally and is therefore named growth tensor. Hence, the following decomposition holds

$$\mathbf{F} = \mathbf{F}_N\mathbf{G} \tag{10}$$

Then there are two things to determine, first how the natural configurations evolve, i.e., characterizing the evolution of the growth tensor $\mathbf{G}$, then how the material behaves from each natural configuration. The multiplicative decomposition of the deformation gradient has been used in several applications, in particular, heart mechanics [173,174], aneurism formation [167], remodelling of arteries [175,176,168,172], and soft tissues in general [177,121].

Coming back to the growth of multicellular spheroids in particular, Ambrosi and Mollica [178,179] used the theory of evolving natural configurations, splitting the evolution into growth and elastic deformations. In their model the interactions with the ECM and extracellular liquid are neglected so that the tumour is described as a one-constituent compressible elastic body.

This approach was generalized in [17] to the multiphase framework, taking into account the internal re-organization and ECM deformation. Here the evolution of the natural configuration is related to two different contributions: growth (or remodelling) and cell re-organization because of the experimental observation that adhesion bonds between cells have a finite strength and might break or build up during the evolution.

This gives rise to an elasto-visco-plastic description for the cell population and a compressive elastic description for the ECM. In addition, one needs to describe how the natural configuration evolves in time due to growth and to internal re-organization, so that the decomposition

$$\mathbf{F} = \mathbf{F}_N\mathbf{F}_P\mathbf{G} \tag{11}$$

is introduced where $\mathbf{F}_P$ describes the internal re-organization of cells.

The idea is then that the rearrangement of adhesion bonds during the deformation of the cell aggregate is related to the existence of a yield stress in the macroscopic constitutive equation. Hence, for a moderate amount of stress, the cell aggregate deforms elastically, while above a yield value τ_s the cell aggregate undergoes an internal re-organization which is modelled at a macroscopic level as a viscoplastic deformation.

In the limit of small deformation with respect to the natural configuration, the proposed constitutive model for the excess stress reads

$$\lambda\dot{\boldsymbol{\tau}}_c + \left[1 - \frac{\tau_s}{f(\boldsymbol{\tau}_c)}\right]_+ \boldsymbol{\tau}_c = 2\eta\left(\mathbf{D} - \frac{1}{3}(\operatorname{tr}\mathbf{D})\mathbf{I}\right) \tag{12}$$

where $\boldsymbol{\tau}_c$ is the excess stress, $\mathbf{D}$ is the rate of strain tensor, $[\cdot]_+$ stands again for the positive part of the bracket, τ_s is the yield stress, f is an invariant measure of the stress, λ is the relaxation time, and η is the viscosity.

As shown in [180], the model reproduces very well several experiments performed by two different teams, in particular the behaviour under steady shear reported by [58], and the compression experiments performed in [181–184]. Very recently the model has been extended to explain the behaviour of a single cell deformed by the action of an optical tweezer [185]. Here the adhesive behaviour is related to the links acting among the microtubules and the actin filaments.

Contemporarily, several authors (e.g., [186,187]) developed an approach based on thermodynamic arguments, stating an *a priori* dissipative principle that distinguishes between standard and accretive forces. In this way one obtains a constitutive relation providing a direct coupling between stress and growth in terms of an Eshelby-like tensor [188]. This approach was for instance applied to bones [189].

The two approaches above present differences and similarities that have been nicely pointed out [190] and [191].

This can also be applied to model the active behaviour of tissues, like heart [174] and muscles [192]. For instance, Stolarska et al. [193], using concepts very close to those presented above, propose to decompose the deformation gradient into an active and a passive part.

