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## **Tensional Homeostasis at Different Length Scales**

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## Abstract

Tensional homeostasis is a phenomenon of fundamental importance in mechanobiology. It refers to the ability of organs, tissues, and cells to respond to external disturbances by maintaining a homeostatic (set point) level of mechanical stress (tension). It is well documented that breakdown in tensional homeostasis is the hallmark of progression of diseases, including cancer and atherosclerosis. In this review, we surveyed quantitative studies of tensional homeostasis with the goal of providing characterization of this phenomenon across a broad range of length scales, from the organ level to the subcellular level. We considered both static and dynamics approaches that have been used in studies of this phenomenon. Results that we found in the literature and that we obtained from our own investigations suggest that tensional homeostasis is an emergent phenomenon driven by collective rheostatic mechanisms associated with focal adhesions, and by a collective action of cells in multicellular forms, whose impact on tensional homeostasis is cell type-dependent and cell microenvironment-dependent. Additionally, the finding that cadherins, adhesion molecules that are important for formation of cell-cell junctions, promote tensional homeostasis even in single cells, demonstrates their relevance as a signaling moiety.

## 1 Introduction

### 1.1 The concept and a definition of tensional homeostasis

Tissues and cells exhibit the remarkable ability to adapt to changes in their mechanical environment. This allows them to maintain their mechanical stress (or tension) steady and stable, at a homeostatic (set point) level, which is essential for normal physiological functions and for protection against various diseases. This phenomenon is referred to as *tensional homeostasis*. Here we define tensional homeostasis as *the ability to maintain a consistent level of tension, with a low variability around a set point, across multiple length scales*.

### 1.2 Physiological background

Homeostasis is a fundamental concept in physiology, which refers to the ability of living systems to maintain stability of their internal environment (e.g., body temperature, blood pressure, blood sugar level, body's PH balance, water balance, etc.), and a breakdown of homeostasis is the hallmark of a disease progression. In the late 1800s, French physiologist Claude Bernard promulgated the idea of the consistency in the internal environment, or the *milieu intérieur*.<sup>1</sup> This idea refers to the interstitial fluid and its physiological capacity to ensure protective stability for the tissues and organs of multicellular organisms in response to disturbances from the environment. Bernard's work laid the groundwork for the development of the principle of homeostasis, the term coined by American physiologist Walter B. Cannon in the late 1920s. According to Cannon, homeostasis describes the "fairly constant or steady state, maintained in many aspects of the bodily economy even when they are beset by conditions tending to disturb them".<sup>2</sup> Cannon also referred to the ability of the body to maintain homeostasis as the "Wisdom of the Body".<sup>3</sup> In the early 2000s, this concept was extended to the

cellular level by American physiologist and bioengineer Shu Chien, who referred to the ability of cells to maintain homeostasis as the “Wisdom of the Cell”.<sup>4</sup>

In mechanobiology of cells, because of the pluripotent role of mechanical stress, homeostasis is associated with the cell’s ability to maintain its endogenous cytoskeletal mechanical tension stable. Brown and colleagues referred to this phenomenon as “tensional homeostasis”.<sup>5</sup> Banes and colleagues were among the first who recognized the importance of stable cytoskeletal tension for the cell’s normal physiological functions.<sup>6</sup> For example, maintenance of tensional homeostasis in the endothelium in the face of combined effects of shear flow and stretch of the blood vessel downregulates pro-inflammatory and proliferative pathways. A breakdown in endothelial homeostasis increases the risk of atherosclerosis.<sup>4</sup> In the epithelium, tensional homeostasis is maintained by “mechanoreciprocity” between cell-generated contractile forces and exogenous forces.<sup>7</sup> Loss of this mechanoreciprocity promotes progression of diseases, including cancer.<sup>7,8</sup>

The concept of homeostasis is closely associated with the ability of a living system to respond to external disturbances by maintaining a set point physiological value, using a negative feedback loop, around which the normal range fluctuates. A negative feedback loop is a reaction of a living system that causes a decrease in function in response to some kind of stimulus in order to maintain homeostasis (e.g., thermoregulation). While tensional homeostasis at the organ and tissue level is associated with a set point stress (e.g., in the vasculature, the stress in the vessel wall is determined by the normal blood flow and the normal blood pressure), tensional homeostasis of cells has neither a specific set point value of cytoskeletal tension, nor a specific “normal” range of tensional fluctuations. The reason is that the level of cell contractility varies among different cell types and among same cell types under different physiological and

pathophysiological conditions. Thus, it has been widely assumed that a good indicator of tensional homeostasis is the cell's ability to return to a basal tension in response to external disturbances.<sup>5,9-13</sup> The concept of homeostasis as the ability of a living system to maintain a set point physiological value has been challenged by evolutionary scientist John S. Torday, who argued that this idea represented a quasi-static point of view, which was merely concerned with maintaining the *status quo*, "like a household thermostat".<sup>14</sup> This investigator described homeostasis as a dynamic phenomenon, which was "constantly oscillating around a set-point, monitoring the cellular environment, always ready to reset itself, but also to provide the reference point for a change if necessary for survival in an ever-changing environment".<sup>14</sup> In accordance with this point of view, recent studies of tensional homeostasis have focused on the dynamic nature of the cytoskeletal tension.

It has been shown that cellular traction forces, which arise at the cell-substrate interface as a result of tension generated by the contractile cytoskeleton (CSK), exhibit erratic temporal fluctuations.<sup>15-18</sup> If the range of these fluctuations becomes large in comparison with the mean traction force, cells cannot maintain tensional homeostasis, like when fluctuations of body temperature exceed their normal physiological range the body cannot maintain its homeostatic temperature of 37°C. This fluctuating nature of cellular traction forces prompted us to define tensional homeostasis as the cell's ability to maintain a consistent level tension with a low level of temporal fluctuations.<sup>17,18</sup>

Tensional homeostasis has been studied in a variety of contexts. One group of studies has been focused on the conceptual understanding of mechanisms by which cells maintain tensional homeostasis,<sup>6,19-21</sup> and/or how breakdown of tensional homeostasis leads to progression of various diseases.<sup>4,7,8,22,23</sup> The other group of studies, which are quantitative in nature, have

been focused on two aspects of tensional homeostasis. One is the ability of cells to return to the baseline tension in response to external disturbances, such as mechanical stretch.<sup>5,9,-13,24,25</sup> The other is the ability of cells to attenuate temporal fluctuations of cytoskeletal tension.<sup>17,18,26-29</sup>

Based on the previous studies, one may say that tensional homeostasis is conceptually well understood, but not well characterized in a quantitative manner. For example, using a continuum mechanics analysis of experimental data from the literature, Humphrey concluded that tensional homeostasis spans multiple length scales, from the subcellular to the organ level, and multiple time scales, from minutes to months.<sup>20,21</sup> However, quantitative data in support of these conclusions are scarce. In this review, we surveyed quantitative studies of tensional homeostasis with the goal of providing characterization of this phenomenon across several length scales, including the organ and tissue level, multicellular forms, single cells, and the subcellular level. Results that we found in the literature and that we obtained from our own investigations suggest that tensional homeostasis is an emergent phenomenon, driven by collective rheostatic subcellular mechanisms and by a collective action of cells in multicellular forms. Importantly, the extent of the impact of those mechanisms appears to be cell type-dependent.

## **2 Metrics of tensional homeostasis**

Tension, in general, refers to both tensile forces, which are vectors, and tensile stresses, which are tensors. In order to quantitatively describe tensional homeostasis, scalar metrics of forces and stresses need to be defined. These scalar metrics may differ depending on the length scale, on the types of experimental techniques used, and whether they describe homeostasis under static or under dynamic conditions. We first describe how scalar metrics of tension are

used in static and dynamic studies of tensional homeostasis regardless of the length-scale and of the experimental techniques.

Let  $S(t)$  indicate a generic scalar metric of tension and its dependence on time ( $t$ ). If under static or quasi-static external disturbances (e.g., step stretch or slowly varying cyclic stretch)  $S(t)$  returns to its baseline value over time, this indicates tensional homeostasis (Fig. 1A).<sup>12,13</sup> If  $S(t)$  is innately dynamic (e.g., exhibits temporal fluctuations) and/or if dynamic external disturbances are applied (e.g., cyclic stretch), then some metric describing the extent of dynamic fluctuations of  $S(t)$  needs to be defined. The coefficient of variation ( $CV$ ) has been used to quantitate the extent of these fluctuations.<sup>18,27-29</sup> It is indicative of the extent of variability of  $S(t)$  relative to its time-average mean ( $\langle S \rangle$ ) (Fig. 1B). Mathematically,  $CV$  is defined as the standard deviation [ $\sigma(S)$ ] of  $S(t)$  divided by  $\langle S \rangle$ , i.e.,

$$CV = \frac{\sigma(S)}{\langle S \rangle}, \quad (1)$$

Based on our definition of tensional homeostasis, the smaller the value of  $CV$ , the closer is the system to the state of tensional homeostasis. Although this approach does not specify a threshold value of  $CV$  below which tensional homeostasis is achieved, it does permit quantitative comparison to determine how different factors, such as multicellularity, cell type, substrate stiffness, or applied stretch impact tensional homeostasis.

## 2.1 Metrics of tension at different length scales

At the organ and tissue levels, quantitative metrics of mechanical stress are determined based on externally applied loads and organ/tissue geometry using a macroscopic approach of continuum mechanics (e.g., solving the initial-boundary value problem).<sup>21,30</sup>



In tissue constructs, where many cells are seeded in a hydrogel, tension is generated within the gel by contractile cells which apply traction forces to the fibrous backbone of the gel. This tension can be measured at the construct boundary as a force whose magnitude is used as a scalar metric in the studies of tensional homeostasis.<sup>5,10,31</sup>

At the level of multicellular structures (e.g., monolayers, clusters of cells) and the single cell, quantitative scalar metrics of cytoskeletal tension are determined from measurements of traction forces that are applied to focal adhesions (FAs). All traction force vectors within a single cell or a cluster of cells define the traction vector field. Two commonly used scalar metrics of the traction field are the magnitude of the traction field ( $T$ ), defined as the sum of the magnitudes (norms) of all traction force vectors within a cluster at a given time. The other is the magnitude of the traction moment, or the contractile moment ( $M$ ). It is defined as the trace of the first moment tensor of the traction field and is equal to the sum of the dot products of the traction force vectors and the corresponding position vectors of the point of force application at a given time.<sup>32,33</sup> Note that  $M$  is directly proportional to the product of the mean normal cytoskeletal stress (i.e., mean tension) times the cell volume. To the extent that cell volume changes very little during the experiments,  $M$  is indicative of the cytoskeletal tension.

Since cytoskeletal stiffness ( $G$ ) is closely associated with cytoskeletal tension,<sup>34</sup> it has been used as a scalar metric of tension in studies of tensional homeostasis at the single cell level.<sup>9,35</sup> Note that cells have an intrinsic component of stiffness, which is not tension-dependent. However, it is a smaller fraction of the total stiffness.<sup>36</sup>

At the FA level, scalar metrics of tension are either the magnitude (norm) of the traction force vector ( $F$ ) acting on the FA, or the magnitude (norm) of the displacement vector ( $u$ ) of the FA (i.e., local deformation).

Our survey of studies of tensional homeostasis at different length scale begins at the organ and tissue level and moves down in scales. At the organ and tissue level, mechanical stresses are often described by mathematically transparent equations that provide an insight into how changes in organ/tissue geometry interplay with changes in external loading in order to maintain tensional homeostasis.

### 3. Tensional homeostasis in the vasculature

An example of tensional homeostasis at the organ-to-tissue level is maintenance of constant stress in the blood vessel wall. Here we adopt the analysis of Humphrey<sup>20,21</sup> to explain this phenomenon. Blood vessels are exposed to mechanical loading due to blood flow, including shearing and blood pressure, which itself varies temporally. As a result, two primary mechanical stresses acting on the vessel wall arise, include the flow-induced shear stress ( $\tau$ ), applied tangentially to the luminal surface of the vessel, and the pressure-induced hoop stress ( $\sigma_\theta$ ), applied in the circumferential direction, as shown in Fig. 2. Furthermore, as a result of blood vessel development, the axial stress ( $\sigma_z$ ) arises in the vessel and acts along its longitudinal axis, (Fig. 2). The mean values of these stresses are given by the following equations,<sup>20,21,30</sup>

$$a) \quad \tau = \frac{4\mu Q}{\pi R^3}; \quad b) \quad \sigma_\theta = \frac{p}{h/R}; \quad c) \quad \sigma_z = \frac{F_z}{2\pi R h(1+h/2R)}, \quad (2)$$

where  $\mu$  is the blood viscosity,  $Q$  is the mean flow rate,  $R$  is the luminal radius,  $p$  is the transmural pressure,  $h$  is the wall thickness, and  $F_z$  is the axial force. Quantitatively, both  $\sigma_\theta$  and  $\sigma_z$  are on the order of  $10^2$  kPa, whereas  $\tau$  is much smaller, order of 1.5 Pa. According to Eq. 2a, in order to maintain constant  $\tau$  in response to a change in  $Q$ ,  $R$  has to change in proportion to

$Q^{1/3}$ . According to Eq. 2b, in order to maintain constant  $\sigma_\theta$  in response to a change in  $p$  and no change in  $Q$ ,  $h$  has to change in proportion with  $p$ . These changes in  $R$  and  $h$ , which are governed by changes in  $Q$  and  $p$ , respectively, would also cause changes in  $\sigma_z$  (Eq. 2c). Thus, in order to maintain constant  $\sigma_z$ ,  $F_z$  has to change.

Experimental data have supported these theoretical predictions. First, Langille and colleagues found that in response to a decrease in  $Q$ , arteries initially (first 3 days) decrease their  $R$  due to vasoactivities and after that  $R$  changes via remodeling ( $\sim$  two weeks), thus maintaining constancy of  $\tau$ .<sup>37</sup> Second, the aortic media is composed of concentric layers of lamellar units (mostly elastin and collagen). In mammals, an increase in  $R$  also causes a proportional increase in the number of lamellar units and therefore, a proportional increase in  $h$ . However, tension per lamellar unit remains constant ( $\sim 2$  N/m on average), suggesting a constant  $\sigma_\theta$  during development.<sup>38,39</sup> Conversely, wall thinning has been observed in response to a drop in  $p$  caused by artery cuffing,<sup>40</sup> thus maintaining constancy of  $\sigma_\theta$ . Finally, constancy of  $\sigma_z$  is governed by  $F_z$ . This force is not measurable *in vivo*, but it is associated with the axial prestretch of the blood vessel, which is set during development and which is measurable after excision of the blood vessel. This prestretch varies among species<sup>30</sup> and along the vascular tree.<sup>41</sup>

Equations Eq. 2a-c describe idealized conditions of static equilibrium of a linearly elastic vessel wall, which does not interact with the surrounding perivascular tissue. In reality, however, the diameter of blood vessels changes during the cardiac cycle,<sup>42</sup> and the tissue is non-linear and inelastic.<sup>30,43</sup> Nevertheless, the above analysis provides a good insight into macroscopic mechanisms that govern tensional homeostasis in the blood vessel wall.

