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Mechanical Stability: A Construction Principle for Cells

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Abstract

The glassy wormlike chain model is a highly successful phenomenological model recently introduced to describe anomalously slow subdiffusive dynamics in biopolymer networks and living cells. Here we extend this model by proposing a generic scheme how to include nonlinear plastic effects by introducing the possibility of force-dependent opening and closing of internal bonds. Further, we discuss physiological implications of this bond kinetics. Stability arguments lead us to the postulation of a "physiological sheet" in the parameter space. This sheet defines the set of parameters characterizing cells which are flexible enough to perform biological tasks while still being able to bear external perturbations characteristic of their surroundings and their internally generated prestress without damage. At the end of this contribution, we speculate about the connection between prestress and cell stiffness and about the mechanism by which the cell adapts to its mechanical environment.

1 Introduction

To get a qualitative impression of the mechanical response of living tissue simply pinch your own cheeks. Only quite recently, it has become possible to track the characteristic mechanical properties of living tissue [1] down to the level of individual cells. Among the most salient reproducible features observed are a quite broad linear response followed by strain stiffening [2], slow anomalous dynamics and power-law rheology [3], inelastic creep [4], and a fluidization response to transient stretch [5]. With regard to the emergent character and the striking simplicity and universality of these patterns the usual biological approach — namely to examine the specific interactions of a large number of molecules communicating with each other via chemical signaling — appears somewhat inappropriate in this context. Many physicists believe instead that it makes more sense to search for an underlying mechanism able to generate all of the above mentioned properties in a natural way from a minimum number of generic principles. In particular, based

on a large body of data, Fabry, Fredberg and coworkers [3] have first argued that a generic energy landscape picture originally developed to account for the subdiffusive dynamics of inanimate soft glassy materials [6] might well provide such a powerful unifying principle able to explain many of the characteristic mechanical features of living cells [7]. In a similar spirit, the glassy wormlike chain (GWLC) model was recently proposed [8] as a possible ansatz to establish a more direct link between these observations and the underlying molecular structure of the cytoskeleton. It suggests that the wormlike chain (WLC), a generic polymer model that has been highly successful in the quantitative prediction of single molecule dynamics [9] might provide a key to predict the macroscopic dynamical properties of biopolymer networks[10] and whole cells [11] on the basis of certain generic features of the (effective) interactions between protein fibers in the cytoplasm.

In this article, we extend this approach somewhat further with the intension to account for the intrinsically inelastic (as opposed to merely viscoelastic) character of the mechanics of living cells and tissues. Inspired by the mentioned experimental observations, we introduce a simplistic model to condense what we consider the most salient generic features of the mutual interactions of cytoskeletal protein fibers into a small number of relevant parameters. Our model makes use of binding and unbinding events of internal bonds and thus bears some similarity to the two-spring model for focal adhesions [12, 13]. We identify constraints that should be obeyed by these parameters in physiologically functional "rest states", roughly defined in an operational way as states adherent cells attain under physiological conditions when no distinct external stimulus is present. A strong correlation between the parameters of the functional state and the cell's actively generated prestress is established. On the basis of our model we arrive at a simple functional expression for this correlation, which we expect to hold with great generality for adherent cells.

2 Generic Features of Cytoskeletal Interactions: A Simple Model

The effective interactions between cytoskeletal polymers just alluded to are largely unknown. Specializing our discussion to the most prominent stress bearing element, F-actin, one can say that fiber-fiber interactions *in vivo* are mediated by a large number of actin binding proteins, which comprise several types of reversible crosslinkers favoring different types of filament assembly [14, 15, 16] and molecular motors [17], which are able to strain or even move adjacent actin filaments on demand. Even pure actin solutions in physiological buffer turn sticky at low temperatures [10], let alone non-physiological conditions, under which probably all proteins can be forced to aggregate. It is crucial to realize that the cytoplasm of a living cell has to maintain a robust yet somewhat subtle balance between the mutual attractions and repulsions of typically thousands of different types of proteins that should neither undergo flocculation nor complete disintegration even under strongly varying ambient conditions. A plausible way to achieve such a "loosely condensed