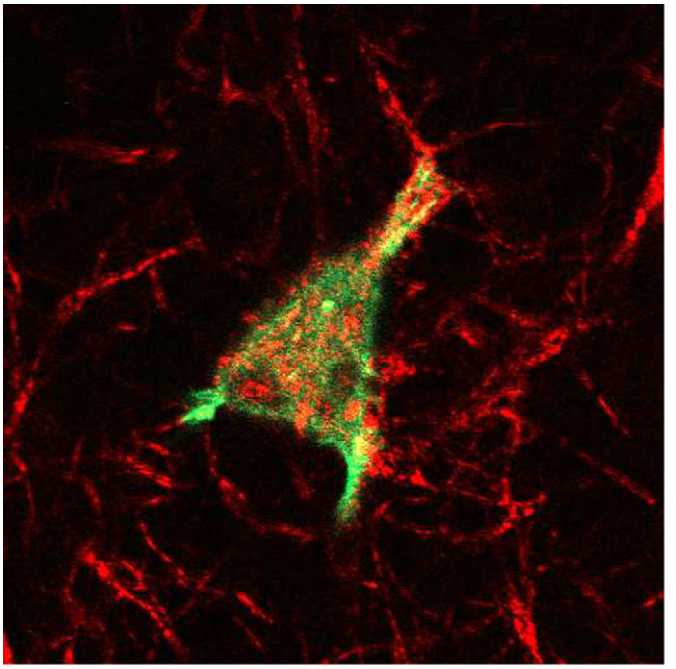

Fig. 6. CHO cell (green) embedded in a collagen matrix (red) together with culture medium. One can notice the contacts between the cell and the collagen as well as the elongated shape of the cell [194].

However, as already remarked in the previous section, tissues are made of several constituents and, in particular, in addition to cells, the behaviour of the extracellular matrix plays a relevant role in the description of the mechanical behaviour of the tissue. The continuous remodelling of the extracellular matrix has been already emphasized [194–196]. In particular, Iordan et al. [194] studied the interactions between the cells and the surrounding ECM (Fig. 6): they showed that the mechanical properties of the collagen gel can change because of the ability of cells to pull and adhere to the ECM, and exert traction forces leading to inhomogeneities of the collagen.

4. New challenges – conclusions

If a single message was to be retained out of this review, it would be that there remains a huge gap between having a detailed view of the cell's innermost molecular essence and understanding the complexity of its individual and collective mechanical behaviour. At the submicron scale, the tremendous development of cell biology has brought a wealth of informations on the molecular composition, properties and coordinated mechanisms within the cell. At the cell and tissue scales, ever improving microrheometry techniques have allowed to shed light on the nonlinear behaviour that arises from this organization. However, our understanding of the emergence of such properties from the molecular ingredients we see remains limited to ad-hoc models of a few emblematic behaviours, far from any predictive science of the living matter.

What could be a successful approach for addressing this issue? Obviously enough, it is to integrate the microscale phenomena systematically as a basis for all macroscale behaviours. It has been suggested [197] that the necessary horizon was to couple such a micro–macro simulation of the mechanics to simulations of the gene transcription and full machinery of biochemical reactions. One should nevertheless be wary to keep the outcome of the models employed within the grasp of understanding, so that the underlying function of each element is clearly brought to light. Well-defined test-cases however are already within the reach of such approaches, as reviewed in [18]. For example, one can relate the microscopic detachment-formation of bonds at the molecular scale to describe how cells bind or roll along a substrate [73,198] or how the molecular machinery comes into play to push the membrane forward in lamellipodia. Another important promising issue is to link the cellular (individual cell properties such as adhesive and mechanical effects [199]) and macroscopic scales (tissues) through homogenization methods (e.g., [200]). Coupling observations of the dynamics of cytoskeleton or sub-elements [201] under controlled applied mechanical stress in order to explore

the relationships between mechanical properties and microstructural changes is next on the shortlist of present day challenges. It is within reach in conventional rheology [2] but requires more sophisticated techniques to have access to such properties with cellular systems [22,202].

On another level, further experimental efforts are needed to bring experimental set-ups from the artificial setting of glass coverslips closer to the physiological reality. Experiments performed under shear flow conditions [203] are an example of this enterprise. Another issue is the transposition of experiments performed on two-dimensional, nonphysiological substrates to three-dimensional settings involving complex fiber networks, reproducing the extracellular matrix. Migration for instance has been extensively studied on flat surfaces [204–206], but more recent three-dimensional assays [207] show differences in the very mechanism cells use to migrate in complex ECM networks [208,209]. In tissues, cell interactions with the ECM while under stress remain to be characterized, as cells are able to remodel their environment extensively. This implies to master 3D observations using confocal microscopy [208] which have been only used at the cell level but deserve to be used at a larger scale in order to obtain sufficient data for developing new models [210].