## Summary

Blood vessels maintain constant mechanical stress in the vessel wall in response to changes in blood flow and pressure by changing their luminal radius and their wall thickness through vasoactivity and tissue remodeling, as well as by developing longitudinal pre-strain. However, the macroscopic approach used in the above analysis does not tell us how mechanics of cells associated with blood vessels (e.g., endothelial cells, smooth muscle cells, and fibroblasts) contribute to tensional homeostasis of the vessel. In order to get insight into the contribution of cellular mechanics to tensional homeostasis of tissues, we next discuss stability of mechanical stress in tissue constructs in response to external stretch.

## 4. Tensional homeostasis of tissue constructs subjected to quasi-static stretch

Tissue constructs are made of three dimensional hydrogel blocks seeded with a large number of cells. Those cells do not establish cell-cell contacts, rather they form FAs almost exclusively with the surrounding hydrogel. The contractile forces generated by the cells are transmitted via FAs to the hydrogel block causing it to shrink. If the shrinkage is opposed by applying physical constraints to the construct's boundaries, tension (prestress) is developed within the construct. This tension is applied to the physical constraints at the boundaries, where it can be measured (Fig. 3).

Delvoye and colleagues first demonstrated that *in vitro* collagen tissue constructs seeded with living cells (human or calf skin fibroblasts) actively maintain a steady level of contractile force developed in the constructs.<sup>31</sup> The investigators placed those constructs into a device for measuring tension developed by the contractile fibroblasts. After tension reached a plateau, they

increased/decreased it by increasing/decreasing the length of the construct. They observed that the constructs restored the original level of contractile tension in the gel within 1 h. However, their results were interpreted merely as a viscoelastic relaxation of the constructs.

Seven years later, Brown and colleagues reported a similar study where they used collagen gel-fibroblast constructs (human dermal fibroblasts) to investigate the effect of tensional load application on fibroblast-generated tension.<sup>5</sup> They applied a quasi-static cyclic uniaxial stretch (15 min loading-15 min rest-15 min unloading-15 min rest) to the construct and measured the force response, using their own tensioning-culture force monitor device.<sup>44</sup> During the rest period following loading, tension within the construct decreased, whereas during the rest period following unloading, tension increased (Fig. 4A). To rule out the possibility that viscoelastic relaxation of the collagen gel was responsible for the observed change in tension in the constructs, the investigators performed control experiments on collagen gels without cells. They found that the tension in the cell-free gel did not change systematically during the rest following loading and during the rest following unloading (Fig. 4B). Consequently, they concluded that the observed changes in tension in cell-seeded gels were almost entirely due to cells themselves. Based on those observations, the investigators hypothesized that the fibroblasts in the gel were modulating their contractility to counteract the applied load such to maintain tension within the gel at a baseline level. They coined the term tensional homeostasis to describe this phenomenon.

Ezra and co-workers used the same approach as Brown and colleagues to study tensional homeostasis in healthy tarsal plate fibroblasts and in tarsal plate fibroblasts harvested from patients with the floppy eyelid syndrome (FES).<sup>10</sup> Their results indicated that constructs with both healthy and FES cells were capable of returning to their baseline tension after application of

external stretch, which is the hallmark prediction of tensional homeostasis. Interestingly, the set point tension in the FES constructs was elevated relative to the healthy ones by nearly five times. Together, these findings suggest that FES is not associated with a breakdown of tensional homeostasis, like in some other diseases. Instead, FES fibroblasts are capable of adapting in order to continue maintaining tensional homeostasis, but around a new set point tension.

Based on their findings, Brown and colleagues raised a question: what mechanisms allow cells to recover their baseline tension relatively quickly in response to applied stretch?<sup>5</sup> The investigators ruled out the possibility that viscoelastic relaxation of the cells drives stress relaxation of the construct, claiming that the bidirectional nature of tension recovery (in response to loading and in response to unloading) was not consistent with viscoelasticity. (While this argument is not quite correct, the viscoelastic relaxation of the cells was probably not very prominent, considering the quasi-static nature of the experiments.) Instead, the investigators speculated that the driving mechanism of tensional homeostasis might be an interplay that includes mechanical balance between intracellular forces, borne by the CSK, and extracellular traction forces, borne by the extracellular matrix (ECM), on one hand, and assembly and disassembly of cytoskeletal biopolymers, on the other hand. Here we interpret their argument using the cellular tensegrity model.

According to the cellular tensegrity model, cytoskeletal contractile (tensile) forces borne by actin filaments are resisted partly internally, by compression-bearing microtubules, and partly externally, by traction forces, applied to the ECM via FAs, as shown schematically in Fig. 5.<sup>45</sup> During stretching of the ECM (Fig. 5A), microtubules are relieved from compression causing a transfer of the portion of cell's contractile forces that was balanced by microtubules to the ECM and thus, traction forces increase. In the absence of compression, microtubules polymerize,

grow in length, and eventually reengage in the force balance, causing thereby traction forces to decrease (negative feedback). During unloading (Fig. 5B), compression in microtubules increases and consequently the contribution of traction forces in balancing contractile forces in the actin filaments decreases. Increased compression on microtubules causes their buckling and disassembly, which results in a transfer of contractile forces to the ECM and consequently an increase in traction forces (negative feedback). While this explanation appears plausible, it also retains a possibility that during loading-unloading of the collagen gel constructs cytoskeletal tension of internalized fibroblasts may not have changed and that the changes in traction forces, which define tension in the collagen gel matrix, may have resulted solely from stretch-unstretch-induced assembly and disassembly of microtubules.

## Summary

Collagen gel constructs seeded with fibroblasts exhibited the tendency to return to the baseline tension in response to applied quasi-static stretch, which is the basic prediction of tensional homeostasis. Interestingly, this behavior is not affected by pathophysiological conditions such as FES, although the baseline tension is elevated. The observed ability of constructs to regain the baseline tension in response to sustained stretch cannot be explained by viscoelastic relaxation of either the collagen gel or of the internalized fibroblasts. Instead, it may reflect changes in the force balance between intracellular contractile forces and extracellular traction forces mediated by assembly and disassembly of compression-bearing microtubules.

In collagen gel constructs, embedded fibroblasts are isolated and do not interact with each other. In order to gain an insight into how cell-cell interactions may affect tensional homeostasis, we next discuss studies of tensional homeostasis of confluent multicellular clusters.

## 5 Attenuation of tensional fluctuations in multicellular clusters

Cellular traction forces exhibit erratic temporal fluctuations.<sup>15-18</sup> These fluctuations presumably originate from the dynamics of the contractile machinery of the CSK and/or from the dynamics of FAs. Consequently, the traction field of the cell fluctuates with time. For achieving and maintaining tensional homeostasis, these fluctuations should not become excessive and therefore, mechanisms that attenuate traction fluctuations need to be understood.

One way that cells may attenuate fluctuations of traction forces is if they are organized in multicellular clusters, where the number ( $N$ ) of traction forces (i.e., the number of FAs) is greater than in single cells. Based on the central limit theorem, increasing  $N$  would lead to attenuation of temporal fluctuations of the traction field, providing that traction forces are independent of each other.

To study the effect of multicellularity on attenuation of traction field fluctuations, we used micropatterned traction microscopy.<sup>17,18</sup> Traction forces were measured in cells plated on soft polyacrylamide gels whose apical surface was micropatterned by a regular array of fibronectin dots. Those dots were loci where cells form FAs. By observing displacements of the dots in response to cell contraction and from known elastic properties of the gel, we could compute traction forces applied to individual FAs.<sup>32,46</sup> Measurements were carried out in single cells and in confluent multicellular clusters of either bovine aortic endothelial cells (BAECs), mouse embryonic fibroblasts (MEFs), or bovine vascular smooth muscle cells (BVSMCs), for 2 h, with a 5 min sampling rate.<sup>17,18</sup> No external forces were applied. We used the magnitude of the traction field,  $T(t)$ , as a scalar metric and obtained the corresponding coefficient of variation,  $CV_T$  according to Eq. 1.



In BAECs,  $CV_T$  decreased with increasing  $N$ , whereas in MEFs,  $CV_T$  exhibited no significant changes with increasing  $N$ .<sup>27</sup> In BVSMCs,  $CV_T$  did not change significantly with increasing  $N$  below  $N \approx 80$ , and precipitously decreased for  $N > 80$  (Fig. 6). Since the observed  $CV_T$  vs.  $N$  relationships were qualitatively different between the cell types, they could not be explained by the central limit theorem. Thus, we provided an alternative explanation.

From the definition of the coefficient of the coefficient of variation (Eq. 1), it follows that

$$CV_T = \frac{\sqrt{\sum_{i=1}^N \sigma^2(F_i) + \sum_{i \neq j=1}^{N \times (N-1)} \text{cov}(F_i, F_j)}}{\sum_{i=1}^{N_{FA}} \langle F_i \rangle}, \quad (3)$$

where  $F_i(t)$  indicates a traction force applied to the  $i$ -th FA,  $\sigma^2(F_i)$  is its variance, and  $\text{cov}(F_i, F_j)$  is the covariance between a pair of distinct traction forces.<sup>27</sup> The covariance  $\text{cov}(F_i, F_j)$  is indicative of temporal correlation between pairs of traction forces. If forces were not correlated, then the covariance term in Eq. 3 is zero, and the central limit theorem holds. If they were positively or negatively correlated, then the covariance term is positive or negative, respectively, and the central limit theorem does not apply.

Our correlation analysis on living cells indicated that traction forces in single cells and in clusters of cells were positively correlated. In BAECs, the normalized global correlation coefficient (i.e., the average Pearson's correlation coefficient of each pair of forces among  $N$  forces) decreased with increasing  $N$ , whereas in MEFs, the correlation was weaker and changed little with increasing  $N$  (Fig. 6).<sup>27</sup> These results were consistent with the observed  $CV_T$  vs.  $N$  relationships of these two cell types. The mechanistic underpinning of the difference in the correlation between traction forces in BAECs and MEFs is not well understood.

From a physiological point of view, the observed difference in the behaviors of BAECs and BVSMCs on one hand, and MEFs on the other hand, appear reasonable. In the case of BAECs and in BVSMCs, clustering would be favorable for achieving tensional homeostasis since these cells *in vivo* form multicellular structures in blood vessels (the endothelial monolayer of BAECs and the medial layer of BVSMCs), where a very large number of cells (i.e., large  $N$ ) tends to maintain low traction field fluctuations. In MEFs, however, which exhibit relatively low traction field fluctuations in comparison with BAECs, cell clustering appears to have a little effect on tensional homeostasis. This, in turn, suggests that MEFs are capable of achieving tensional homeostasis at a single cell level, which is consistent with the fact that these cells *in vivo* do not form large clusters and monolayers.

### **5.1 The role of cell-cell adhesions on tensional homeostasis: the contribution of E-cadherin**

The observation that stabilization of tension fluctuations in BAECs can be achieved in multicellular clusters,<sup>17,18</sup> brings up a question about the impact of cell-cell interactions on tensional homeostasis. It has been reported that tension fluctuations in monolayers of endothelial cells were predictive of gap formation and loss of barrier function,<sup>47</sup> suggesting that these fluctuations need to be attenuated in order to maintain intercellular force transmission and therefore, tensional homeostasis in the monolayer. To explain how intercellular force transmission may impact tensional homeostasis in multicellular clusters, we consider a simple conceptual model.

Consider a one-dimensional array of mechanically jointed blocks (“cells”), representing a cluster of confluent cells, and of disjointed blocks, representing a cluster of non-confluent cells (Fig. 7). Traction forces are applied to each cell and at equilibrium all forces have to be

balanced.<sup>26</sup> In the jointed model, which allows cell-cell force transmission, traction forces are balanced at the whole cluster level, not at the single cell level. Any disturbance that will alter a traction force in an individual cell would be countered by changes of traction forces in the remaining cells in order to reestablish force balance and tensional homeostasis in the cluster. In the disjointed model, which does not allow cell-cell force transmission, traction forces have to be balanced at the single cell level. Thus, any disturbance of equilibrium in an individual cell will not affect equilibrium of the remaining cells and, hence, there will be no action of those cells to reestablish tensional homeostasis in the cluster.

Cadherins are cell-cell adhesion molecules that play a major role in cell-cell force transmission. They exist in many forms across cell types. These proteins are mechanosensitive, as they have both the ability to sense and adapt to changes in environmental forces and to be used as contractile force transducers between adjacent cells.<sup>48-51</sup> The main cadherin molecule expressed in the epithelium is E-cadherin. In epithelial cancers, E-cadherin is downregulated or completely lost.<sup>52,53</sup> This loss of E-cadherin has been often associated with the breakdown of tensional homeostasis in breast cancer.<sup>7,8</sup> On the other hand, it has been shown in healthy epithelial cells that E-cadherin is under constitutive tension even in the single cell state.<sup>54</sup> This, in turn, indicates that E-cadherin may not need cell-cell junctions to be mechanically coupled to the cellular contractile machinery. Therefore, E-cadherin may affect tensional homeostasis in epithelial cells even in the absence of adjacent cells. We studied the effect that the presence of functional E-cadherin in epithelial cells has on tensional homeostasis.

Using micropatterned traction microscopy, we measured temporal fluctuations of cellular traction forces in single cells and in clusters of gastric adenocarcinoma (AGS) cells stably expressing E-cadherin (E-cad cells) and cells without the molecule (Mock cells). Measurements

were carried out over 1 h, at a 5 min sampling rate.<sup>18</sup> We found that E-cadherin was an important regulator of tensional homeostasis, even in the absence of cadherin engagement with neighboring cells, which demonstrated its relevance not only as a structural molecule but also as a signaling moiety. In particular, we found that single AGS E-cad cells were more contractile (Fig. 8A) and exhibited significantly lower values of  $CV_T$  than single AGS Mock cells (Fig. 8B). These results suggest that E-cadherin may impact tensional homeostasis in single cells, although they do not experience cell-cell contact. We observed similar trends in cell clusters. The lower values of  $CV_T$  in E-cad single cells and clusters than in Mock single cells and clusters are the result of both lower variability of  $T$ , i.e., lower variance, and of greater contractility, i.e., greater magnitude of  $\langle T \rangle$ , in the former than in the latter. Thus, the presence of E-cadherin promotes attenuation of the traction field temporal fluctuations in AGS single cells and clusters and is therefore beneficial for homeostasis. Interestingly, in both E-cad and Mock cells,  $CV_T$  was  $\sim 1.5$  times lower in the clusters than in the single cells (Fig. 8B). This, in turn, suggests that cell-cell force transmission via E-cadherin may have a little impact on attenuation of traction field fluctuations in E-cad clusters, although intercellular force transmission is also possible through physical contacts of adjacent cells in both E-cad and Mock clusters. Instead, the lower values of  $CV_T$  in clusters than in single cells, in both E-cad and Mock cells, probably reflect a greater number of FAs in the clusters (like in BAECs).