state" might be by strongly anisotropic ("patchy") short ranged interactions. In situations where, as in the cytoplasm of a cell, a large number of dissolved protein fibers interact strongly, and one is interested in the overall mechanical behavior, it should then be possible to model the fiber-fiber interaction as (*i*) strongly repulsive below a hard core diameter representing the excluded volume, (*ii*) attractive just somewhat beyond that range in order to effectively represent the sticky patches, and (*iii*) repulsive at an even longer range to represent an overall electrostatic stabilization. Finally, any two filaments in the cytoskeleton are subject to confinement by a chemical potential effectively representing the mentioned loosely condensed state of the cytoplasm.

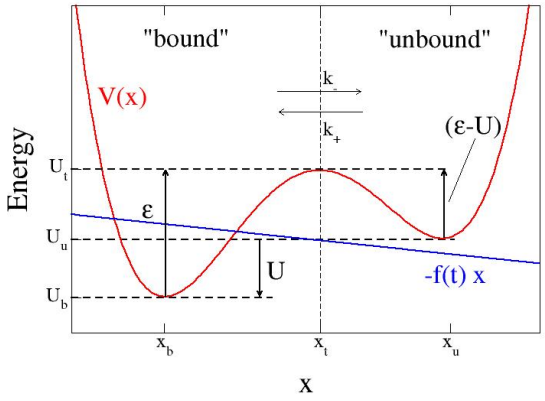

Figure 1: Schematic sketch of a hypothetical interaction potential argued to represent generic features of the effective interactions of cytoskeletal filaments (not to scale for better readability).

A sketch of a toy model of such an effective potential meant to describe the binding and unbinding of adjacent cytoskeletal fibers and including all of these features is shown in fig. 1. The height of the energy barrier is $\mathcal{E}k_BT$ and we included the possibility of a difference U in the binding energies of "bound" and "unbound" states. The positions of the corresponding energy minima are labeled by x_u and x_b, respectively, the position of the barrier maximum or transition state is referred to as x_b. At this point, it is not necessary to speculate about the appropriate values for these positions. The scales in the sketch were chosen for better readability and are not meant to mimic physical reality. In the following sections, we explore two major implications of such a generic interaction potential for the mechanical properties of the cell, namely the GWLC and the bond kinetics.

3 The Glassy Wormlike Chain

The viscoelastic dynamics of a single stiff biopolymer is very well described by the wormlike chain (WLC) model; for a recent review, see [9]. To account for the *effects of interactions between polymers onto the viscoelastic dynamics of a test polymer*, a simple phenomenological scheme has recently been proposed [8]. The basic idea is that the thermal undulations of the polymer backbone can relax freely if their wavelength is smaller than the mean distance Λ between adjacent bonds with the background network. This means that for the short wavelengths, the WLC applies. For the longer wavelength undulations to relax, a number of bonds proportional to the wavelength has to break open (and reform) first. The glassy wormlike chain (GWLC) model assigns an Arrhenius factor $\exp[\mathcal{E}(\lambda/\Lambda - 1)]$ to the corresponding increase in the bare WLC relaxation time. Bending undulations of wavelength $\lambda = 2\pi/q$ are thus assumed to relax with a characteristic time $\tau_q^{GWLC} = \tau_q \exp[\mathcal{E}(\lambda/\Lambda - 1)]$, where τ_q is the WLC relaxation time for a free polymer in pure solvent. This prescription gives rise to a viscoelastic relaxation and dynamic response that appears WLC-like for short times, but exhibits a strongly subdiffusive asymptotics for long times. This slowdown is not of the sort that could be subsumed into a simple rescaling of the time axis. It is much more extreme. In fact, as evident from the small apparent subdiffusive exponent [11], the whole relaxation is stretched such that the overall relaxation and dynamic response becomes essentially logarithmically slow (corresponding to an exponentiated time axis).

