Soft Matter

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COMMENT

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Cite this: *Soft Matter*, 2022, **18**, 680

Reply to the 'Comment on "Tensional homeostasis at different length scales" by J. Humphrey and C. Cyron, *Soft Matter*, 2022, 18, DOI: 10.1039/D1SM01151K'

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Received 17th October 2021,
Accepted 16th December 2021

DOI: 10.1039/d1sm01495a

rsc.li/soft-matter-journal

Drs Humphrey and Cyron wrote a commentary regarding our review article entitled "Tensional homeostasis at different length scales" that was published in *Soft Matter*, 2020, **16**, 6946–6963. These authors brought up some valid concerns to which we would like to respond. Their first concern is related to our remark regarding equations that we used to describe homeostasis in blood vessels, where we stated that those equations were limited only to linearly elastic materials. We were wrong, and we agree with the authors that these equations hold for all cylindrical vessels regardless of their material properties. Their second concern is related to tensional homeostasis at the subcellular level. Drs Humphrey and Cyron disagree with our substantiated claim that tensional homeostasis breaks down at the level of focal adhesions (FAs) of a living cell. In our reply, we provided several pieces of evidence that demonstrate that tensional homeostasis depends upon FA size, FA maturity and FA force dynamics and thus, tensional homeostasis cannot hold in all FAs across a cell. In summary, we are grateful for the opportunity to reply to the commentary of Drs Humphrey and Cyron. Moreover, we are excited that this topic has become an important focus in the biomechanics and mechanobiology communities, and we feel strongly that critical feedback is necessary to move this field forward.

1. Introduction

We thank Drs Humphrey and Cyron for their Commentary regarding our review article on "Tensional homeostasis at different length scales".[1] They brought up some valid points to which we would like to respond. We are also excited that this topic has become an important focus in the biomechanics and mechanobiology communities, and we feel strongly that critical feedback is necessary to move this field forward.

2. Stresses in cylindrical tubes

Considering the concern of Drs Humphrey and Cyron regarding the equations for the hoop stress and the axial stress that we used to describe tensional homeostasis in blood vessels, we agree with their comments. We were indeed incorrect in pointing out that those equations represent "*static equilibrium of a linearly elastic vessel wall*". They represent equilibrium of any cylindrical vessel regardless of its material properties. However, our description of tensional homeostasis in the blood vessel wall that is based on those equations is not influenced by our erroneous remark regarding the material properties of the wall.

3. Tensional homeostasis in the vasculature

The other concerns of Drs Humphrey and Cyron are primarily centered around tensional homeostasis at the focal adhesion (FA) level. Before we address those concerns, we would like to address the comment regarding tensional homeostasis in the vasculature. Drs Humphrey and Cyron interpreted our parenthetical remark that the set point stress in the vasculature implies that the stress in the blood vessel walls is homogeneous throughout the vascular tree. What we meant was that stress in the blood vessels was largely determined by blood pressure, blood flow, and blood vessel geometry. Under normal physiological conditions blood (arterial) pressure and flow are maintained at a narrow range. Thus, blood vessels of similar geometry (diameter and wall thickness) will have similar stresses.

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4. Tensional homeostasis in FAs

Homeostasis at the FA level rests upon the premise that FAs carry the same stress across a cell.[2,3] This is supported by the observations that traction forces applied to FAs are linearly correlated with surface areas of FAs, across a broad range of FA sizes, implying a constant FA stress.[4–6] However, as we pointed out in our review article,[1] exceptions to this premise are notable.

First, it has been shown that a linear correlation between traction forces and FA size breaks down for very small FAs (<1 μm^2)[5,6] and for very large FAs (>7.5 μm^2), so-called super FAs,[6] where the corresponding stresses are much larger than the stress in the region where FA traction forces are linearly correlated with the FA size.

Second, Stricker and co-workers found that only immature FAs exhibit a linear correlation between their size and their traction force, whereas such correlation does not exist in mature FAs.[7] In particular, these authors observed that: "*. . .no robust correlation exists between FA size and traction force across an entire cell. . . . We find that that even similarly sized FAs do not exert a constant stress. Instead, a strong positive correlation between FA size and traction stress persists only during the initial stages of myosin-mediated FA maturation. After this period, the FA size remains constant, whereas the local traction stress can either increase or decrease depending on the proximity of the FA to the cell edge. (. . .) We show that mature FAs can withstand as much as sixfold increases in their endogenous tension without subsequent changes in size. Together, our data show that the strong correlations between FA size and traction stress occur only during the initial stages of myosin-mediated maturation*." These results provide further evidence that FAs do not maintain constant stress across the entire cell. In their Commentary, Drs Humphrey and Cyron made only a parenthetic remark regarding the study of Stricker and co-workers, by pointing out that those investigators observed the absence of a strong correlation between traction forces and FA size in the case of small FAs (<2 μm-length). However, they left out a major finding of this study, namely that in mature FAs no strong positive correlation occurs between traction forces and FA size across an entire cell, including FAs whose size is greater than 2 μm. Thus, the claim made by Drs Humphry and Cyron that a positive linear correlation between the FA force and FA size (and thereby tensional homeostasis in FAs) existed over "a central range of FAs" – namely FAs whose area is between 1 μm^2 and 7.5 μm^2 – is not tenable in the case of mature FAs.