Previous studies have shown that there is a cross-talk between cadherins and integrins as both molecules are coupled to the actin CSK. This crosstalk also affects tension generation in the CSK,<sup>55,56</sup> which could serve as a possible explanation for the differences in  $T$  of cells that express E-cadherin and those that do not.

## Summary

Attenuation of traction field temporal fluctuations may be achieved through cell clustering only in certain cell types, like in BAECs, BVSMCs, and AGS cells, and not in other cell types, like in MEFs. Temporal correlation between traction forces may affect the ability of multicellular clusters to attenuate traction field fluctuations. The presence of E-cadherin at the adherens junctions of AGS cells helps to attenuate tensional fluctuations even at the single cell level, without formation of cell-cell adherens junctions, indicating the relevance of E-cadherin as a signaling molecule for achieving tensional homeostasis.

The above studies describe attenuation of temporal fluctuations of the traction field of multicellular clusters in the absence of external disturbances. While understanding how clusters achieve tensional homeostasis in the absence of external perturbations is important, it is a fundamental and more compelling biological problem to understand how cells restore tensional homeostasis in response to external perturbations. This problem is discussed in the next section, where tensional homeostasis of single cells exposed to external stretch is described.

## 6 Tensional homeostasis of single cells

Studies of tensional homeostasis in single cells are centered around the application of various modes of mechanical stretch to the cell, with the aim of testing whether the cell can regain its baseline tension. It appears that the ability of the cell to regain the baseline tension depends on the mode of stretching. Application of uniaxial static stretch to the single cell leads to the phenomenon known as *tensional buffering*.<sup>12</sup> Tensional buffering is different from tensional homeostasis in the sense that, under applied stretch, tension relaxes to a stable, steady

state value which is greater than the baseline tension. On the other hand, in response to the equibiaxial static or transient stretch, cells are capable of recovering the baseline tension and therefore of achieving tensional homeostasis. Finally, in response to the application of periodic (dynamic) uniaxial stretch at physiological frequencies, cellular traction forces realign in the direction perpendicular to stretch axis and the baseline tension is regained. Below we discuss several studies that exemplify tensional buffering and tensional homeostasis under different stretching conditions and which utilize different experimental approaches.

### **6.1 Static uniaxial stretch of single cells**

Webster and colleagues studied tensional homeostasis in isolated NIH-3T3 fibroblasts during uniaxial stretch.<sup>12</sup> They applied stretch directly to the cell which was attached on one end to a fixed surface and on the other end to a flexible cantilever beam that was mounted on a feedback controlled atomic force microscope (Fig. 9A). As the cell spread, it exerted contractile force on the cantilever beam until it reached a steady set point value within ~40 min and then stayed constant (> 30 min). A uniaxial step displacement of 1  $\mu\text{m}$  was then applied to the cell via the cantilever beam and it was observed that the force rapidly relaxed and then stabilized at a value higher than the baseline value (Fig. 9B). When the same deformation was applied as a ramp displacement at a slow rate (0.1  $\mu\text{m}/\text{min}$ ), the baseline force did not change (Fig. 9C). When it was applied at a higher rate (1  $\mu\text{m}/\text{min}$ ), the force increased and stayed nearly constant after the ramp displacement was over (Fig. 9D). These responses did not depend on the stiffness of the cantilever beam and there were no modifications in the FAs in response to fast and slow rates of loading. However, overexpression of actin crosslinker  $\alpha$ -actinin resulted in a significant increase of a set point force after both slow and high rates of deformation. Taken together, these results indicated that fibroblasts did not conform to the tensional homeostasis hypothesis. Rather

than maintaining constant baseline tension, the cells altered tension in a deformation-rate-dependent manner. The authors referred to this phenomenon as “tensional buffering”. They suggested that it allowed cells to modulate their tension according to the rate of deformation that cell experience, like, for example, slow deformations during growth and fast deformations during injury.

Mizutani and co-workers also studied the response of single NIH-3T3 fibroblasts subjected to static uniaxial stretch (8% strain), that was applied to the cell via a flexible silicone membrane substrate.<sup>9</sup> As a quantitative metric of tension, they used cell stiffness,  $G(t)$ , measured by mechanical scanning probe microscopy. The investigators observed tensional buffering rather than tensional homeostasis, which was consistent with the observations of Webster and co-workers.<sup>12</sup> The stretch applied to the cells did not affect organization of cytoskeletal contractile actin stress fibers (SFs). When the investigators repeated the experiments in cells treated by a Rho-kinase inhibitor Y-27632 that abrogates contractile stress generation, they observed no changes in  $G(t)$  in response to applied stretch. Based on these observations, the investigators concluded that the changes of  $G(t)$  in response to substrate deformation were not controlled by cytoskeletal remodeling, but rather by tensile forces generated within the SFs.

## 6.2 Static and transient equibiaxial stretch of single cells

Weng and colleagues studied the response of different cell types (rat embryo fibroblasts, human skin fibroblasts, and human mesenchymal stem cells) to equibiaxial step stretch using stretchable micropost arrays (Fig. 10A,B).<sup>13</sup> These are arrays of identical flexible microposts that act as cantilever beams. The tips of the microposts are loci where cells form FAs. In response to applied traction forces, microposts bend and from the tip displacements and known elastic and geometrical properties of the microposts, the traction forces are calculated.

Stretchable micropost arrays allow measurements of changes of the traction forces in response to applied stretch. Simultaneously with the traction measurements, the investigators recorded clustering of yellow fluorescent protein paxillin, which is a component of the FA protein complex. They used the latter measurements as a quantitative metric of the FA size. They found that in response to the applied equibiaxial step stretch (8% strain), both the magnitude of the traction field,  $T(t)$ , and the additive size of all FAs of the single cells promptly increased and then slowly relaxed to their respective baseline values within 30 min of stretch application (Fig. 10C,D). However, on the level of individual FAs, they did not observe such behavior. Following the onset of stretch, some FAs exhibited relaxation and some reinforcement of the magnitude of their traction forces,  $F(t)$ , and of their size (Fig 10E,F). These observations suggest the absence of tensional homeostasis at the FA level. To determine how these heterogeneous, non-homeostatic responses of individual FAs might contribute to tensional homeostasis at the whole cell level, the investigators grouped individual FAs on the basis of their baseline traction force magnitudes. This analysis yielded a spectrum of graduated paths (“rheostasis”) of both FA traction forces and of FA sizes from which the tensional homeostasis of the whole cell emerged. Pharmacological perturbations of the CSK with drugs that either inhibit acto-myosin contractility or alter cytoskeletal lattice integrity, and with an antibody that molecularly modulated integrin catch-slip bonds biased the rheostasis and induced non-homeostasis of FAs, but not of cytoskeletal tension, suggesting a unique sensitivity of FAs in regulating homeostasis.

Trepat and co-workers measured changes in stiffness of isolated human airway smooth muscle cells in response to equibiaxial transient stretch (4 s duration) applied to the cell by a flexible substrate.<sup>35</sup> They measured cell stiffness,  $G(t)$ , using oscillatory magnetic twisting cytometry. The investigators observed that after stretch application, the  $G(t)$  promptly decreased



and then slowly recovered to its baseline value within  $\sim 16$  min. Interestingly, the rate of stiffness recovery increased with increasing strain amplitude (ranging from 2.5% to 10%). The investigators attributed the prompt decrease of  $G(t)$  in response to stretch to a phenomenon called fluidization of the CSK, which is presumably caused by a fast disassembly of the cytoskeletal contractile machinery. They attributed the gradual recovery of the baseline  $G(t)$  to a phenomenon called resolidification of the CSK, which is presumably caused by a slow reassembly of the contractile machinery. Fluidization and resolidification are phenomena that characterize rheology of soft glassy materials, which gained a great interest in cell biomechanics in the early 2000s.<sup>35,57-60</sup> Regardless of the underlying mechanisms, the study of Trepap and colleagues showed that in response to transient equibiaxial stretch, single airway smooth muscle cells were capable of regaining their baseline  $G(t)$  and thus achieving tensional homeostasis.

### **6.3 Periodic (dynamic) uniaxial stretch of single cells**

A ubiquitous phenomenon observed in various cell types is that in response to unidirectional sinusoidal stretch of the substrate, the cell body realigns globally, and the cytoskeletal SFs realign locally. If the stretching frequency is very low ( $\ll 1$  Hz), the realignment is along the direction of the stretch axis,<sup>44,61</sup> and if the stretching frequency falls in the range of pulsating heart, the realignment is away from the stretch axis.<sup>62-68</sup> The prevailing interpretation of these observations has been that the SF realignments reflect the cell's tendency to achieve tensional homeostasis. That is, SFs reorient along the direction where they are unaffected by the applied cyclic stretch and thus can maintain optimal (homeostatic) tensile force. A number of theoretical models were advanced to explain this phenomenon.<sup>11,24,25,69-73</sup> Below we discuss two of those models which were focused on tensional homeostasis.

De and colleagues modeled cells as contractile elastic dipoles (i.e., a pair of force vectors of the same magnitude, acting at different points, along the same line of action, in the opposite directions) that can change their contractility by reorganizing SFs and FAs in response to external forces.<sup>24,25</sup> They assumed that external forces are generated by stretching of the elastic substrate. In the absence of any stretch, the contractile dipole forces are resisted by traction forces and the cell is in the optimal (homeostatic) force condition. Any change of this condition caused by applied stretch forces would generate additional internal forces in SFs, which would have to adapt in order to maintain homeostatic level of force. At low stretch frequencies, cells have sufficient time to adapt their contractile SFs in order to maintain the homeostatic force condition. Consequently, the force dipole orients along the stretch axis. At high stretch frequencies, however, the force dipole cannot follow the quickly varying stretch forces to establish its set point. Consequently, the dipole tends to orient perpendicular to the stretch axis so that its forces remain unaffected by the stretch and thus maintains tensional homeostasis.

Kaunas and colleagues modeled a single SF as a series of identical sarcomeres. The SF is attached to the substrate at the endpoints.<sup>11</sup> Each sarcomere consists of individual actin-myosin units. The velocity of myosin shortening vs. force relationship is consistent with Hill's model. In the absence of stretch, the force in the SF is determined by its passive stiffness and the stall force of myosin units. When a low frequency cyclic stretch is applied to the substrate, the SF changes its unloaded reference length through myosin sliding to maintain tension at its baseline value. When higher stretch frequencies are applied, myosin cannot respond quickly enough and the SF behaves as an elastic spring. Thus, the SF must reorient away from the stretch axis to avoid cyclic variations in tension. This reorientation continues until the SF aligns perpendicularly to the stretch axis in order to achieve steady, homeostatic tension.

Despite the great interest in stretch-induced reorientation of cells and SFs, there were no experimental data showing that cells indeed achieve tensional homeostasis following reorientation. In 2012, Krishnan and colleagues studied the effect of periodic uniaxial stretch on the traction field reorientation of human umbilical vein endothelial cells (HUVECs).<sup>16</sup> Pure uniaxial stretch was applied to single cells cultured on polyacrylamide gels substrate by a parallel plate indentation. Trapezoid strain pulses ( $\sim 1$  s loading, 3 s hold,  $\sim 1$  s unload) of different amplitudes and at different frequencies were applied over 2 h. Traction forces were measured during unloading, by observing motion of microbeads embedded in the gel, using Fourier Transform Traction Cytometry.<sup>33</sup> The traction moment tensor was used as metric of the traction field. It was observed that when 10% strain pulses were applied at  $\sim 50$  s intervals, the traction field reoriented in the direction perpendicular to the stretch axis within 60 min and remained such thereafter (Fig. 11A). Although the cycling frequency in these experiments was low ( $\sim 0.02$  Hz), it has been shown that SF realignment is more responsive to the strain rate than to stretch frequency per se, and that trapezoid pulses can cause reorientation at frequencies as low as 0.01 Hz.<sup>74</sup> We have recently carried out a secondary analysis of these data in order to investigate whether the traction field fluctuations became attenuated as the traction field reoriented.<sup>28</sup> Results of our analysis are described below.

We computed the magnitude of the traction moment,  $M(t)$ , and the corresponding coefficient of variation,  $CV_M$ , during the first hour of stretching, when the traction field underwent reorientation, and during the second hour, when the traction field was completely aligned perpendicularly to the stretch axis and did not change its orientation anymore. We found that  $CV_M$  obtained during the first hour was significantly greater, by nearly a factor of two, than  $CV_M$  obtained during the second hour. Since traction forces are innately dynamic, they continued

to exhibit temporal fluctuations after their reorientation was completed. Importantly,  $CV_M$  obtained during the second hour was not significantly different from  $CV_M$  obtained in the absence of stretch, suggesting that once the reorientation was completed, the traction field attained its baseline state (Fig. 11B). The mean contractile moment  $\langle M \rangle$ , on the other hand, did not change significantly during this two time intervals (Fig. 11C). Therefore, according to Eq. 1, the lower value of  $CV_M$  during the second hour had to result from a decrease in the variance of  $M(t)$ . This decrease of the variance was presumably caused by reduced variability of traction forces as they realigned along the direction perpendicular to the stretch axis, where they were little affected by the cyclic stretch. Our analysis provided the first direct experimental evidence that stretch-induced traction field reorientation was associated with the cell's tendency to achieve tensional homeostasis.