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References

[1] A. Vaziri, A. Gopinath, Nat. Materials 7 (1) (2008) 15.
[2] R.G. Larson, The Structure and Rheology of Complex Fluids, Oxford University Press, New York, 1999.
[3] C. Verdier, J. Theor. Medicine 5 (2) (2003) 67.
[4] P.A. Pullarkat, P.A. Fernández, A. Ott, Physics Reports 449 (2007) 29.
[5] R.B. Bird, R.C. Armstrong, O. Hassager, Dynamics of Polymeric Liquids, vol. 1. Fluid Mechanics, 2nd ed., John Wiley and Sons, 1987.
[6] P. Sollich, F. Lequeux, P. Hébraud, M.E. Cates, Phys. Rev. Lett. 78 (10) (1997) 2020.
[7] H.H. Winter, J. Non-Newtonian Fluid Mech. 68 (1997) 225.
[8] L.I. Paladé, V. Vernay, P. Attané, Rheol. Acta 35 (1996) 265.
[9] P.G. de Gennes, J. Chem. Phys. 55 (2) (1971) 572.
[10] M. Doi, S.F. Edwards, The Theory of Polymer Dynamics, Oxford University Press, 1986.
[11] P. Sollich, Phys. Rev. E 58 (1) (1998) 738.
[12] M. Baumgaertel, A. Schausberger, H.H. Winter, Rheol. Acta 29 (1990) 400.
[13] M. Balland, N. Desprat, D. Icard, S. Féréol, A. Asnacios, J. Browaeys, S. Hénon, F. Gallet, Phys. Rev. E 74 (2) (2006) 021911.
[14] L.E. Malvern, Introduction of the Mechanics of a Continuous Medium, Prentice Hall Inc., 1969.
[15] F. Mollica, L. Preziosi, K.R. Rajagopal, Modeling of Biological Materials, Modeling and Simulation in Science, Engineering and Technology, Birkhäuser, Boston, 2007.
[16] Y.C. Fung, Biomechanics. Mechanical Properties of Living Tissues, 2nd ed., Springer-Verlag, New York, 1993.
[17] D. Ambrosi, L. Preziosi, Biomech. Model. Mechanobiol. 8 (2009) 397.
[18] A. Chauvière, L. Preziosi, C. Verdier, Cell Mechanics. From Single Scale-Based Models to Multiscale Modeling, Chapman & Hall/CRC, 2010, in press.
[19] B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, J.D. Watson, Molecular Biology of the Cell, 3rd ed., Garland Publishing, 1994.
[20] R. Merkel, P. Nassoy, A. Leung, K. Ritchie, E. Evans, Nature 397 (6714) (1999) 50.
[21] O. Thoumine, A. Ott, O. Cardoso, J.J. Meister, J. Biochem. Biophys. Methods 39 (1–2) (1999) 47.
[22] E. Canetta, A. Duperray, A. Leyrat, C. Verdier, Biorheology 42 (5) (2005) 321.
[23] D. Leckband, Annu. Rev. Biophys. Biomol. Struct. 29 (2000) 1.
[24] A. Ashkin, J.M. Dziedzic, Proc. Natl. Acad. Sci. USA 86 (1989) 7914.
[25] S. Hénon, G. Lenormand, A. Richert, F. Gallet, Biophys. J. 76 (2) (1999) 1145.
[26] E. Helfer, S. Harlepp, L. Bourdieu, J. Robert, F.C. MacKintosh, D. Chatenay, Phys. Rev. E 63 (2001) 021904.
[27] J. Gück, R. Ananthakrishnan, H. Mahmood, T.J. Moon, C.C. Cunningham, J. Käs, Biophys. J. 81 (2001) 767.
[28] H. Zhang, K.K. Liu, J. Royal Soc. Interface 5 (24) (2008) 671.
[29] G. Binnig, C.F. Quate, C. Gerber, Phys. Rev. Lett. 56 (9) (1986) 930.
[30] R.E. Mahaffy, C.K. Shih, F.C. MacKintosh, J. Käs, Phys. Rev. Lett. 85 (4) (2000) 880.
[31] J. Alcaraz, L. Buscemi, M. Grabulosa, X. Trepat, B. Fabry, R. Farré, D. Navajas, Biophys. J. 84 (3) (2003) 2071.
[32] B.T. Marshall, M. Long, J.W. Piper, T. Yago, R.P. McEver, C. Zhu, Nature 423 (6936) (2003) 190.
[33] G.I. Bell, M. Dembo, P. Bongrand, Biophys. J. 45 (6) (1984) 1051.