The GWLC has been shown to capture very well a large variety of experimental observations such as the dynamic structure factor of pure actin solutions [10] and the apparent power-law rheology of adherent cells [11]. Both of these observations concern the anomalous Browninan dynamics in the *linear* response regime. However, there are some further consequences of the hypothetic interaction potential discussed above, which have so far not been exploited in the GWLC model, and which we expect to become highly relevant for the mechanical and dynamical response of stiff polymer networks and the cytoskeleton of living cells if pushed into the regime of *nonlinear* response. Not only does one have to extend the discussion of the viscoelastic dynamics within GWLC to strong forces, then [10, 11]. One also needs to take into account the *bond breaking kinetics under mechanical load*, which will give rise to irreversible *inelastic or plastic deformations* (as opposed to purely viscoelastic deformations), which have indeed lately been shown to play a crucial role in single cell mechanics [18, 4]. This will be addressed in the following section.

4 Bond Kinetics under Load

To simplify the following discussion, we refrain, for the moment, from giving a formulation of the full problem including the polymeric nature of the cytoskeletal filaments, but focus on the crucial bond formation and bond breaking mechanisms alone. To this end, we turn back to the simple potential model outlined in Sec. 2.

Of course, to eventually arrive at a complete faithful description of the mechanical behavior of cytoskeletal filament networks and whole cells, the inelastic bond breaking dynamics will have to be integrated with the viscoelastic aspects contained in the GWLC model as well as with some aspects of network geometry [19] into a somewhat cumbersome formalism. Yet, some key elements of the inelastic response of such a "complete" mechanical minimal model can already be delineated by studying the bond formation process in isolation, which is the strategy pursued in the remainder.

To start with, consider, in a mean-field approximation, the potential sketched in Fig. 1 as the energy landscape explored by an individual test polymer. In the same spirit as in the conventional tube model [20] or in the GWLC [8], the other polymers are meant to be subsumed into the mean-field potential. We now make use of this assumption, and imagine our energy landscape to be populated by an *ensemble* of bonds, the fractions ν_b and ν_u of which are in the bound and unbound state, respectively. Assuming that the cell is not currently undergoing a remodeling phase, both fractions are connected by a conservation equation

$$\nu_\mathrm{b} + \nu_\mathrm{u} = 1, \tag{1}$$

dictated by the overall network geometry (or, equivalently, by the patchy character of the sticky interactions mentioned above). Therefore, we will restrict ourselves to the discussion of one variable $\nu \equiv \nu_\mathrm{b}$, the relative occupation of the bound state. This is obviously a crucial parameter for the elasticity of the network. If virtually no bond is formed, the network will turn into a very weakly interacting viscous and stress softening filament solution [19, 20]. If, on the other hand, all possible bonds are formed, it turns into an effectively elastic and strongly stress stiffening network [21] with little flexibility and a minimum of viscous creep.

A single polymer intersection can switch between the bound and unbound state with transition rates k_+ and k_-. Assuming a simple first-order kinetics, these rates determine the steady-state value

$$\nu^* = \frac{k_+}{k_- + k_+}. \tag{2}$$

of ν. Apart from the dependence on the potential parameters already introduced, the rates will be sensitive to any internal or external forces present. Such forces might be generated by molecular motors, polymerization forces, or induced by external mechanical deformations of the cell. One can imagine various ways of how the force can influence the potential. Here, we choose the simplest and most commonly assumed possibility[22], namely a tilting of the energy landscape. Furthermore, we assume that the force favors unbinding, i.e. it tilts the potential in the mathematically negative sense of rotation. For sufficiently slowly changing forces, we can use Kramers' escape theory to express the rates as

$$k_+ = \tau_0^{-1} \exp\left[-(\mathcal{E} - U + f(x_\mathrm{u} - x_\mathrm{t})/kT\right] \tag{3}$$

and

$$k_- = \tau_0^{-1} \exp\left[-(\mathcal{E} - f(x_\mathrm{t} - x_\mathrm{b})/kT\right]. \tag{4}$$

The steady-state value of the occupation density (eq. (2)) now reads

$$\nu^* = \frac{1}{1 + \exp\left[-(U - (x_\mathrm{u} - x_\mathrm{b})f)/kT\right]} \tag{5}$$

The overall time constant τ_0 will depend on the local diffusion constant and on the particular shape of the potential in the minimum. For simplicity, we assumed the same constant τ_0 for both states.