## Summary

Single cells do not necessarily achieve homeostasis in response to applied stretch. The type of stretch (uniaxial vs. biaxial) and the mode of stretch (step, transient, ramp, oscillatory) play a role in the cell's ability to regain the baseline tension following the stretch application. In response to the equibiaxial step and transient stretch, cytoskeletal tension returns to its baseline, pre-stretch value (tensional homeostasis), whereas under the uniaxial step and ramp stretch it does not (tensional buffering). In response to the uniaxial periodic stretch, the cell can achieve tensional homeostasis as a result of reorientation of its traction forces in the direction perpendicular to the stretch axis. There is no mechanistic explanation for the difference in the cellular responses to the static uniaxial vs. equibiaxial stretch. A possible reason may be that under uniaxial static stretch, contractile SFs would tend to align in the direction of stretch, thereby increasing the set point tension in this direction. Under the equibiaxial static stretch, SFs

would not realign, and thus the set point tension would not change following the stretch application. From the mechanistic point of view, the ability of the cell to achieve homeostasis under static equibiaxial stretch appears to be an emergent phenomenon driven by graduated dynamics (rheostasis) of traction forces of individual FAs. The ability of the cell to achieve homeostasis under transient equibiaxial stretch, can be explained by fluidization and resolidification of its contractile machinery. The ability of the cell to achieve homeostasis under the uniaxial periodic stretch can be explained by realignment of its SFs in the direction perpendicular to the stretch axis.

The study of Weng and colleagues showed that the ability of single cells to achieve tensional homeostasis in response to a step stretch did not imply tensional homeostasis at the FA level.<sup>13</sup> In the next section, we discuss studies of static and dynamics aspects of tensional homeostasis of FAs in the absence of external disturbances.

## **7 Tensional homeostasis at the subcellular level**

Studies of tensional homeostasis at the subcellular level have been centered around homeostasis of individual FAs. Several independent studies reported that the magnitude of traction forces increased in a direct proportion with the size (i.e., projected area) of the corresponding FAs.<sup>75-77</sup> These observations have been cited by some investigators as an evidence of tensional homeostasis at the level of the FA.<sup>11,20,21,24,25</sup> They argued that the observed proportionality between the traction force and the FA area implied that the stress (i.e., force to area ratio) transmitted by the FA was maintained at a set point value (e.g., ~5.5 kPa for human foreskin fibroblasts), regardless of the force magnitude and the corresponding FA size.<sup>75</sup>

However, this view has been challenged by the following observations. First, small FAs ( $< 1 \mu\text{m}^2$ ) develop very large traction forces that do not correlate linearly with the FA size.<sup>76,77</sup> Second, in cells with large, “supermature” FAs that can transmit stresses which are several times higher ( $\sim 12$  kPa in REF-52 myofibroblasts) than forces transmitted by normal size FAs, traction force increases faster than linearly with increasing FA size.<sup>77</sup> Finally, a strong positive correlation between the FA size and the corresponding traction force exists only during maturation of FAs, whereas in mature FAs such correlation is not observed, suggesting that mature FAs may not transmit a constant stress.<sup>78</sup> We have already discussed in the previous section that during static equibiaxial static stretch, tensional homeostasis could not be achieved at the FA level, and that tensional homeostasis at the whole cell level emerged through rheostasis of traction forces of individual FAs.<sup>13</sup> Below we discuss the dynamic aspect of tensional homeostasis at the FA level.

### 7.1 Attenuation of temporal fluctuations of traction forces of individual focal adhesions

Our group was also interested in determining if temporal fluctuations of FA traction forces could be used to interpret features that lead to or limit tensional homeostasis. Using micropatterned traction microscopy, we obtained time lapses of FA traction forces in single BAECs and BVSMCs and in clusters of these cells, from which we calculated the coefficient of variation of FA traction forces ( $CV_F$ ) according to Eq. 1. We found that as the time-average force magnitude ( $\langle F \rangle$ ) increased,  $CV_F$  changed very little until  $\langle F \rangle$  reached a threshold value, after which  $CV_F$  rapidly decreased with further increase of  $\langle F \rangle$  (Fig. 12A).<sup>29</sup> This behavior has been observed in both single cells and in clusters (Fig. 12A) and did not change with the cell type (Fig. 12C). Changes of substrate stiffness between 3.6 to 30 kPa had a little effect on the shape of the observed  $CV_F$  vs.  $\langle F \rangle$  relationship, except that the curves was shifted to the right nearly in

proportion with the higher substrate stiffness (Fig. 12A). Since traction forces were calculated as the product of the substrate stiffness and the displacement of FAs caused by the corresponding traction force, we obtained that  $CV_F$  vs. the average FA displacement magnitude ( $\langle u \rangle$ ) relationships. We found that these relationships were little dependent on the substrate stiffnesses (Fig. 12B). The threshold of  $\langle u \rangle$  after which  $CV_F$  rapidly decreases was between 1 and 2  $\mu\text{m}$ . The rapid decrease of  $CV_F$  after the forces and displacements reached the threshold is not well understood. One possibility may be that after crossing the threshold, the dynamics of actin-myosin bonds<sup>79</sup> and/or of molecular clutches of FAs<sup>80-82</sup> slows down as forces/displacements approach the stalled limit.

The behavior observed at the single FA level was not observed at the whole cell and cluster level (Fig. 12D). In particular, we did not observe a threshold in the  $CV_T$  vs.  $\langle T \rangle$  relationship. This may be explained by several competing influences that simultaneously exist at the whole cell/cluster level. An increasing number of FAs causes an increase in  $\langle T \rangle$ , which causes  $CV_T$  to decrease (see Eq. 1). However, this decrease is impeded by a heterogeneous distribution of traction forces within cells/clusters. Forces of larger magnitudes are located primarily near the cell/cluster edges and forces of lower magnitudes are located primarily in the interior of the cell/cluster.<sup>16,17,33,46,75,83-85</sup> With increasing cluster size, the number of FAs at the cluster edge would decrease relative to the number of FAs in the cluster interior (i.e., with increasing cluster size the cluster circumference grows slower than its projected area). Thus, one would expect that the number of forces of lower magnitudes would increase relative to the number of forces of greater magnitudes. Since forces of lower magnitudes exhibit greater values of  $CV_F$  than forces of greater magnitudes, one would expect that  $CV_T$  would increase with increasing number of FAs. Finally, a positive correlation between traction forces enhance  $CV_T$ .

Together, these competing influences that exist at the cell/cluster level suggest that  $CV_T$  is emergent property that result from the FA dynamics. This appears to be consistent with the concept of rheostasis proposed by Weng and colleagues, where rheostasis at the level of FAs drives homeostasis of the whole cell.<sup>13</sup>

The most intriguing aspect of the above results is that that the substrate displacement is a unifying feature that determines FA tensional stability. Beyond 1  $\mu\text{m}$  of substrate displacement, FAs, regardless of cell type, whether associated with single cells or cell clusters, or substrate stiffness, exhibited a precipitous drop of  $CV_F$ . Traction force also exhibited a threshold in  $\langle F \rangle$  below which  $CV_F$  was nearly constant and above which it rapidly decreased, although this threshold value increased with increasing substrate stiffness. These results suggest that at the FA level, tensional homeostasis may be determined by a set point displacement rather than a set point force. These findings may be consistent with the results of LaCroix and colleagues, who developed tension sensors for FA protein vinculin that required different force magnitudes to achieve the same level of extension. Using these vinculin sensors, these investigators demonstrated that highly loaded FAs on the periphery of MEFs exhibited conserved gradients of vinculin extension, rather than force.<sup>86</sup>

Temporal fluctuations of forces or displacements of individual FAs did not appear to depend on the substrate stiffness and on the cell type. These findings suggest that cells use FAs to sense stiffness of their microenvironment in a manner that is independent of the cell type, substrate stiffness, and whether cells are isolated or organized into multicellular forms. Thus, in order to achieve and sustain tensional homeostasis in a microenvironment, cells also need to serve as mechanosensors.<sup>87</sup> Indeed, some investigators have associated tensional homeostasis with the cell's ability to sense rigidity of its microenvironment.<sup>7,8,23,88</sup> Since rigidity sensing is



closely associated with the level of cell contractility, it can be used to describe the ability of a cell to adapt its contractile machinery to maintain tension on different stiffnesses.

The studies of tensional homeostasis at the FA level discussed in this section utilized measurements of displacement (deformation) of the FAs caused by the applied traction forces. These forces were then calculated from measured displacements using models from the theory of elasticity (e.g., in the case of the micropost arrays method, forces are calculated using the model of deflection of the cantilever beam due to the force applied at the tip; and in the case of micropatterned traction microscopy, forces are calculated from the model of deformation of an elastic half-space due to a point force acting on its apical surface). However, the FA is composed of nanometer-sized proteins and, as such, requires special tools for determination of forces across molecules. Forster Resonance Energy Transfer (FRET)-based tools have emerged that can indicate separation of fluorophores with high precision at the nanometer scale, and models of polymer elasticity along with experimental validation can be used to convert these separation values to force estimates on molecules.<sup>86</sup> The simplest model of a single FA may be an integrin linking the matrix to an accessory molecule such as talin, which is a multidomain protein that can link integrins via its N-terminal FERM domain to F-actin via its C-terminal rod domains.<sup>89</sup> Each link in this chain has been shown to bear mechanical load, including individual integrins,<sup>90,91</sup> talin,<sup>92,93</sup> and vinculin.<sup>94</sup> Although temporal variations in tension have not been quantified with these approaches, they do serve as a foundation for understanding the mechanobiology of FAs, which is necessary for a comprehensive understanding of tensional homeostasis.

## Summary

Tensional homeostasis does not necessarily exist at the level of individual FAs. At the whole cell level, tensional homeostasis emerges through graduated dynamics (rheostasis) of traction forces of individual FAs. The most striking feature of FA dynamics is that the substrate displacement resulting from FA traction forces is a unifying feature that determines FA tensional stability. Beyond approximately 1  $\mu\text{m}$  of substrate displacement, FAs exhibit a precipitous drop in temporal fluctuations of traction forces, regardless of cell type, single cell or cluster of cells, or of substrate stiffness. This threshold, however, disappears at the whole cell/cluster level because traction force distribution is heterogeneous. These findings suggest that traction force dynamics collectively determines whether cells or cell clusters develop tensional homeostasis.

## 8 Conclusions

In this article, we presented a critical review of quantitative studies of tensional homeostasis at different length scales. Major take-home messages of this survey are:

- tensional homeostasis is cell type-dependent;
- tensional homeostasis is length scale-dependent;
- tensional homeostasis is microenvironment-dependent;
- cadherins promote tensional homeostasis.

Tensional homeostasis is an emergent phenomenon across several length scales. At the whole cell level, it emerges through collective rheostatic subcellular mechanisms at the FA level. At the cell cluster level it emerges through increasing number of cells (i.e., increasing number of FAs) in the cluster. These are cell-type dependent processes as well as the length-scale

dependent processes. External disturbance from the cell's microenvironment also influence tensional homeostasis. In response to the applied step and transient equibiaxial stretch, cells are capable of regaining their baseline tension, whereas in response to the uniaxial step stretch they are not. Instead, they attain a higher-level tension – a phenomenon referred to as tensional buffering, rather than tensional homeostasis. On the other hand, in response to uniaxial periodic stretch, cell tend to achieve tensional homeostasis through reorientation their contractile SFs away from the stretch axis. While external stretch has a marked impact on tensional homeostasis, substrate rigidity seem to have a little effect on the ability of FAs to attenuate their traction force variability.

Except in few cases, we still know very little about biophysical mechanisms and molecular pathways that cells use to achieve and maintain tensional homeostasis. While we have identify some physical factors that affect homeostasis, like viscoelastic relaxation, cytoskeletal resolidification, number of FAs, correlation between traction forces, and the presence of cadherins, we do not understand their mechanistic underpinnings. Why does an increasing number of FAs promote homeostasis in some cell types and not in others? Why is correlation between traction forces greater in some cell types than in others? Why does uniaxial stretch lead to buffering, whereas equibiaxial stretch leads to homeostasis? By what mechanisms do cadherins promote homeostasis even in the absence of cell-cell junctions? All these questions need to be answered before we will be able to understand the role of tensional homeostasis in health and in diseases.

## **8.1 Outlook**

Future studies of homeostasis should be aimed at answering the above questions. For example, focused work will continue to interrogate the molecular mechanism of the acto-myosin

machinery that both allows fluctuations of traction forces, which are necessary for rigidity sensation, but also is equipped to maintain tension at a set level. Interestingly, the biophysical underpinnings of myosin, actin, and crosslinkers, such as  $\alpha$ -actinin, remain identical in each cell type. However, the intracellular architecture of these structures along with their relative amounts and expression levels likely have a dramatic impact on tension fluctuations at FAs. Indeed, the actin CSK is itself distributed among multiple networks in cells,<sup>95</sup> and these networks may be quite variable from one cell type to the next. In addition, non-covalent bonds that stabilize these tension-generating structures, as well as stabilize their adhesion to the surrounding extracellular matrix, have lifetimes defined by probability functions that could explain tension fluctuations, and this hypothesis has been explored in depth by Odde's group.<sup>80-82</sup>

In this article, we considered tensional homeostasis across a broad range of length scales. We did not consider how time scales affect tensional homeostasis. Some of the studies that we discussed indicated the importance of the time scale for tensional homeostasis. For example, the short-time response of blood vessels to a decrease in blood flow is governed by vasoactivities, whereas the long-long time response is governed by tissue remodeling.<sup>37</sup> Second, Webster and colleagues showed that stress recovery during uniaxial stretch of cell is rate of deformation-dependent.<sup>12</sup> Third, reorientation of SFs in response to uniaxial stretch depends on the frequency and on the rate of stretching.<sup>11,24,25,66,74</sup> The mere fact that most tissues and cells exhibit time-dependent viscoelastic or poroelastic behaviors strongly suggests that their response to external disturbances, and therefore their ability to achieve tensional homeostasis, are dependent on time scales over which their material responses are observed. However, many soft tissues<sup>96,97</sup> and cells<sup>57-60,98</sup> exhibit a ubiquitous power-law viscoelastic behavior, which is indicative of the timescale-independence, suggesting that tensional homeostasis may be also timescale-

independent. Thus, future studies need to address the issue of the effect of the time scale on the ability of living systems to achieve tensional homeostasis. For example, in our studies of temporal fluctuations of the traction field and of traction forces, we used a 5-min sampling rate. However, we do not know whether a change of the sampling rate may impact our conclusions regarding the cell's ability to attenuate temporal fluctuations of tractions.

We have considered two distinct aspects of tensional homeostasis; one is the ability of the cell to recover the baseline tension following external mechanical disturbances, and the other is the ability of the cell to attenuate temporal fluctuations of cytoskeletal tension. Future investigations should be directed towards understanding mechanisms that can explain how cells can maintain low level of temporal fluctuations around a stable set point tension. Merging these two aspects of tensional homeostasis may provide a unique insight into the fundamental mechanism of evolution – how homeostasis can act simultaneously as both a stabilizing agent and as the determining mechanism for evolutionary change.<sup>14</sup>

## **Conflicts of interest**

There is no conflict to declare.