[34] M. Benoit, D. Gabriel, G. Gerisch, H.E. Gaub, Nat. Cell Biol. 2 (6) (2000) 313.
[35] X. Zhang, A. Chen, D.D. Leon, H. Li, E. Noiri, V.T. Moy, M.S. Goligorsky, Am. J. Physiol. Heart Circ. Physiol. 286 (1) (2004) H359.
[36] B. Fabry, G.N. Maksym, J.P. Butler, M. Glogauer, D. Navajas, J.J. Fredberg, Phys. Rev. Lett. 87 (14) (2001) 148102.
[37] N. Wang, J.P. Butler, D.E. Ingber, Science 260 (1993) 1124.
[38] V. Laurent, E. Planus, R. Fodil, D. Isabey, Biorheology 40 (2003) 235.
[39] D. Stamenovic, N. Rosenblatt, M. Montoya-Zavala, B.D. Matthews, S. Hu, B. Suki, N. Wang, D.E. Ingber, Biophys. J. 93 (8) (2007) L39.
[40] E. Evans, Biophys. J. 13 (1973) 941.
[41] E. Evans, A. Yeung, Biophys. J. 56 (1) (1989) 151.
[42] A. Yeung, E. Evans, Biophys. J. 56 (1) (1989) 139.
[43] O. Thoumine, A. Ott, J. Cell Sci. 110 (Pt 17) (1997) 2109.
[44] N. Desprat, A. Richert, J. Simeon, A. Asnacios, Biophys. J. 88 (3) (2005) 2224.
[45] M. Duszyk, B. Schwab, G.I. Zahalak, H. Qian, E.L. Elson, Biophys. J. 55 (4) (1989) 683.
[46] W.H. Goldmann, Biotechnol. Lett. 22 (2000) 431.
[47] D. Riveline, E. Zamir, N.Q. Balaban, U.S. Schwarz, T. Ishizaki, S. Narumiya, Z. Kam, B. Geiger, A.D. Bershadsky, J. Cell Biol. 153 (6) (2001) 1175.
[48] T.G. Mason, D.A. Weitz, Phys. Rev. Lett. 74 (7) (1995) 1250.
[49] D. Mizuno, C. Tardin, C.F. Schmidt, F.C. Mackintosh, Science 315 (5810) (2007) 370.
[50] J.C. Crocker, M.T. Valentine, E.R. Weeks, T. Gisler, P.D. Kaplan, A.G. Yodh, D.A. Weitz, Phys. Rev. Lett. 85 (4) (2000) 888.
[51] B.D. Hoffman, G. Massiera, K.M.V. Citters, J.C. Crocker, Proc. Natl. Acad. Sci. USA 103 (27) (2006) 10259.
[52] P. Bursac, G. Lenormand, B. Fabry, M. Oliver, D.A. Weitz, V. Viasnoff, J.P. Butler, J.J. Fredberg, Nat. Materials 4 (7) (2005) 557.
[53] X. Trepat, G. Lenormand, J.J. Fredberg, Soft Matter 4 (2008) 1750.
[54] D. Stamenovic, Nat. Materials 5 (8) (2006) 597.
[55] A. Boulbitch, Z. Guttenberg, E. Sackmann, Biophys. J. 81 (5) (2001) 2743.
[56] U. Seifert, R. Lipowski, Phys. Rev. A 42 (8) (1990) 4768.
[57] Z. Guttenberg, B. Lorz, E. Sackmann, A. Boulbitch, Europhys. Lett. 54 (6) (2001) 826.
[58] A. Iordan, A. Duperray, C. Verdier, Phys. Rev. E 77 (1) (2008) 011911.
[59] R.A. Foty, M.S. Steinberg, Dev. Biol. 278 (1) (2005) 255.
[60] D.A. Beysens, G. Forgacs, J.A. Glazier, Proc. Natl. Acad. Sci. USA 97 (17) (2000) 9467.
[61] C. Zhu, J. Biomech. 33 (1) (2000) 23.