We are aware of the fact that the applied force also may change the coordinates x_b, x_t, and x_u [23], which gives rise to a more complicated force dependence. The essence of our argument would not be affected, however, so that we stick to the above simple expressions. All that is said in the following could easily be adapted to more general force dependencies.

5 The Physiological Sheet of Mechanical Stability

In the preceding section, we have introduced a simplistic effective description of a generic physical structure that we argue to be highly relevant to cell mechanics, in particular to reversible bond formation between cytoskeletal filaments. The physical effect of mechanical force onto the bond formation was outlined. All the complicated specific interactions usually emphasized when characterizing biological processes have been neglected or were effectively subsumed into the shape of the potential sketched in fig. 1. Therefore, this description might so far also be valid for an inanimate network of sticky fibers (e.g. carbon nanotubes). In fact, as pointed out in the preceding section, we refrain from including the physical properties of *filamentous* networks in the discussion, at the present stage. Hence, the reader may well wonder what kind of useful predictions we may hope to make at all from such a simple model. We now want to exploit a very general feature of biological systems, namely that their design has been subject to natural selection. Evolution will select for the mechanical properties of a cell and its cytoskeleton such that they are capable of withstanding the stress actively generated by the cell itself — the so-called *prestress* [24] — and in its environment, e.g. by the surrounding tissue. At the same time the cell has to be flexible enough to be able to move and reshape (or even divide), and to maintain its connectivity with its possibly dynamic surroundings. For example, a lung cell has to sustain a persistent periodic stretch of up to ten percent without being damaged or otherwise impaired in its biological function, throughout its whole lifetime. In fact, most of our cells experience at least some mechanical repercussions of the pulsating blood flow in our veins. Translated into the language of our generic potential, this means that under physiological conditions, given a prestress f_0, the parameters U and $x_\mathrm{u} - x_\mathrm{b}$ should under most circumstances better take values such that the ensemble of bonds is neither completely trapped in

the unbound state (disintegration of the cell) nor completely trapped in the bound state (no plasticity or mobility). See fig. 2 for a schematic illustration. Now, this

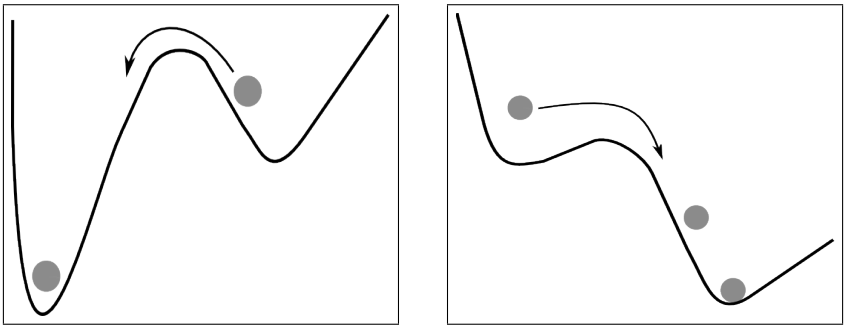

Figure 2: Improper dependence of the potential parameters on the prestress leads to a trapping of the ensemble either in the closed state (left panel) or in the open state (right panel).

implies that if a cell is in a functional rest state as introduced above, with parameters f_0, U_{f_0}, and $(x_\mathrm{u} - x_\mathrm{b})_{f_0}$, and another cell is functional under a different prestress f_1, we expect the parameters of the second cell have to obey the relation

$$U_{f_0} - (x_\mathrm{u} - x_\mathrm{b})_{f_0} f_0 \simeq U_{f_1} - (x_\mathrm{u} - x_\mathrm{b})_{f_1} f_1. \tag{6}$$