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## References

1. C. Bernard, *Leçons sur les phénomènes de la vie communs aux animaux et aux végétaux*. (Deuxième édition conforme à la première édition de 1878) Paris, Librairie J.-B. Baillié et Fils, 1885.
2. W. B. Cannon, *Physiol. Rev.*, 1929, **9**, 399-431.
3. W. B. Cannon, *The Wisdom of the Body*, New York, Norton, 1932.
4. S. Chien, *Am. J. Physiol. - Heart Circ. Physiol.*, 2007, **292**, H1209–H1224.
5. R. A. Brown, R. Prajapati, D.A. McGrouther, I.V. Yannas, and M. Eastwood, *J. Cell. Physiol.*, 1998, **175**,323–332.
6. A. J. Banes, M. Tsuzaki, J. Yamamoto, T. Fischer, B. Brigman, T. Brown, and L. Miller, *Biochem. Cell Biol.*, 1995, **73**, 349-365.
7. D. T. Butcher, T. Alliston, and V. M. Weaver, *Nat. Rev. Cancer*, 2009, **9**, 108-122.
8. M. J. Paszek, N. Zahir, K. R. Johnson, J. N. Lakins, G. I. Rozenberg, A. Gefen, C. A. Reinhart-King, S. S. Margulies, M. Dembo, D. Boettiger, D. A. Hammer, and V. M. Weaver, *Cancer Cell*, 2005, **8**, 241-254.
9. T. Mizutani, H. Haga, and K. Kawabata, *Cell Motil. Cytoskeleton*, 2004, **59**, 242-248.
10. D. G. Ezra, J. S. Ellis, M. Beaconsfield, R. Collin, and M. Bailly, *IOVS*, 2010, **51**, 3853-3863.
11. R. Kaunas, H. J. Hsu, and S. Deguchi, *Cell Health Cytoskeleton*, 2011, **3**, 13-22.
12. K. D. Webster, W. P. Ng, and D. A. Fletcher, *Biophys. J.*, 2014, **107**, 146-155.

13. S. Weng, Y. Shao, W. Chen, and J. Fu, *Nat. Mater.*, 2016, **15**, 961-966.
14. J. S. Torday, *Biology*, 2015, **4**, 573-590.
15. S. V. Plotnikov, A. M. Pasapera, B. Sabass, B., and C. M. Waterman, *Cell*, 2012, **151**, 1513-1527.
16. R. Krishnan, E. P. Canović, A. L. Iordan, K. Rajendran, G. Manomohan, A. P. Pirentis, M. L. Smith, J. P. Butler, J. J. Fredberg, and D. Stamenović, *Am. J. Physiol. Cell Physiol.*, 2012, **303**, C368-C375.
17. E. P. Canović, A.J. Zollinger, S.N. Tam, M.L. Smith, and D. Stamenović, *Am. J. Physiol. Cell Physiol.*, 2016, **311**, C528-C535.
18. A. J. Zollinger, H. Xu, J. Figueiredo, J. Paredes, R. Seruca, D. Stamenović, and M. L. Smith, *Cell. Mol. Bioeng.*, 2018, **11**, 175-184.
19. G. Bazoni, and D. Dejana, *Physiol. Rev.*, 2004, **84**, 869-901.
20. J. D. Humphrey, *Hypertension*, 2008, **52**, 195-200.
21. J. D. Humphrey, *Cell Biochem. Biophys.*, 2008, **50**, 53-78.
22. P. P. Provenzano, and P. J. Keely, *J. Cell Sci.*, 2011, **124**, 1195-1205.
23. S. Sen, W. P. Ng, and S. Kumar, *J. R. Soc. Interface*, 2012, **9**, 1311-1317.
24. R. De, and S. A. Safran, *Phys. Rev. E*, 2009, **78**, 031923.
25. R. De, A. Zemel, and S. A. Safran, *Nat. Phys.*, 2007, **3**, 655-659.
26. S. N. Tam, M. L. Smith, and D. Stamenović, *Int. J. Numer. Meth. Biomed. Eng.*, 2017, **33**, e02801.

27. J. Li, P. E. Barbone, M. L. Smith, and D. Stamenović, *J. Biomech.*, 2020, **100**, 109588.
28. D. Stamenović, R. Krishnan, E. P. Canović, and M. L. Smith, *J. Biomech.*, 2020, **105**, 109770.
29. H. Xu, S. Donegan, J. M. Dreher, A. J. Stark, E. P. Canović, D. Stamenović, and M. L. Smith, *Acta Biomater.*, 2020, doi.org/10.1016/j.actbio.2020.06.043.
30. J. D. Humphrey, *Cardiovascular Solid Mechanics: Cells, Tissues, and Organs*, 2020, New York: Springer Verlag, 2002.
31. P. Delvoye, P. Wiliquet, J.-L. Leveque, B. V. Nusgens, and C. M. Lapiere, *J. Investig. Dermatol.*, 1991, **97**, 898–902.
32. S. R. Polio, K. E. Rothenberg, D. Stamenović, and M. L. Smith, *Acta Biomater.*, 2012, **8**, 82–88.
33. J. P. Butler, I. M. Tolić-Nørrelykke, B. Fabry, and J. J. Fredberg, *Am. J. Physiol. Cell Physiol.*, 2002, **282**, C595-C605.
34. N. Wang, I. M. Tolić-Nørrelykke, J. Chen S. M. Mijailovich, J. P. Butler, J. J. Fredberg, and D. Stamenović, *Am. J. Physiol. Cell Physiol.*, 2002, **282**, C606-C616.
35. X. Trepas, L. Deng, S. S. An, D. Navajas, D. J. Tschumperlin, W. T. Gerthoffer, J. P. Butler, and J. J. Fredberg, *Nature*, 2007, **447**, 592-595.
36. N. Rosenblatt, N., S. Hu, B. Suki, N. Wang, and D. Stamenović, *Ann. Biomed. Eng.*, 2007, **35**, 224-234.



37. B. L. Langille, M. P., Bendeck, and F. W. Keeley, *Am. J. Physiol. Heart Circ. Physiol.*, 1989, **256**, H931–H939.
38. H. Wolinsky, H., and S. Glagov, *Circ. Res.*, 1967, **20**, 99–111.
39. H. Wolinsky, *Circ. Res.*, 1970, **27**, 531–538.
40. I. M. Bayer, S. L. Adamson, and B. L. Langille, *Arterioscler. Thromb. Vasc. Biol.*, 1999, **19**, 1499–1505.
41. H. C. Han, and Y. C. Fung, *J. Biomech.*, 1995, **28**, 637–641.
42. H. C. Chen, V. Patel, J. Wiek, S. M. Rassam, and E. M. Kohner, *Eye*, 1994, **8**, 97-103.
43. Y. C. Fung, *Biomechanics: Mechanical Properties of Living Tissues* (2nd edn.). New York: Springer-Verlag, 1993
44. M. Eastwood, R. Porter, U. Khan, G. McGrouther and R. Brown, *J Cell. Physiol.*, 1996, **166**, 33–42.
45. D. Stamenović, and D. E. Ingber, *Soft Matter*, 2009, **5**, 1137 – 1145.
46. S. R. Polio, H. Parameswaran, E. P. Canović, C. M. Gaut, D. Aksyonova, D. Stamenović, and M. L. Smith, *Integr. Biol.*, 2014, **6**, 357-365.
47. E. T. Valent, G. P. van Nieuw Amerongen, V. W. M. van Hinsbergh, and P. L. Hordijk, *Exp. Cell Res.*, 2016, **347**, 161–170.
48. T. Lecuit, and A. S. Yap, *Nat. Cell Biol.*, 2015, **17**, 533-539.

49. C. Collins, A. K. Denisin, B. L. Pruitt, and W. J. Nelson, *Proc. Natl. Acad. Sci. USA*, 2017, **114**, E5835–E5844.
50. I. Muhamed, J. Wu, P., Sehgal, X. Kong, N. Wang, D. E. Leckband, *J. Cell. Sci.*, 2016, **129**, 1843–1854.
51. F. van Roy, and G. Berx. The cell-cell adhesion molecule E-cadherin. *Cell. Mol. Life Sci.*, 2008, **65**, 3756–3788.
52. J. Figueiredo, O. Söderberg, J. Simões-Correia, K. Grannas, G. Suriano, and R. Seruca, *Eur. J. Hum. Genet.*, 2013, **21**, 301–309.
53. B. Mayer, J. P. Johnson, F. Leidl, K. W. Jauch, M. M. Heiss, F. W. Schildberg, W. Birchmeier, and I. Funke, *Cancer Res.*, 1993, **53**, 1690–1695, 1993.
54. N. Borghi, M. Sorokina, O. G. Shcherbakova, W. I. Weis, B. L. Pruitt, W. J. Nelson, and A. R. Dunn. *Proc. Natl. Acad. Sci. USA*, **2012**, 109, 12568–12573, 2012.
55. C. M. Nelson, D. M. Pirone, J. L. Tan, and C. S. Chen, *Mol. Biol. Cell*, 2004, **15**, 2943–2953.
56. K. L. Mui, C. S. Chen, and R. K. Assoian, *J. Cell Sci.*, 2016, **129**, 1093–1100.
57. B. Fabry, G. N. Maksym, J. P. Butler, M. Glogauer, D. Navajas, and J. J. Fredberg, *Phys. Rev. Lett.*, 2001, **87**, 148102.
58. G. Lenormand, E. Millet, B. Fabry, J. P. Butler, and J. J. Fredberg, *J. R. Soc. Lond. Interface*, 2004, **1**, 91-97.
59. P. Bursac, G. Lenormand, B. Fabry, M. Oliver, D. A. Weitz, V. Viasnoff, J. P. Butler, and J. J. Fredberg, *Nat. Mater.*, 2005, **4**, 557-561.

60. L. Deng, X. Trepate, J. P. Butler, E. Millet, K. G. Morgan, D. A. Weitz, and J. J. Fredberg, *Nat. Mater.*, 2006, **5**, 636-640.
61. A. M. Collinsworth, C. E. Torgan, S. N. Nagda, R. J. Rajalingam, W. E. Kraus, and G. A. Truskey, *Cell Tissue Res.*, 2000, **302**, 243-251, 2000.
62. K. Hayakawa, A. Hosokawa, K. Yabusaki, and T. Obinata, *Zool. Sci.*, 2000, 17,617-624.
63. K. Hayakawa, K., N. Sato, and T. Obinata, *Exp. Cell Res.*, 2001, **68**, 104-114.
64. J. H.-C. Wang, P. Goldschmidt-Clermont, J. Wille, and F. C.-P. Yin, *J. Biomech.*, 2001, **34**, 1563-1572.
65. J. H.-C. Wang, P. Goldschmidt-Clermont, and F. C.-P. Yin, *Ann. Biomed. Eng.*, 2000, **28**, 1165-1171.
66. R. Kaunas, P. Nguyen, S. Usami, and S. Chien, *Proc. Natl. Acad. Sci. USA*, 2005, **102**, 15895-15900.
67. R. Kaunas, S. Usami, and S. Chien, *Cell. Signal.*, 2006, **18**, 1924-1931.
68. K. Kurpinski, J. Chu, K. Hashi, and S. Li, *Proc. Natl. Acad. Sci. USA*, 2006, **103**, 16095-16100.
69. J. H.-C. Wang, *J. Theor. Biol.*, 2000, **202**, 33-41.
70. K. A. Lazopoulos, and A. P. Pirentis. *Int. J. Solids Struct.*, 2007, **44**, 8285-8296.
71. Z. Wei, V. S. Deshpande, R. M. McMeeking, and A. G. Evans, *J. Biomech. Eng.*, 2008, **130**, 031009.

72. D. Stamenović, K. A. Lazopoulos, A. Pirentis, and B. Suki, *Cell. Mol. Bioeng.*, 2009, **2**, 475-485.
73. A. P. Pirentis, E. Peruski, A. L. Iordan, and D. Stamenović. *Cell. Mol. Bioeng.*, 2011, **4**: 67-80.
74. A. Tondon, H.-J. Hsu, and R. Kaunas, *J. Biomech.*, 2012, **45**, 728-735.
75. N. Q. Balaban, U. S. Schwartz, D. Riveline, P. Goichberg, G. Tzur, I. Sabanay, D. Mahalu, S. Safran, A. Bershadsky, L. Addadi, and B. Geiger, *Nat. Cell Biol.*, 2001, **3**, 466-472.
76. J. L. Tan, J. Tien, D. M. Pirone, D. S. Gray, K. Bhadriraju, and C. S. Chen, *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 1484-1489.
77. J. M. Goffin, P. Pittet, G. Csucs, J. W. Lussi, J.-J. Meister, and B. Hinz, *J. Cell Biol.*, 2006, **172**, 259-269.
78. J. Stricker, Y. Aratyn-Schaus, P. W. Oakes, and M. L. Gardel, *Biophys. J.*, 2011, **100**, 2883-2893.
79. H. Parameswaran, K. R. Lutchen, and B. Suki, *J. Appl. Physiol.*, 2014, **116**, 825-834.
80. C. E. Chan, and D. J. Odde, *Science*, 2008, **322**, 1687-1691.
81. B. L. Bangasser, and D. J. Odde, *Cell. Mol. Bioeng.*, 2013, **6**, 449-459.
82. B. L. Bangasser, S. S. Rosenfeld, and D. J. Odde, *Biophys. J.*, 2013, **105**, 581-592.
83. M. Dembo, and Y.-L. Wang, *Biophys. J.*, 1999, **76**, 2307-2316.

84. V. Maruthamuthu, B. Sabass, U. S. Schwarz, and M. L. Gardel, *Proc. Natl. Acad. Sci. USA*, 2011, **108**, 4708-4713.
85. A. F. Mertz, Y. Che, S. Banerjee, J. M. Goldstein, K. A. Rosowski, S. F. Revilla, C. M. Niessen, M. C. Marchetti, E. R. Dufresne, and V. Horsley, *Proc. Natl. Acad. Sci. USA*, 2013, **110**, 842-847.
86. A. S. LaCroix, K. E. Rothenberg, M. E. Berginski, A. N. Urs, and B. D. Hoffman, *Methods Cell Biol.*, 2015, **125**, 161-186.
87. B. Suki, H. Parameswaran, C. Alves, A. D. Araújo, and E. Bartolák-Suki. In: *Multi-scale Extracellular Matrix Mechanics and Mechanobiology*. Studies in Mechanobiology, Tissue Engineering and Biomaterials, vol. 23, ed. Y. Zhang, Springer, 2020, pp. 83-12.
88. S. Lin, M. C. Lampi, C. A. Reinhart-King, G. Tsui, J. Wang, C. A. Nelson, and L. Gu. *Biomech. Model. Mechanobiol.*, 2018, **17**, 951-959.
89. D. A. Calderwood, I. D. Campbell, and D. R. Critchley, *Nat. Rev. Mol. Cell Biol.*, 2013, **14**, 503–517.
90. M. Morimatsu, A. H. Mekhdjian, A. S. Adhikari, and A. R. Dunn, *Nano Lett.*, 2013, **13**, 3985–3989.
91. Y. Zhang, C. Ge, C. Zhu, and K. Salaita, *Nat. Commun.*, 2014, **5**, 5167.
92. A. Kumar, M. Ouyang, K. Van den Dries, E. J. McGhee, K. Tanaka, M. D. Anderson, A. Groisman, B. T. Goult, K. I. Anderson, and M. A. Schwartz, *J. Cell Biol.*, 2016, **213**, 371-383.