[62] C. Verdier, C. Couzon, A. Duperray, P. Singh, J. Math. Biol. 58 (2009) 235.
[63] G.I. Bell, Science 200 (4342) (1978) 618.
[64] V. Ramachandran, T. Yago, T.K. Epperson, M.M.A. Kobzdej, M.U. Nollert, R.D. Cummings, C. Zhu, R.P. McEver, Proc. Natl. Acad. Sci. USA 98 (18) (2001) 10166.
[65] K.E. Caputo, D.A. Hammer, Biophys. J. 89 (1) (2005) 187.
[66] C. Pozrikidis, J. Fluid Mech. 440 (2001) 269.
[67] I. Cantat, C. Misbah, Phys. Rev. Lett. 83 (1) (1999) 235.
[68] T. Biben, C. Misbah, Phys. Rev. E 67 (3) (2003) 031908.
[69] J. Etienne, A. Duperray, Biophys. J. (under review), submitted for publication.
[70] W. Helfrich, Z. Naturforsch. 28C (1973) 693.
[71] O.Y. Zhong-can, W. Helfrich, Phys. Rev. A 39 (10) (1989) 5280.
[72] M.P. Sheetz, J.E. Sable, H.G. Döbereiner, Annu. Rev. Biophys. Biomol. Struct. 35 (2006) 417.
[73] W. Shyy, M. Francois, H.S. Udaykumar, N. N'dri, R. Tran-Son-Tay, Appl. Mech. Rev. 54 (5) (2001) 405.
[74] M. Herant, V. Heinrich, M. Dembo, J. Cell Sci. 118 (2005) 1789.
[75] M.R. King, D.A. Hammer, Proc. Natl. Acad. Sci. USA 98 (26) (2001) 14919.
[76] M. Dembo, D. Torney, K. Saxman, D. Hammer, Proc. R. Soc. London B 234 (1988) 55.
[77] S. Sukumaran, U. Seifert, Phys. Rev. E 64 (2001) 011916.
[78] D. Wachsstock, W. Schwarz, T. Pollard, Biophys. J. 66 (1994) 801.
[79] P. Sollich, in: R.G. Weiss, P. Terech (Eds.), Molecular Gels – Materials with Self-Assembled Fibrillar Networks, Springer-Verlag, Netherlands, 2006, pp. 161–192.
[80] G.T. Charras, M. Coughlin, T.J. Mitchison, L. Mahadevan, Biophys. J. 94 (2008) 1836.
[81] M. Dembo, F. Harlow, Biophys. J. 50 (1986) 109.
[82] F. Guilak, V.C. Mow, J. Biomech. 33 (2000) 1663.
[83] K. Kruse, J.F. Joanny, F. Jülicher, J. Prost, K. Sekimoto, Phys. Rev. Lett. 92 (2004) 078101.
[84] J. Condeelis, Annu. Rev. Cell Biol. 9 (1993) 411.
[85] T.P. Stossel, Science 260 (1993) 1086.
[86] T.J. Mitchison, L.P. Cramer, Cell 84 (1996) 371.
[87] M.P. Sheetz, D.P. Felsenfeld, C.G. Galbraith, Trends Cell. Biol. 8 (1998) 51.
[88] T.D. Pollard, G.G. Borisy, Cell 112 (2003) 453.
[89] H.P. Grimm, A.B. Verkhovsky, A. Mogilner, J.J. Meister, Eur. Biophys. J. 32 (2003) 563.
[90] K. Kruse, J.F. Joanny, F. Jülicher, J. Prost, Phys. Biol. 3 (2006) 130.
[91] C.T. Mierke, D. Rösel, B. Fabry, J. Brábek, Eur. J. Cell Biol. 87 (2008) 669.
[92] D. Ambrosi, A. Duperray, V. Peschetola, C. Verdier, J. Math. Biol. 58 (2009) 163.