There are various ways to interpret this condition physiologically. In any case, we will arrive at the conclusion that the prestress is correlated with structural parameters in some way, after some major internal structural remodelling processes have come to a temporal rest (that might typically be stable for many minutes to hours), and to a lesser extent also during such processes. Let us consider the example where $(x_\mathrm{u} - x_\mathrm{b})$ is fixed by geometry. Then, U would have to be correlated with the rest prestress f_0 according to

$$U \propto f_0^\xi, \tag{7}$$

with $\xi \approx 1$. The larger the range of prestresses for which eq. (7) is supposed to be valid, the closer the value of the exponent ξ should be to one. If, on the other hand, U is for instance thought to be prescribed by the specific interaction enthalpy of some crosslinking molecule, the inverse relationship must hold for $(x_\mathrm{u} - x_\mathrm{b})$. If all parameters are variable, we can define a "physiological sheet" in our three-dimensional parameter space spanned by U_{f_0}, $(x_\mathrm{u} - x_\mathrm{b})_{f_0}$, and f_0 (see fig. 3). More generally, one will speak of a hypersurface in some d-dimensional reduced parameter space (d not necessarily equal to 3). Each point in this space represents a cell with the corresponding parameter values. Cells lying on the surface have parameter values "adapted to their prestress" in the above sense, i.e., they are functional and selected by evolution. A cut of the sheet along the U-axes gives a curve according

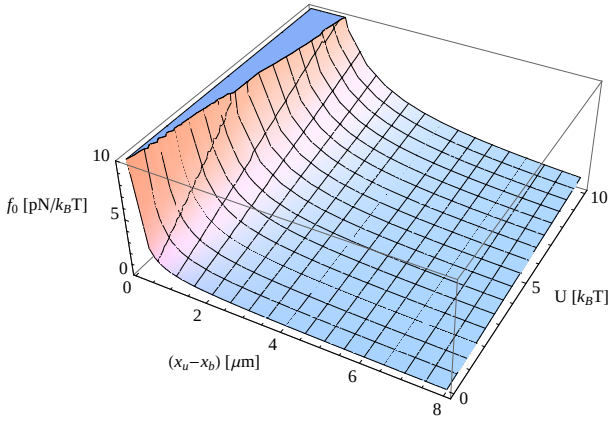

Figure 3: Physiological sheet in parameter space

to eq. (7), a cut along the $(x_\mathrm{u} - x_\mathrm{b})$-axes gives the prestress dependence for fixed U. Cells with rest parameters off the surface or cells that are less efficient in reaching the physiological sheet after a perturbation, are, within our simplified discussion, not well adapted to perform their biological function. These cells will have a higher probability to be evolutionary suppressed and should therefore less likely be found in living organisms. We thus expect results from experiments done under physiological conditions to cluster on the physiological sheet unless driven away from it by a sudden violent external stimulus.

As the parameters of our hypothetical potential are assumed to determine the macroscopic properties of the network, the existence of the physiological sheet will also lead to a dependence of the prestress on, e.g., the stiffness of the cell. To see this, consider the balancing condition eq. (6). From the above discussion we cannot expect this condition to be an exact equality: In the case of fixed prescribed $x_\mathrm{u} - x_\mathrm{b}$ eq. (7) holds, and the prestress has to grow approximately linear with U. The prefactor of this linear dependence, however, is not determined by our argument and in general the prestress dependence of U_{f_0} and $(x_\mathrm{u} - x_\mathrm{b})_{f_0} f_0$ need not cancel exactly. The consequence is that we have a relation between the steady-state occupation density (which should be positively correlated to the stiffness) and the prestress. The question whether increasing fraction of closed bonds ν^* correlates with increasing or decreasing prestress is readily decided by applying the definition of the physiological sheet: as the stability condition prohibits trapping in the completely disintegrated or unbound state, the bound state has to survive the lowering of the barrier by the prestress. Translated into the languague of our parameters, this minimum stability requirement means that $(x_\mathrm{u} - x_\mathrm{b}) f_0$ has to grow at least a little bit weaker than U. It can easily be checked in eq. (5) that this leads to an increase of ν^* with increasing prestress and vice versa. In other words, *a stiffening of the cell correlates with increasing prestress.*