93. Kumar, K. L. Anderson, M. F. Swift, D. Hanein, N. Volkmann, and M. A. Schwartz, *Biophys. J.*, 2018, **115**, 1569-1579.
94. Grashoff, B. D. Hoffman, M. D. Brenner, R. Zhou, M. Parsons, M. T. Yang, M. A. McLean, S. G. Sligar, C. S. Chen, T. Ha, and M. A. Schwartz, *Nature*, 2010, **466**, 263–266.
95. S. Lee, E. Kassianidou, and S. Kumar, *Mol. Biol Cell*, 2018, **29**, 1992-2004.
96. Suki, A. L., Barabasi, and K. R. Lutchen. *J. Appl. Physiol.*, 1994, **76**, 2749-2759.
97. S. Nicolle, P. Vezin, and J.-F. Paliarne, *J. Biomech.*, 2010, **43**, 927-932.
98. B. D. Hoffman, and J. C. Crocker. *Annu. Rev. Biomed. Eng.*, 2009, **11**, 259-288.

## Figure captions

**Figure 1.** A cartoon depiction of tensional homeostasis under static and under dynamic conditions. A) In response to step stretch (lower panel) metric of tension [ $S(t)$ ] recovers its baseline value over time ( $t$ ) (top panel). B) Temporal fluctuation of  $S(t)$  around its time average  $\langle S \rangle$ . The orange curve represents large fluctuations, away from homeostasis, and the blue curve represents attenuated fluctuations when  $S(t)$  approaches homeostasis.

**Figure 2.** A free-body diagram of the blood vessel indicating shear stress ( $\tau$ ), the circumferential (hoop) stress ( $\sigma_\theta$ ), and the axial stress ( $\sigma_z$ ).

**Figure 3.** A cartoon depiction of a collagen gel (blue) seeded with cells (red). The construct is placed between two posts, one of which is fixed (right) and the other is movable (left). Cell contraction (white arrows) develops prestress (tension) in the gel which acts on the posts (yellow arrows). By increasing or decreasing the distance between the posts, the tension is increased or decreased and those changes are measured by the force transducer.

**Figure 5.** A cartoon depiction of the three-way force balance at the focal adhesion (FA) level. A) In response to stretching of the extracellular matrix (ECM), compression in the microtubule (MT) is relieved, traction forces increase (black arrows) and balance tension in the actin filaments (blue arrows). In the absence of compression, MT polymerizes (addition of gray monomers), grows longitudinally, and eventually regains its compression-supporting role in the force balance, causing the traction forces to decrease. B) In response to unstretching of the ECM, compression in MTs increases (red arrow), causing MT to buckle and disassemble and traction forces to increase. (Reproduced and adapted from Ref. 45 with permission from the Royal Society of Chemistry.)

**Figure 6.** Attenuation of traction field fluctuations with increasing number of focal adhesions ( $N$ ). The coefficient of variation ( $CV_T$ ) of the magnitude of the traction field decreases in BAECs (black solid circles, solid lines) with increasing  $N$ , stays virtually constant in MEFs (red solid circles, solid lines), does not change in BVSMCs (blue solid circles, solid lines) below  $N \approx 80$ , and then precipitously decrease as  $N$  increases. These behaviors of BAECs and MEFs are paralleled with a decrease in the normalized correlation coefficient ( $R_{norm}$ ) between traction forces in BAECs (black open circles, dashed lines) and no significant changes in MEFs (red open circles, dashed lines) as  $N$  increases. (Reproduced and adapted from Reference 27, with permission of Elsevier.)

**Figure 7.** One-dimensional models of a cluster of confluent (jointed) cells (A) and a cluster of non-confluent (disjointed) cells (B). Arrows indicate traction forces. In the jointed cluster model, traction forces are balanced at the whole cluster level, whereas in the disjointed cluster model, traction forces are balanced at the individual cell level.

**Figure 8.** The presence of E-cadherin affects contractility and traction field variability of gastric adenocarcinoma (AGS) cells. A) The time-average magnitude of the traction field ( $\langle T \rangle$ ) in single cells (black bars) and clusters (gray bars) expressing E-cadherin (E-cad) is greater than in single cells and clusters expressing the mock vector (Mock). B) The coefficient of variation ( $CV_T$ ) of  $T(t)$  is significantly greater in Mock cells and clusters than in E-cad cells and clusters. Data are median  $\pm$  median absolute deviation; \*significance  $p < 0.05$ . (These results are obtained by reanalyzing data from Reference 18 by taking into consideration equilibration of the traction field.)



**Figure 9.** Steady-state tension in single fibroblasts is altered by cell displacement in a rate-dependent manner. A) A cartoon depiction of the loading a cantilever beam system applying 1- $\mu\text{m}$  step displacement after the cell has reached steady state. B) Application of step displacement induced a jump in tension, followed by viscoelastic relaxation to a smaller, but significantly higher steady state value compared to before loading. C) Tension increased slightly when a cell was slowly strained at 0.1  $\mu\text{m}/\text{min}$ . D) Tension increased when a cell was quickly strained at 1  $\mu\text{m}/\text{min}$  and the cell remained at higher tension even after the ramp displacement ended. (Reproduced and adapted from Reference 12, with permission of Elsevier.)

**Figure 10.** Dynamics of cytoskeletal tension, focal adhesion (FA) forces and FA sizes during single-cell tensional homeostasis. A) A cartoon depiction of single cell tensional homeostasis. The cellular response to the applied biaxial step stretch comprises of an excitation phase (Phase I) followed by a decay phase (Phase II). B) Scanning electron microscope image showing single REF-52 fibroblast adhering to microposts at the ground state (time 0 min) (scale bar 20  $\mu\text{m}$ ). C) Time lapses of the magnitude of the traction field and D) of the additive size of all FAs of single cells following equibiaxial step stretch of 8%. Both the traction field magnitude and the FA size return to the baseline values within 30 min following the step stretch application. E) Time lapses of the magnitude of four representative individual FA forces and F) of the corresponding FA sizes. Single FAs do not show the tendency to return to the baseline following the stretch application. (Reproduced and adapted from Reference 13, with permission of Springer Nature.)

**Figure 11.** Cartoon depictions of traction field reorientation and the associate changes in the traction field fluctuations in response to periodic uniaxial stretch pulses (left panels). A) The stretching regimen with the onset of stretch marked by the red arrow. B) Following the onset of stretch, the traction forces (dark gray arrows) begin to reorient away from the stretch axis (blue

double headed arrow). As the stretch continues, the traction forces align perpendicularly to the stretch axis. C) During the reorientation, traction field fluctuations increase and then decrease to the level before the onset of stretch once the traction forces align perpendicularly to the stretch axis. A scalar metric of the traction field is the magnitude of the traction moment  $[M(t)]$  normalized by its time ( $t$ ) average ( $\langle M \rangle$ ). Results from the data analysis of uniaxial stretch measurements on single human umbilical vein endothelial cells (HUVECs) (right panels). D) The coefficient of variation of the traction moment ( $CV_M$ ) during the first hour (0-1 h) of stretching is significantly greater than  $CV_M$  obtained during the second hour (1-2 h) of stretching.  $CV_M$  calculated during 1-2 h is not significantly different from  $CV_M$  obtained in the absence of stretch. E) The corresponding  $\langle M \rangle$  obtained during 0-1 h is not significantly different from  $\langle M \rangle$  obtained during 1-2 h. Data are mean  $\pm$  standard error; \* significance  $p < 0.05$ ; NS = no significance. (Panels B and C are obtained from Reference 28 with permission of Elsevier.)

**Figure 12.** Temporal fluctuations of FA traction forces rapidly decrease after reaching a threshold value. A) Relationships between the coefficient of variation ( $CV_F$ ) of the FA forces versus their time-averaged magnitude ( $\langle F \rangle$ ) exhibit a threshold behavior obtained for single BVSMCs (solid circles, solid lines) and for clusters of BVSMCs (open circles, dashed lines). This threshold is shifted to the right as substrate stiffness increases from 3.6 kPa to 30 kPa, but the  $CV_F$  versus  $\langle F \rangle$  relationship does not change. B) Similar behavior is observed in the relationship between  $CV_F$  versus time-average magnitude of FA displacement ( $\langle u \rangle$ ), but in this case, the threshold (between 1 and 2  $\mu\text{m}$ ) does not depend on the substrate stiffness. Different colors correspond to different substrate stiffness (3.6 kPa black, 6.7 kPa red, 13.6 kPa blue, 30 kPa green). C) Single BVSMCs (black) and single BAECs (gray) cultured on 3.6 kPa stiff gels exhibit virtually the same  $CV_F$  versus  $\langle F \rangle$  relationships. D) Coefficient of variation of the

traction field ( $CV_T$ ) does not exhibit threshold with increasing time-averaged magnitude of the traction field ( $\langle T \rangle$ ) in single BVSMCs (black) and in single BAECs (gray). Instead,  $CV_T$  decreases with increasing  $\langle T \rangle$  following roughly a power-law dependence. Measurements are carried out on substrates of 3.6 kPa stiffness. Data are mean  $\pm$  standard error. (Reproduced and adapted from Reference 29 with permission of Elsevier.)