[93] T.A. Springer, Cell 76 (1994) 301.
[94] C. Dong, J. Cao, E.J. Struble, H.H. Lipowsky, Ann. Biomed. Eng. 27 (1999) 298.
[95] C. Johnson-Léger, M. Aurrand-Lions, B.A. Imhof, J. Cell Sci. 113 (2000) 921.
[96] P.P. D'Avino, M.S. Savoian, D.M. Glover, J. Cell Sci. 118 (2005) 1549.
[97] X. He, M. Dembo, Exp. Cell Res. 233 (1997) 252.
[98] J.T. Butcher, A.M. Penrod, A.J. García, R.M. Nerem, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 1429.
[99] N. Desprat, W. Supatto, P.A. Pouille, E. Beaurepaire, E. Farge, Developmental Cell 15 (2008) 470.
[100] D. Choquet, D.P. Felsenfeld, M.P. Sheetz, Cell 88 (1997) 39.
[101] C.S. Chen, J.L. Tan, J. Tien, Annu. Rev. Biomed. Eng. 6 (2004) 275.
[102] H. Fam, J.T. Bryant, M. Kontopoulou, Biorheology 44 (2) (2007) 59.
[103] S.K. Lai, Y.Y. Wang, D. Wirtz, J. Hanes, Adv. Drug. Deliv. Rev. 61 (2) (2009) 86.
[104] J.R. Stokes, G.A. Davies, Biorheology 44 (3) (2007) 141.
[105] G.R. Cokelet, E.W. Merrill, E.R. Gilliand, H. Shin, A. Britten, R. Wells, Trans. Soc. Rheol. 7 (1963) 303.
[106] S. Chien, S. Usami, H.M. Taylor, J.L. Lundberg, M.I. Gregersen, J. Appl. Physiology 21 (1966) 81.
[107] S. Chien, S. Usami, R.J. Dellenback, M.I. Gregersen, Science 157 (1967) 827.
[108] S. Chien, S. Usami, R.J. Dellenback, M.I. Gregersen, L.B. Nanninga, M. Mason-Guest, Science 157 (1967) 829.
[109] M. Casson, in: Rheology of Disperse Systems, Pergamon, Oxford, 1959.
[110] C.W. Macosko, Rheology, Principles, Measurements and Applications, Wiley–VCH, New York, 1994.
[111] P. Snabre, P. Mills, J. Phys. III. France 6 (1996) 1811.
[112] D. Quemada, Eur. Phys. J. AP 1 (1998) 119.
[113] I.M. Krieger, T.J. Dougherty, Trans. Soc. Rheol. III (1959) 137.
[114] C. Misbah, Phys. Rev. Lett. 96 (2) (2006) 028104.
[115] G. Danker, T. Biben, T. Podgorski, C. Verdier, C. Misbah, Phys. Rev. E 76 (2007) 041905.
[116] V. Vitkova, M.A. Mader, B. Polack, C. Misbah, T. Podgorski, Biophys. J. 95 (6) (2008) L33.
[117] A. Drochon, Eur. Phys. J. AP 22 (2003) 155.
[118] R. Chotard-Ghodsnia, C. Verdier, in: F. Mollica, L. Preziosi, K.R. Rajagopal (Eds.), Modeling of Biological Materials, in: Modeling and Simulation in Science, Engineering and Technology, Birkhäuser, 2007, pp. 1–32, chap. 1.
[119] Y.C. Fung, S.Q. Liu, J.B. Zhou, ASME J. Biomech. Eng. 115 (1993) 453.
[120] R.W. Ogden, Non-linear Elastic Deformations, Ellis Horwood, Chichester, UK, 1984.
[121] J.D. Humphrey, Proc. R. Soc. London A 459 (2003) 3.
[122] M. Kohandel, S. Sivaloganathan, G. Tenti, J.M. Drake, Med. Eng. Phys. 28 (5) (2006) 449.
[123] L.R.G. Treloar, The Physics of Rubber Elasticity, Clarendon Press, Oxford, UK, 1975.
[124] K. Miller, Med. Sci. Monit. 6 (1) (2000) 158.
[125] Y.C. Fung, Biorheology 10 (1973) 139.
[126] J.E. Bischoff, Ann. Biomed. Eng. 34 (7) (2006) 1164.
[127] A.D. Drozdov, H. Khanina, Math. Comput. Modelling 25 (2) (1997) 11.
[128] P. Kowalczyk, J. Biomech. 36 (7) (2003) 961.
[129] G.B. Jeffery, Proc. R. Soc. London A 102 (1922) 161.
[130] V.C. Mow, W.M. Lai, Annu. Rev. Fluid Mech. 11 (1979) 247.
[131] V.C. Mow, A. Ratcliffe, S.L.Y. Woo, Biomechanics of Diarthrodial Joints, Springer-Verlag, New York, 1990.
[132] B.R. Simon, Appl. Mech. Rev. 45 (1992) 191.
[133] P. Tracqui, Rep. Prog. Phys. 72 (2009) 056701.
[134] D. Ambrosi, L. Preziosi, Math. Mod. Meth. Appl. Sci. 12 (2002) 737.
[135] C.J.W. Breward, H.M. Byrne, C. Lewis, J. Math. Biol. 45 (2002) 125.
[136] H.M. Byrne, J.R. King, D.L.S. McElwain, L. Preziosi, Appl. Math. Lett. 16 (2003) 567.
[137] H.M. Byrne, L. Preziosi, Math. Med. Biol. 20 (2004) 341.
[138] R. Araujo, D. McElwain, SIAM J. Appl. Math. 65 (2005) 1261.
[139] R.P. Araujo, D.L.S. McElwain, SIAM J. Appl. Math. 65 (2005) 1285.
[140] S. Astanin, L. Preziosi, J. Theor. Biol. 258 (2009) 578.
[141] S. Astanin, A. Tosin, Math. Model. Nat. Phenom. 2 (2007) 153.
[142] C.J.W. Breward, H. Byrne, C. Lewis, Bull. Math. Biol. 65 (2003) 609.
[143] M. Chaplain, L. Graziano, L. Preziosi, Math. Med. Biol. 23 (2006) 197.
[144] S.J. Franks, J.R. King, Math. Med. Biol. 20 (2003) 47.
[145] T.J. Jackson, H.M. Byrne, Math. Biosci. 180 (2002) 307.
[146] L. Preziosi, A. Tosin, J. Math. Biol. 58 (2007) 625.
[147] G. Lemon, J. King, Math. Med. Biol. 24 (2007) 57.
[148] Astanin L. Preziosi, in: N. Bellomo, M. Chaplain, E.D. Angelis (Eds.), Selected Topics on Cancer Modelling: Genesis – Evolution – Immune Competition – Therapy, Birkhäuser, 2008, pp. 223–253.
[149] L. Graziano, L. Preziosi, in: F. Mollica, L. Preziosi, K.R. Rajagopal (Eds.), Modeling of Biological Materials, Birkhäuser, 2007, pp. 267–328.
[150] S.J. Franks, H.M. Byrne, H.S. Mudhar, J.C.E. Underwood, C. Lewis, Math. Med. Biol. 20 (2003) 277.
[151] V. Cristini, H.B. Frieboes, X. Li, J. Lowengrub, P. Macklin, S. Sanga, S.M. Wise, X. Zheng, in: N. Bellomo, M. Chaplain, E.D. Angelis (Eds.), Selected Topics on Cancer Modelling: Genesis – Evolution – Immune Competition – Therapy, Birkhäuser, 2008.