Third, in our own studies of the dynamic nature of FA tension, we showed that temporal fluctuations of FA displacements caused by traction forces become attenuated only after mean displacements reach a threshold of 1–2 μm. This unique relationship between FA fluctuations and FA mean displacements was observed on substrates of different stiffnesses, with two different cell types, in isolated cells and in multicellular clusters, and, importantly, on patterned substrates that limit FA size to a diameter of ~2 μm.[8] This, in turn, indicates that only those FAs where traction forces are sufficiently large to displace FAs beyond the threshold value can reach tensional homeostasis. Drs Humphrey and Cyron completely ignored these results in their Commentary.

Taken together, the results discussed above firmly establish that FAs do not maintain constant stress across a cell. Drs Humphrey and Cyron acknowledge this, suggesting that tensional homeostasis should be "*thought of locally and over appropriate ranges*". These appropriate ranges would presumably include only FAs whose areas linearly correlates with their traction forces, and exclude small FAs, large (super) FAs, mature FAs, and FAs that exhibit large tensional fluctuations. If so, then the idea that tensional homeostasis exists across multiple length scales, from the tissue level to the subcellular level, that has been promulgated by Dr Humphrey,[2,3] does not apply to FAs.

Finally, we would like to address the comment related to the study of Weng and co-workers.[9] We discussed this study in our review paper[1] as evidence of the absence of tensional homeostasis at the FA level. Weng and colleagues measured FA traction force and FA size in cells exposed to static equibiaxial stretch. They found that FAs exhibit "*highly heterogeneous, non-homeostatic behaviors*" of individual traction forces and FA sizes. They also showed that plots of traction force *vs.* FA area relationships of four arbitrarily selected FAs appear to be positively correlated. In their Commentary, Drs Humphrey and Cyron use this as an argument in favor of tensional homeostasis of FAs. By scaling forces with the corresponding area of four FAs, they obtain that FA stresses exhibit less heterogeneous behaviors than the corresponding traction forces. On average, those stresses exhibit a tendency to return to the baseline value following the applied static stretch, which is indicative of tensional homeostasis. However, out of the four FAs, only two appear to show this tendency (purple and blue lines in the figure shown in the Commentary), whereas the other two (green and red lines) do not. Thus, more data from individual FAs are needed in order to reach a tenable conclusion that FAs, which do not exhibit traction force homeostasis, do exhibit stress homeostasis. There are some other issues regarding scaling of traction forces with measured FA area which we address below.

In the study of Weng and co-workers, FAs are formed on the tips of microposts whose diameter is ~1.8 μm, corresponding to the area of ~2.6 μm^2.[9] Considering that on solid substrates the FA area easily exceeds 3 μm^2, it is conceivable that on some of the microposts FAs may cover the entire tip of the micropost throughout the experiments. This, in turn, suggests that the stresses in those FAs may have a similar non-homeostatic behavior as their corresponding forces.

Finally, in most studies, the FA size (area) has been determined from imaging only one of many proteins that comprise FAs (mostly paxillin and vinculin). While the size of a single FA protein may correlate with the corresponding FA force, it may not accurately represent the true area over which FA force is transmitted. Thus, the stresses estimated from these measurements may not be very accurate.

5. Summary

We addressed two main concerns raised in the Commentary of Drs Humphrey and Cyron. One concern is related to the equations describing the hoop and axial stresses in the blood vessel walls, and the other is related to tensional homeostasis at the FA level. We agree with Drs Humphrey and Cyron that the equations describing the mean stresses in the blood vessel walls are universal and therefore, our remark that these equations were derived under the assumption of linear elasticity is wrong. Considering tensional homeostasis at the subcellular level, we provide strong evidence that (i) FAs do not carry a constant uniform stress across a cell and that this stress depends upon the size and maturity of FAs, and (ii) that FA forces exhibit large temporal fluctuations until they reach a threshold value after which those fluctuations exhibit a precipitous drop. Taken together, these findings suggest that tensional homeostasis does not exist in all FAs across a cell. Nevertheless, tensional homeostasis at the whole cell level does exist. It emerges through dynamic rheostasis of all FA forces and FA sizes.[9]

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Supported by NSF grant CMMI-1910401.

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