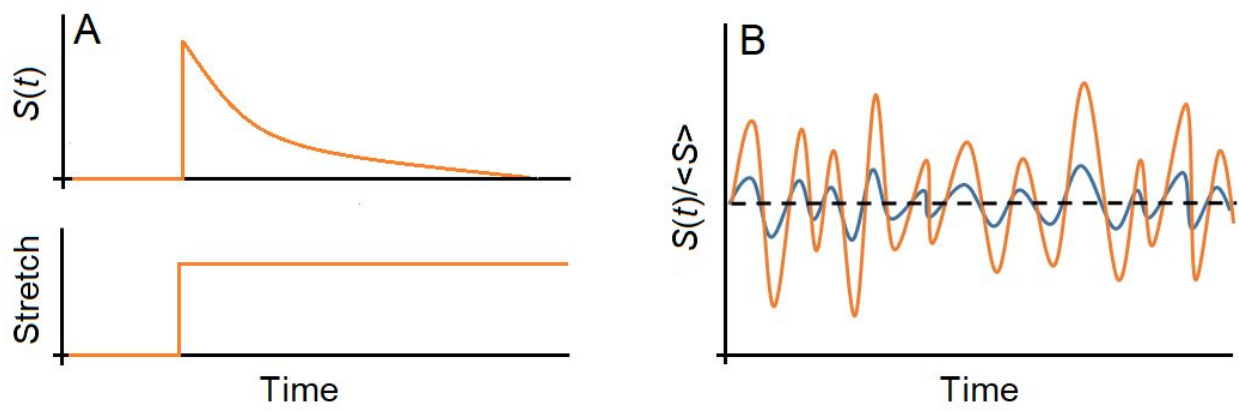


Figure 1

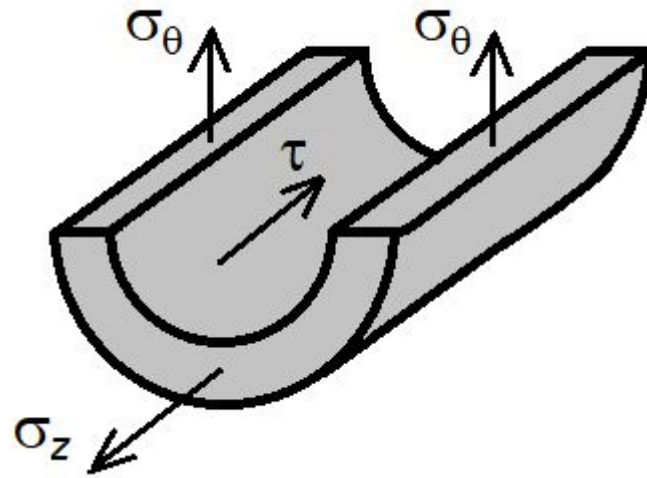


Figure 2

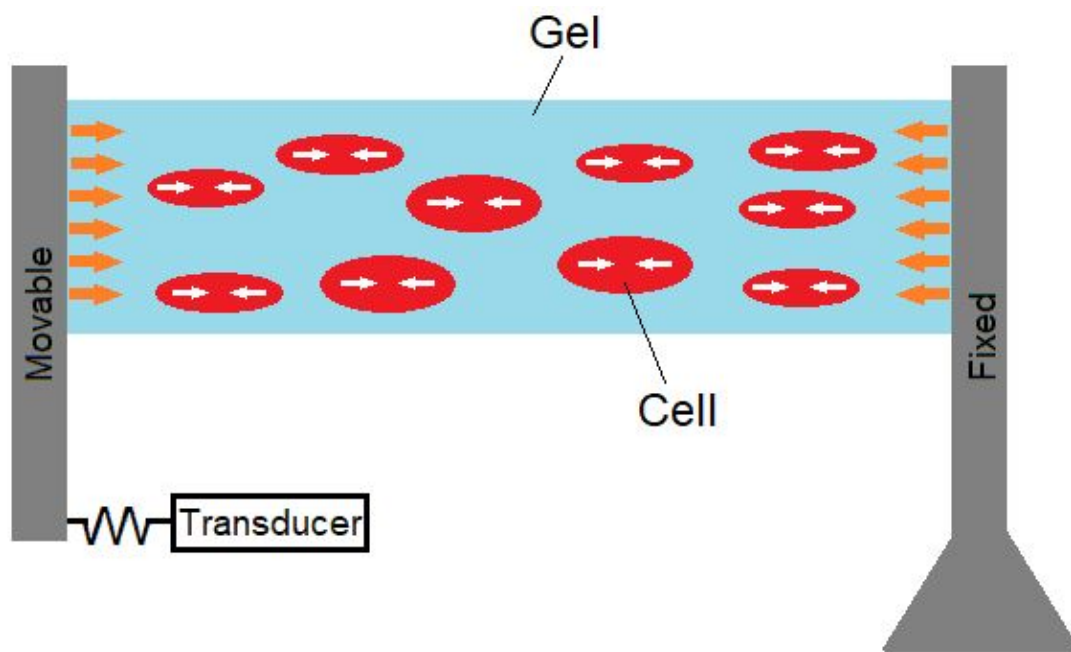


Figure 3

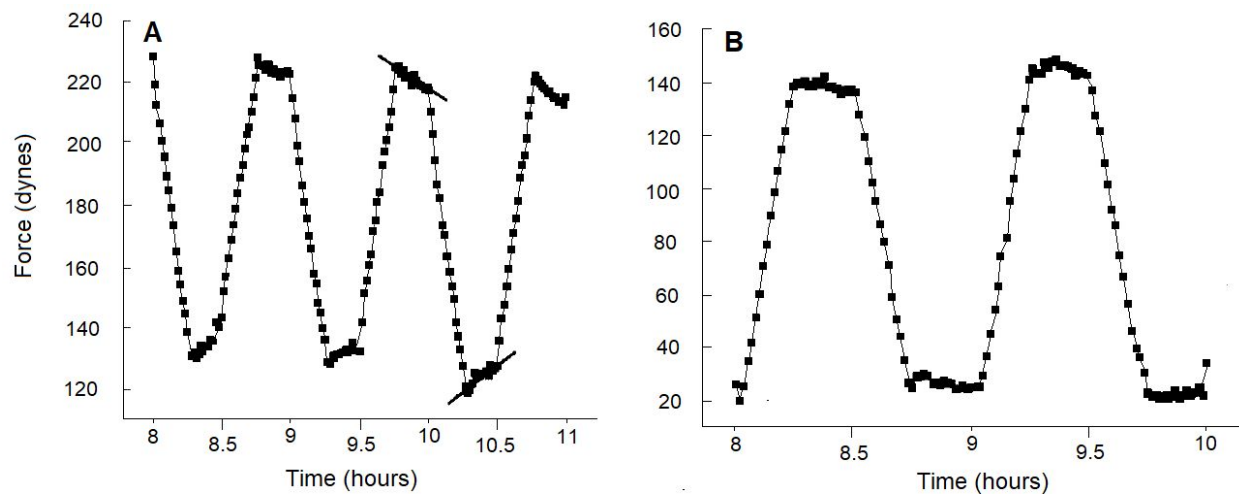


Figure 4

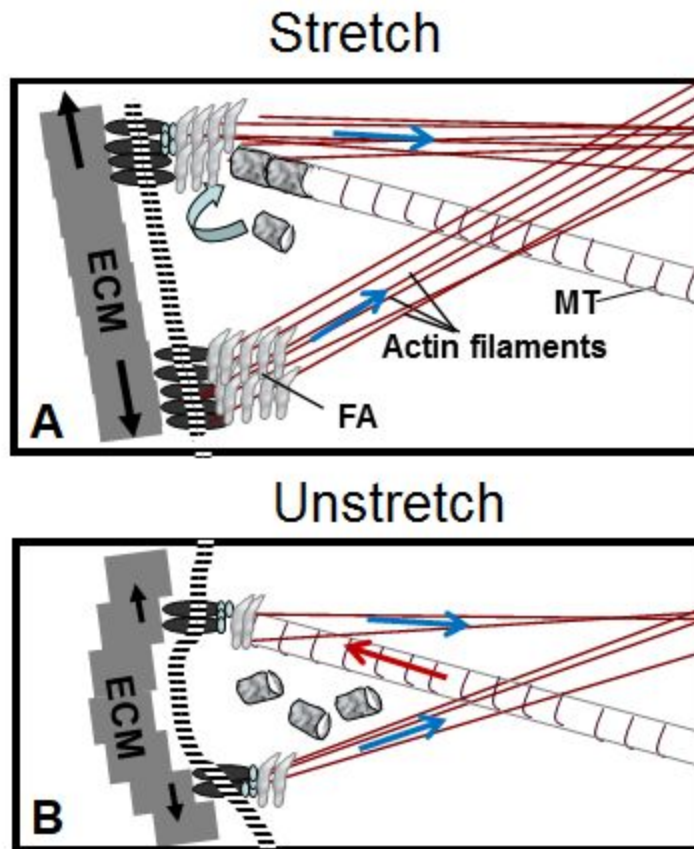


Figure 5



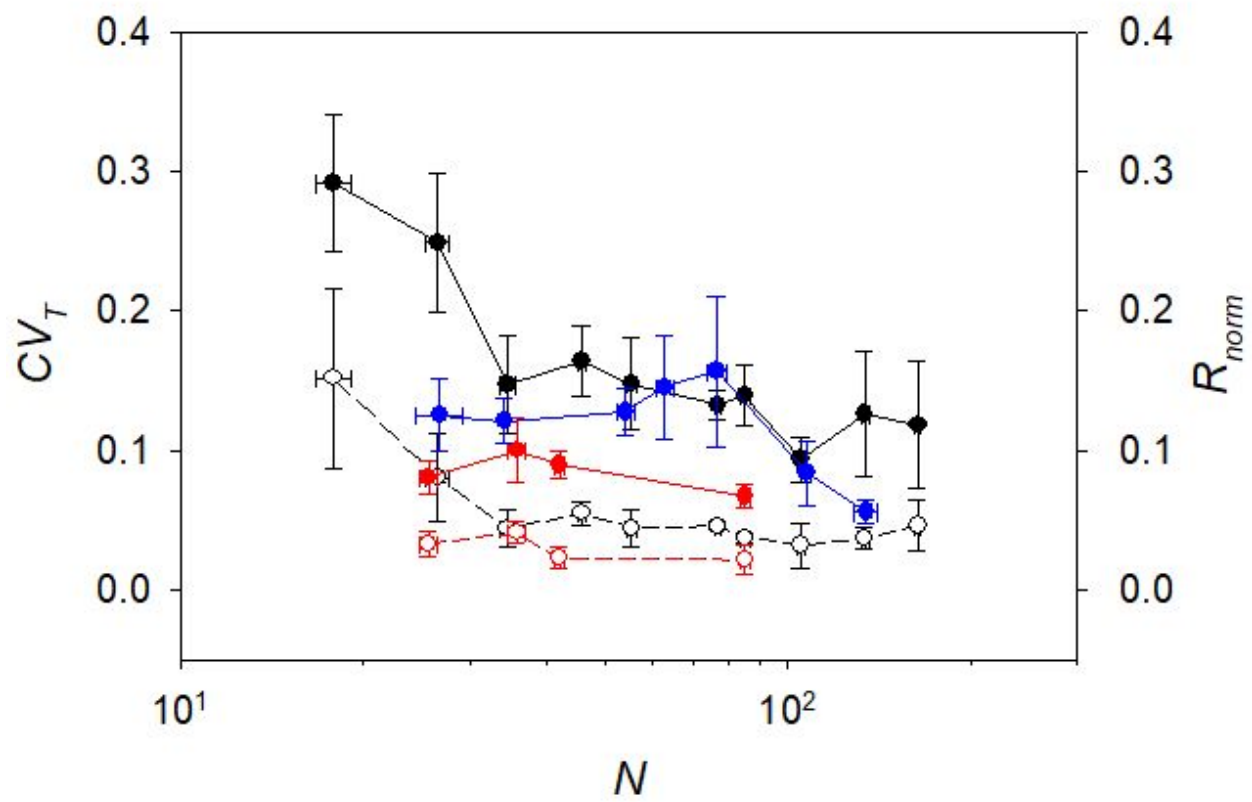


Figure 6

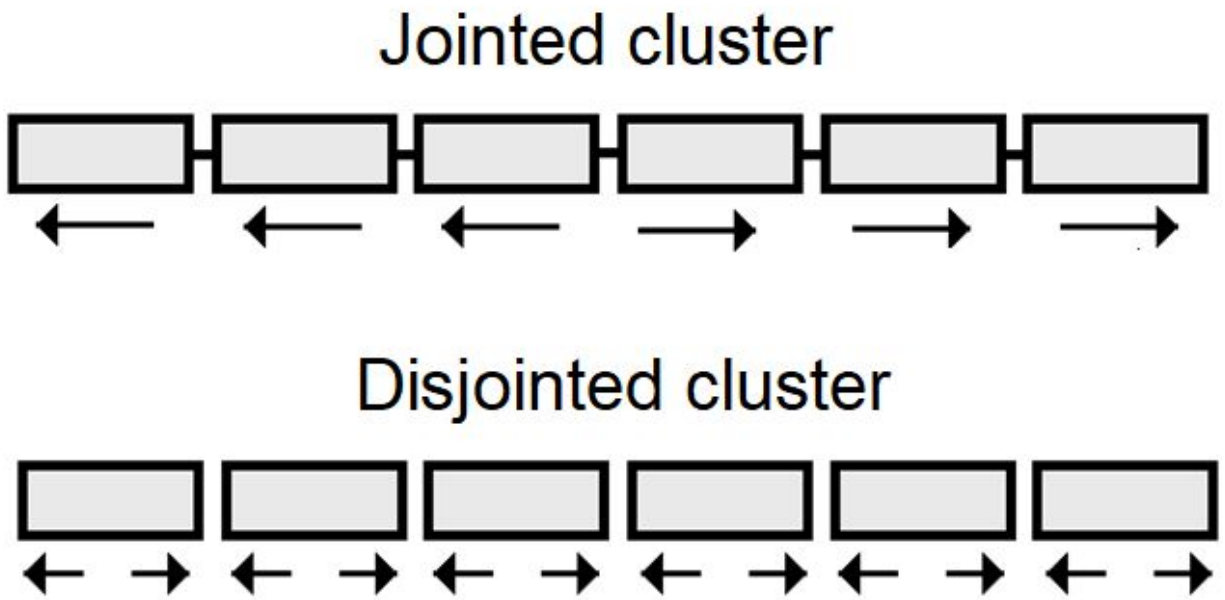


Figure 7

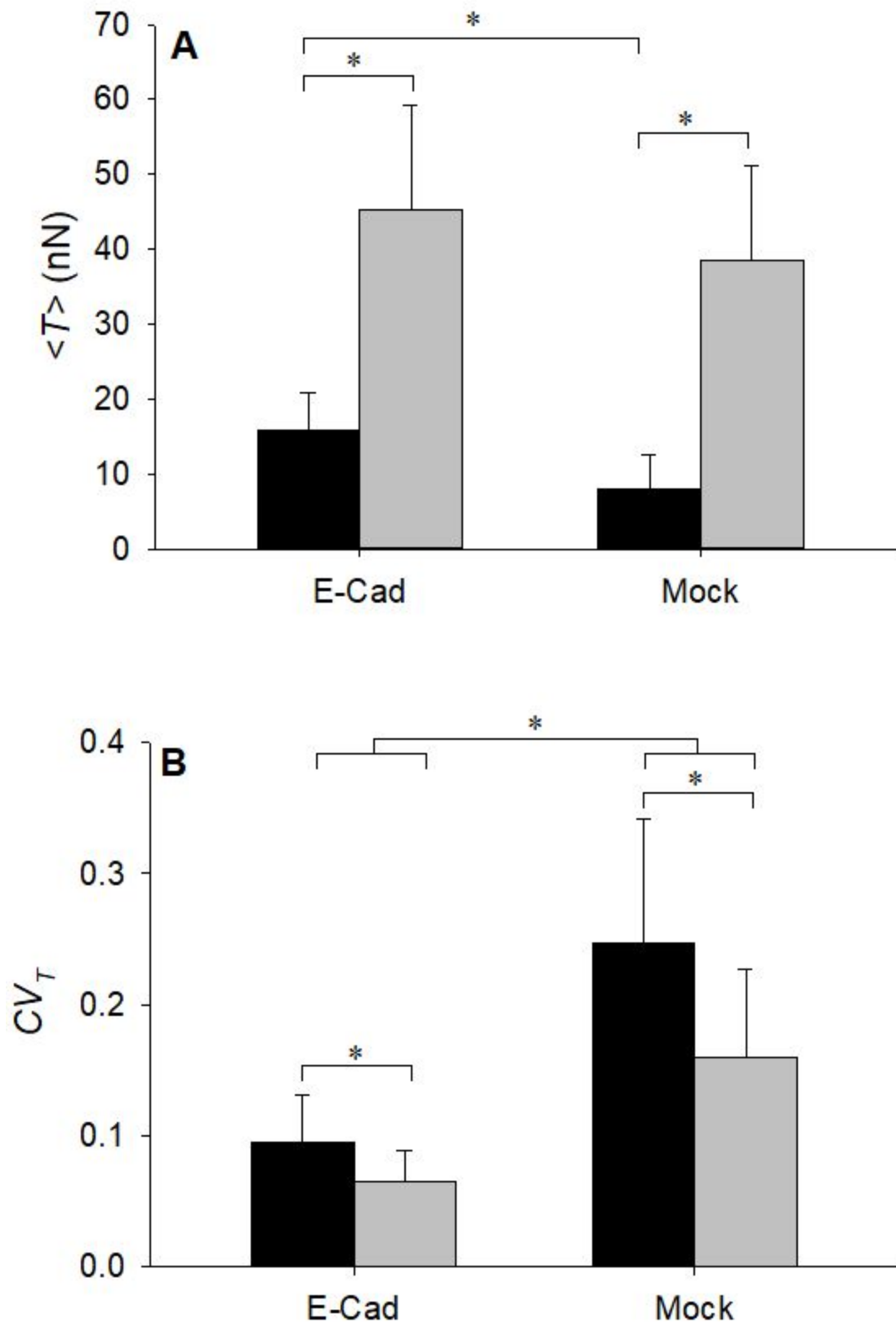


Figure 8

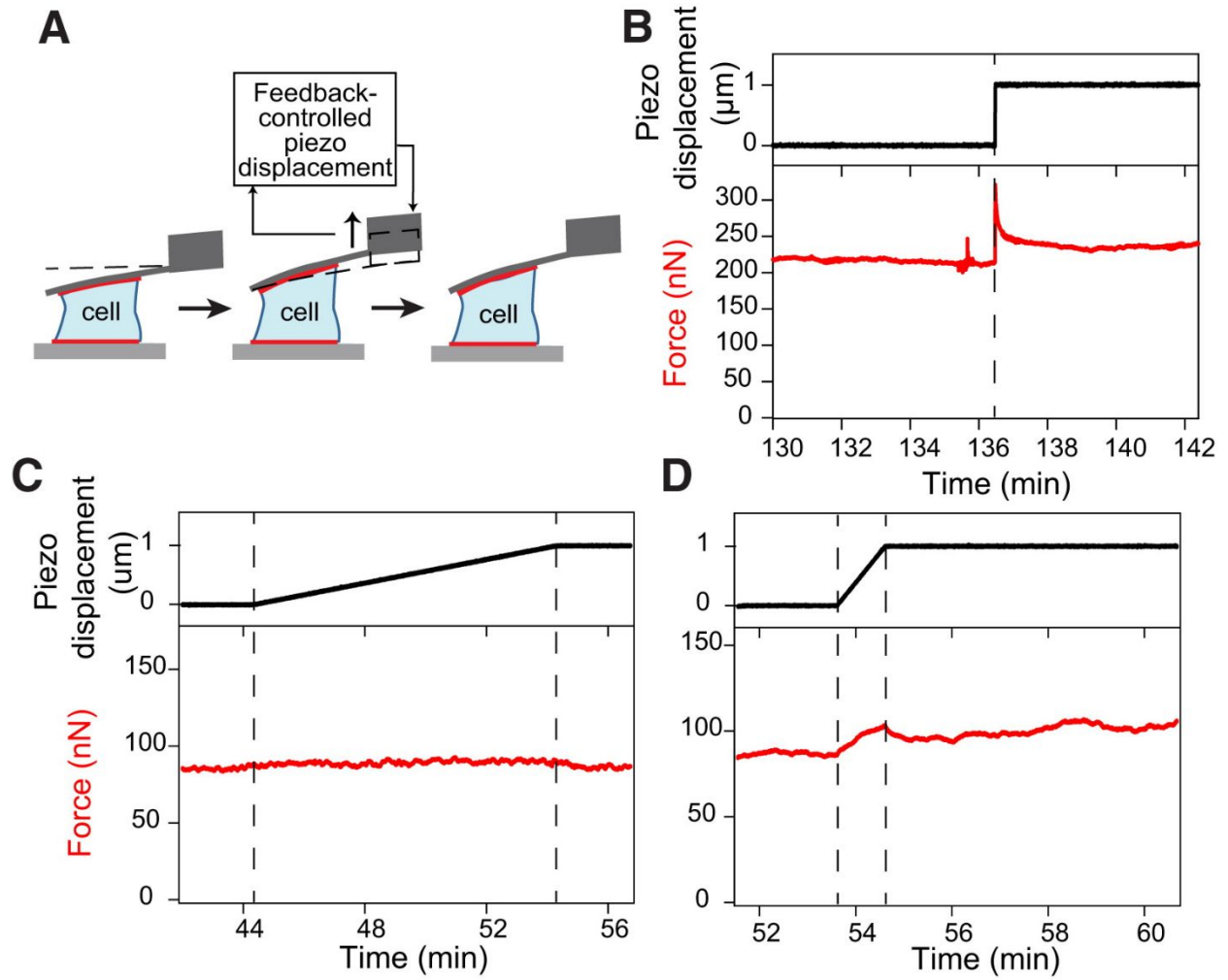