[152] V. Cristini, J. Lowengrub, Q. Nie, J. Math. Biol. 46 (2003) 191.
[153] J.C. Dunn, W.Y. Chan, V. Cristini, J.S. Kim, J. Lowengrub, S. Singh, B.M. Wu, Tissue Eng. 12 (2006) 705.
[154] H.B. Frieboes, J.S. Lowengrub, S. Wiseb, X. Zheng, P. Macklin, E. Bearer, V. Cristini, Neuroimage 37 (2007) S59–S70.
[155] X. Li, V. Cristini, Q. Nie, J. Lowengrub, Discr. Cont. Dyn. Sys. B 7 (2007) 581.
[156] P. Macklin, J. Lowengrub, J. Theor. Biol. 245 (2007) 677.
[157] P. Macklin, S. McDougall, A.R.A. Anderson, M.A.J. Chaplain, V. Cristini, J. Lowengrub, J. Math. Biol. 58 (2009) 765–798.
[158] V. Cristini, X. Li, J.S. Lowengrub, S.M. Wise, J. Math. Biol. 58 (4–5) (2009) 723–763.
[159] S.M. Wise, J. Lowengrub, H.B. Frieboes, V. Cristini, J. Theor. Biol. 253 (2008) 524.
[160] J. Galle, M. Loeffler, D. Drasdo, Biophys. J. 88 (2005) 62.
[161] D. Drasdo, S. Höhme, M. Block, Journal of Statistical Physics 128 (1) (2007) 287–345.
[162] M. Radszuweit, M. Block, J.G. Hengstler, E. Schöll, D. Drasdo, Phys. Rev. E 79 (2009) 051907.
[163] H. Byrne, D. Drasdo, J. Math. Biol. 58 (2009) 657.
[164] J. Galle, L. Preziosi, A. Tosin, Appl. Math. Lett. 22 (2009) 1483.
[165] J. Galle, L. Preziosi, in: F. Giraldez, M.A. Herrero (Eds.), Contemporary Mathematics, American Mathematical Society, 2009.
[166] J.D. Humphrey, K.R. Rajagopal, Math. Mod. Meth. Appl. Sci. 12 (2002) 407.
[167] S. Baek, K.R. Rajagopal, J.D. Humphrey, J. Biomech. Eng. 128 (2006) 142.
[168] J.D. Humphrey, K.R. Rajagopal, Biomech. Model. Mechanobiol. 2 (2003) 109.
[169] I.J. Rao, J.D. Humphrey, K.R. Rajagopal, Comp. Mod. Eng. Sci. 4 (2003) 439.
[170] S.M. Klisch, A. Hoger, Math. Mech. Solids 8 (2003) 377.
[171] E.K. Rodriguez, A. Hoger, A. McCulloch, J. Biomech. 27 (1994) 455.
[172] L.A. Taber, J.D. Humphrey, J. Biomech. Eng. 123 (2001) 528.
[173] I. Lin, L.A. Taber, J. Biomech. Eng. 117 (1995) 343.
[174] L.A. Taber, R. Perucchio, J. Elasticity 61 (2000) 165.
[175] D. Ambrosi, A. Guillou, E.D. Martino, Biomech. Model. Mechanobiol. 7 (2008) 63.
[176] R.L. Gleason, J.D. Humphrey, J. Vas. Res. 41 (2004) 352.
[177] K. Garikipati, E.M. Arruda, K. Grosh, H. Narayanan, S. Calve, J. Mech. Phys. Solids 52 (2004) 1595.
[178] D. Ambrosi, F. Mollica, Int. J. Engng. Sci. 40 (2002) 1297.
[179] D. Ambrosi, F. Mollica, J. Math. Biol. 48 (2004) 477.
[180] L. Preziosi, D. Ambrosi, C. Verdier, J. Theor. Biol. (2009), doi:10:1016/j.jtbi.2009.08.023, in press.
[181] G. Forgacs, R. Foty, Y. Shafrir, M. Steinberg, Biophys. J. 74 (1998) 2227.