To obtain the physiological sheet depicted in fig. 3, we used the particular force dependence introduced in eqs. (3) and (4). The argument of mechanical stability can of course be reiterated for other force dependencies, the shape of the physiological sheet will then be affected, however. To illustrate this effect, it may be useful to consider a more complicated form of the on and off rates. For example, one could imagine a case were the Kramers escape rate is amplified by a linearly position-dependent factor. Then, one might obtain for the steady-state occupation density

$$\nu^* = \frac{1}{1 + \left(1 - \frac{x_\mathrm{u} - x_\mathrm{b}}{x_\mathrm{u}}\right) \exp\left[-[U + f(x_\mathrm{u} - x_\mathrm{b})]/kT\right]}. \tag{8}$$

Obviously, this expression does now depend on the *absolute values* of x_b and x_u and not any more merely on their difference. The physiological sheet is now a three-dimensional hypersurface in a four-dimensional parameter space. The projection of this hypersurface on the U-$(x_\mathrm{u} - x_\mathrm{b})$-$f_0$ space for fixed f_0 is, apart from weak logarithmic corrections, identical to the physiological sheet for the simple force dependence. For even more complicated force dependencies, the relevant parameter space will be of even higher dimension. Also, the projection on the U-$(x_\mathrm{u} - x_\mathrm{b})$-$f_0$ space might generally differ more substantially.

6 Discussion

We have introduced a simple model potential to mimic generic aspects of the molecular interactions between filaments of the cytoskeleton, mainly with its most important stress bearing component, the actin cortex, in mind. After a brief review of the GWLC model that accounts for the *viscoelastic* dynamics of *in vitro* cytoskeletal model systems and even living cells with impressive precision, we proposed to extend this model in order to account also for *inelastic and plastic* processes. Our extension was based on the same schematic interaction potential that motivated the GWLC. We discussed the force dependence of the binding and unbinding rates of the bonds between a test polymer and a surrounding biopolymer network. We used a simple but plausible model for the force dependence of the transition rates. However, even for arbitrarily complicated force dependencies, the general picture should remain valid. Our discussion of forces and interactions, which were already hinted at in less detail in previous work ([25, 8]), suggested a *mechanical stability condition* of fully functional cells at rest. In brief, this condition states that in order to perform its tasks a cell should neither tear itself apart in a destructive way nor behave entirely rigid. We reiterate that our study needs to be completed by blending in the information that the bonds are formed between biopolymers or other fibrous substructures of the cytoskeleton, which could be accomplished by coupling it to the GWLC or even a more complete network model. This would provide a translation of our force parameter f_0 to the backbone tension in the filaments and eventually to the prestress, measured by external devices or inferred by cutting the cytoskeleton and monitoring the retraction [26].

Our discussion assigned a prominent role to the prestress, which is actively generated by the cell and which is assumed to co-evolve with the cytoskeleton during cell development and/or remodelling such as to balance the cell mechanically in (or close to) the physiological sheet at all times. According to our model, the prestress is firmly tied to the mechanical strength of the cell in agreement with experimental observations of a linear correlation of prestress and cell stiffness [27]. A possibly related observation is that the stiffness of the substrate on which the cells are cultured has a strong influence on the amount of internally generated stress [28]. There have been many speculations about the underlying mechanisms [29, 30]. In particular, it has been attributed to the physical conditions in the focal adhesions [31, 13], and it has been asked what might be the microscopic variable actually measured by the cell [32, 33, 34]. Two at the first glance very intuitive candidates for the measured variables are either external deformation [33] or force [34]. With regard to the strongly fluctuating cell strengths, sizes, and geometries, however, it is not clear at all how the cell should be able to measure absolute stresses or strains. The absolute values of these parameters may vary dramatically between cell types [35], throughout a single cell type [2], and even for a single cell that may considerably remodel its cytoskeleton in response to changing external stress or substrate stiffness [36].