Figure 9

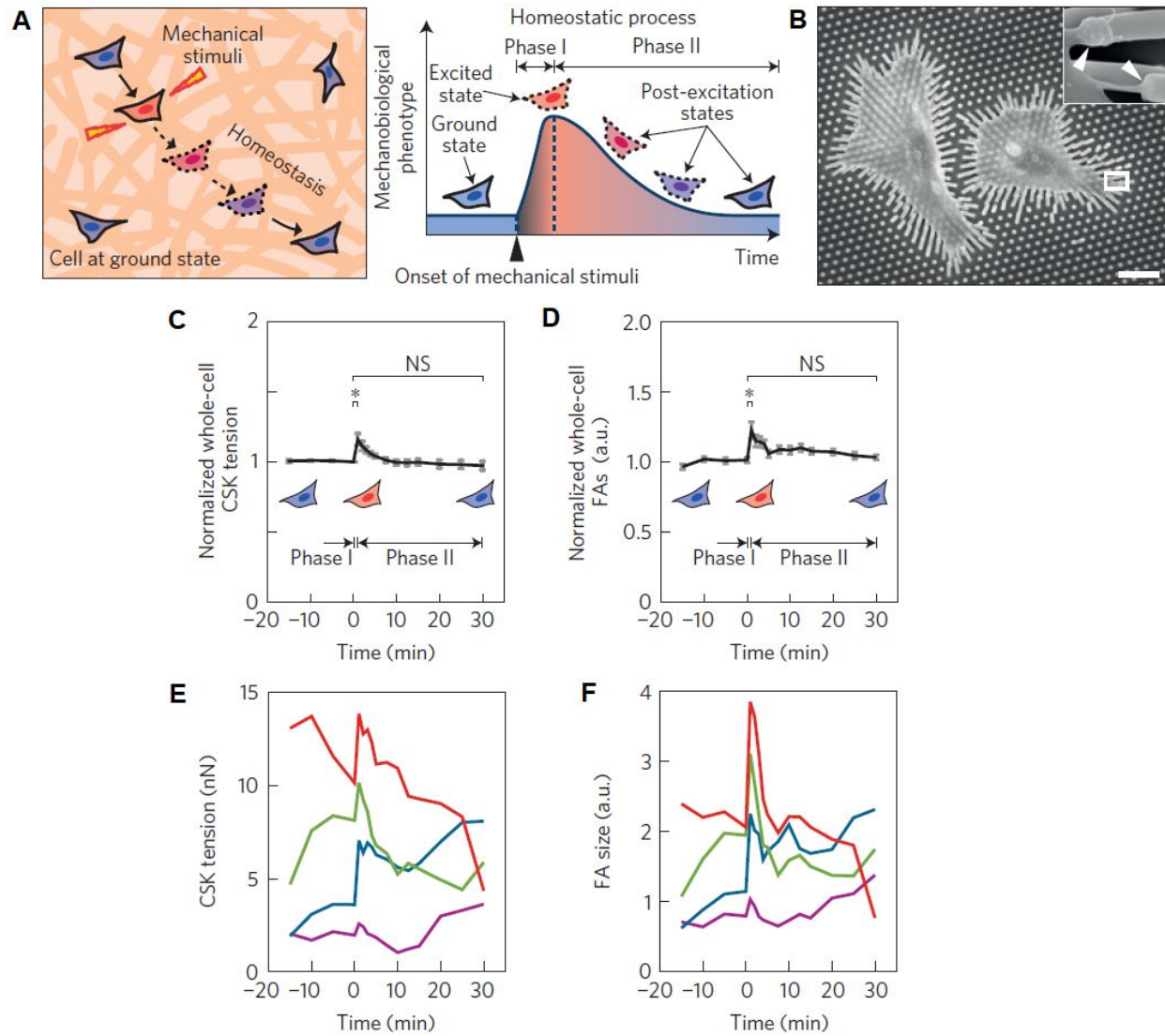


Figure 10

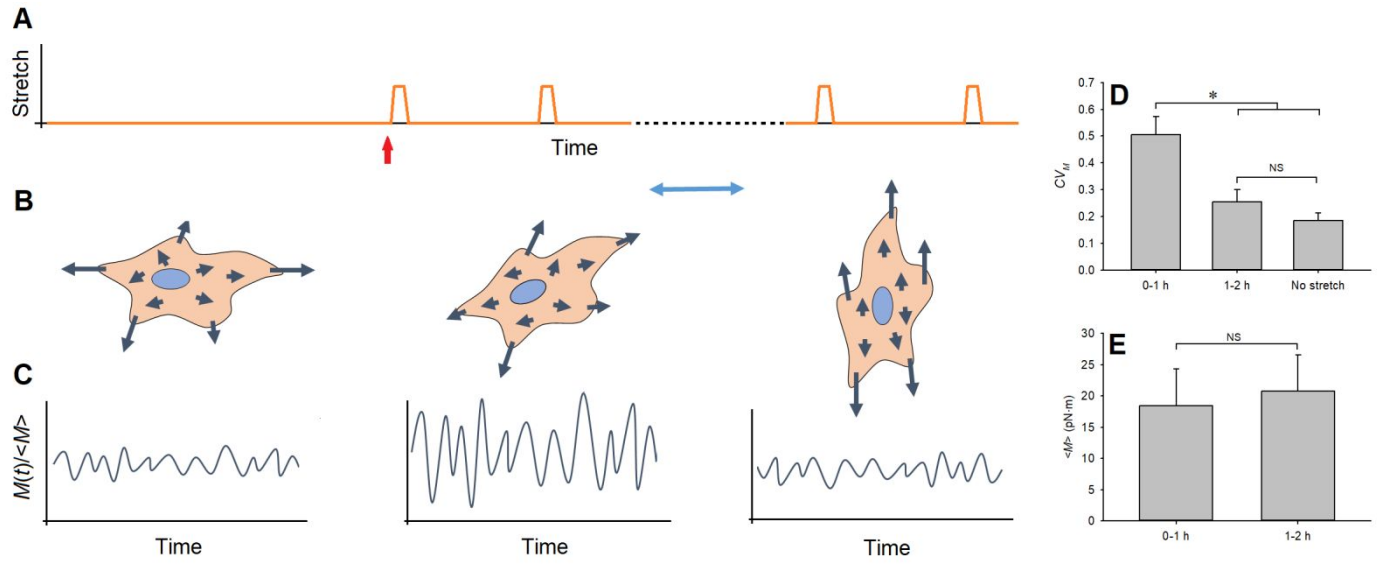


Figure 11

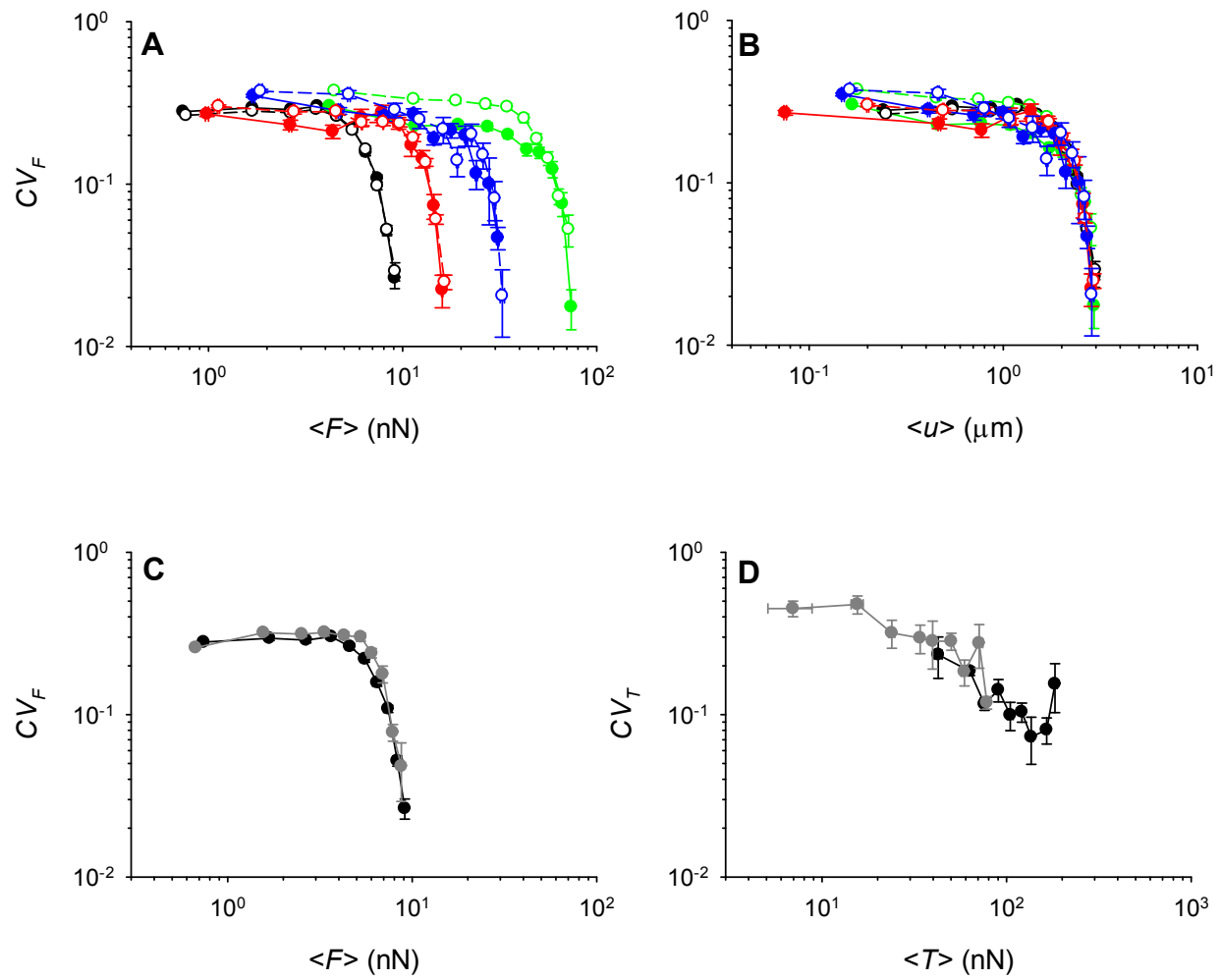
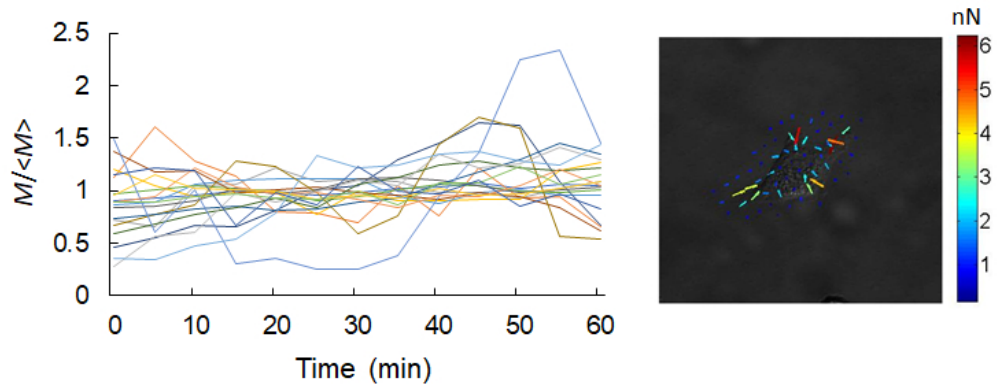


Figure 12



**Graphical abstract.** Traction field temporal fluctuations of bovine aortic endothelial cells; each color corresponds to a single cell (left), and a representative traction field of a single cell (right) (adapted with permission from Elsevier).