[182] R.A. Foty, G. Forgacs, C.M. Pfleger, M.S. Steinberg, Phys. Rev. Lett. 72 (1994) 2298.
[183] R.A. Foty, G. Forgacs, C.M. Pfleger, M.S. Steinberg, Development 122 (1996) 1611.
[184] B.S. Winters, S.R. Shepard, R.A. Foty, Int. J. Cancer 114 (2005) 371.
[185] S. Astanin, K. Müller, J. Käs, J. Galle, L. Preziosi, (2009), submitted for publication.
[186] D. Ambrosi, F. Guana, Math. Mech. Solids 12 (2007) 319.
[187] A.D. Carlo, S. Quiligotti, Mech. Res. Commun. 29 (2002) 449.
[188] D. Ambrosi, K. Garikipati, H. Kuhl, Oberwolfach Report 5 (2009) 2220.
[189] E. Kuhl, A. Menzel, P. Steinmann, Comp. Mech. 32 (2003) 71.
[190] G. Himpel, E. Kuhl, A. Menzel, P. Steinmann, Comp. Meth. Eng. Sci. 8 (2005) 119.
[191] D. Ambrosi, A. Guillou, Cont. Mech. Thermodyn. 19 (2007) 245.
[192] M. Tringelova, P. Nardinocchi, L. Teresi, A. DiCarlo, in: B. Miara, G.E. Stavroulakis, V. Valente (Eds.), Topics on Mathematics for Smart Systems, World Scientific, 2007, pp. 253–270.
[193] M. Stolarska, Y.J. Kim, H. Othmer, Phil. Trans. Roy. Soc. A 367 (2009) 3525–3553.
[194] A. Iordan, Rheological properties of biological materials: From cell suspensions to tissues, Ph.D. thesis, Université Grenoble I, 2008.
[195] E. Kuhl, K. Garikipati, E. Arruda, K. Grosh, J. Mech. Phys. Solids 53 (2005) 1552.
[196] E. Kuhl, G.A. Holzapfel, J. Math. Sci. 42 (2007) 8811.
[197] D. Noble, Science 295 (5560) (2002) 1678.
[198] S.R. Reboux, G.W. Richardson, O.E. Jensen, Proc. Royal Soc. A 464 (2008) 447.
[199] J. Käfer, T. Hayashi, A.F.M. Marée, R.W. Carthew, F. Graner, Proc. Natl. Acad. Sci. USA 104 (47) (2007) 18549.
[200] D. Caillerie, A. Mourad, A. Raoult, ESAIM Math Mod. Num. Anal. 37 (4) (2003) 681.
[201] T.C. Doehring, M. Kahelin, I. Vesely, J. Biomech. Eng. 131 (2009) 061001.
[202] O. Chaudhuri, S.H. Parekh, W.A. Lam, D.A. Fletcher, Nat. Methods 6 (5) (2009) 383.
[203] R. Chotard-Ghodsnia, O. Haddad, A. Leyrat, A. Drochon, C. Verdier, A. Duperray, J. Biomech. 40 (2) (2007) 335.
[204] D.A. Lauffenburger, A.F. Horwitz, Cell 84 (3) (1996) 359.
[205] M.P. Sheetz, D. Felsenfeld, C.G. Galbraith, D. Choquet, Biochem. Soc. Symp. 65 (1999) 233.
[206] J. Condeelis, J.W. Pollard, Cell 124 (2) (2006) 263.
[207] T. Lämmermann, B.L. Bader, S.J. Monkley, T. Worbs, R. Wedlich-Söldner, K. Hirsch, M. Keller, R. Förster, D.R. Critchley, R. Fässler, M. Sixt, Nature 453 (7191) (2008) 51.
[208] P. Friedl, S. Borgmann, E.B. Bröcker, J. Leukoc. Biol. 70 (4) (2001) 491.
[209] P. Friedl, K. Wolf, Nature 3 (2003) 362.
[210] A. Chauvière, T. Hillen, L. Preziosi, Networks Heterog. Media 2 (2) (2007) 333.