From the perspective of the physiological sheet, an alternative interpretation emerges. Could the observed correlation between substrate stiffness, cell strength and prestress be the consequence of a very general and evolutionary strongly conserved optimization involving the physiological sheet? This would go along with a tendency of cells to share the task of shock absorption with the environment. To illustrate this idea and its relation to the concept of the physiological sheet, consider a soft cell (at low prestress, according to the physiological sheet), brought in contact with a stiff substrate. Due to the corresponding "fixed distance" boundary conditions imposed by adhesions on the stiff substrate, thermal or actively generated noise inside the cell as well as external strains translate into large force fluctuations. These have to be absorbed completely inside the cell, increasing the probability of harmful rupture events and loss of integrity. Note that such force fluctuations correspond to transient excursions from the physiological sheet. To counteract these excursions, the natural response of the cell might therefore well be to try and tame these force fluctuations by evolving its state *on the physiological sheet* away from the fixed-distance ensemble and towards a fixed-force ensemble with a *higher prestress and stiffness, but lower relative force fluctuations.* Thereby the cell would be able to trade potentially harmful stress spikes for hydrodynamically overdamped viscoelastic motion and inelastic deformations in the form of reversible bond breaking in a cytoskeletal network maintained at the edge of mobility. Internally, such a state change would involve a reinforcement of the cytoskeleton over timescales much slower than the force fluctuations. Externally it would manifest itself by a tendency towards mechanical impedance matching with the environment, increased prestress, and strong inelastic structural damping — all in agreement with the observations. Intriguingly, recent findings that stem cell fate is to a large extent governed by sub-

strate stiffness [37, 38] suggest that the general tendency of mechanical adaptation is a crucial facet of a much broader assimilation program.

Interestingly, recent studies of suspended cells that are prevented from adhering to a substrate have revealed an intriguing manifestation of what we think can be interpreted as the cell's active search for mechanical stability [39]. The detected highly reproducible spontaneous shape oscillations with periods on the order of a minute hint at a characteristic internal time scale of the internal machinery at work. These observations suggest that the overall regulation mechanism, by which a cell establishes the mechanical stability conditions summarized in the physiological sheet, may fail to reach a stable state once the cell is not able to attach to a substrate — i.e. in the absence of an external force to balance the internal prestress that is always generated to keep the cell at the edge of mobility.

7 Conclusions

In this contribution, we have tried to step back from a thorough and detailed description of the cell and have emphasized instead the broader context of its mechanical stability. Our line of arguments was centered around the fundamental observation that the cell is a plastic (rather than viscoelastic) body. Based on an intuitive qualitative minimal model of the fundamental inelastic mechanism at work in the cytoskeleton, we posed the question how the cell should tune important mechanical quantities in order to stay mechanically functional, which led to the concept of a "physiological sheet" in parameter space.

In particular, we concluded that the cell should exhibit a tendency of adapting its internal mechanical strength to that of the substrate. From our functional point of view, we claimed that the cell does neither want to achieve a particular stress nor a particular strain. The cell might rather aim at optimizing its internal shock absorption, where "internal" comprises the connections to any substrate. Based on the concept of the functional sheet, we pointed out a mechanism how the cell could become more resilient to potentially harmful force spikes of various origin, while maintaining its state at the edge of mobility, i.e., on the physiological sheet. To achieve this while adhering to a stiff substrate, the cell would naturally have to evolve its state on the physiological sheet by both reinforcing its cytoskeleton and increasing its prestress. It is an intriguing question, whether this scheme can be verified on a microscopic level in the living cell.

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