Date: 24th August 18 11:13:13
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Triggered By: Redacted
BCC: Redacted
Subject: Decision on Nature Materials submission NM18061996
Message: 24th August 2018

Dear Dr Adams,

Thank you for submitting your manuscript, "Protrusion-mediated cell adhesion determines viscosity and stiffness in zebrafish blastula". The manuscript has been seen by 3 referees, whose comments are attached below. You will see that whereas they find your work of potential interest, they have raised substantive points that in our view preclude the publication of the manuscript in its present form in Nature Materials.

Should future experiments allow you to address these criticisms in full, we would be happy to look at a revised manuscript (unless, of course, a similar paper is published elsewhere or is accepted for publication in Nature Materials in the meantime). In particular, the most salient points that need to be addressed are the further experimental justification for the claims made, particularly of the accuracy and reproducibility of the model and measurements carried out. Moreover, there is a need for sufficient controls to accurately interpret the data.

It is only fair to say, however, that we would be reluctant to trouble our referees again unless we thought that their comments had been completely addressed. In a case like this, where the revisions are extensive, we would naturally understand if you preferred to submit your manuscript elsewhere.

When you are ready to submit a revised version, please use the link below to submit the manuscript files, including (in a separate document from the cover letter) a point-by-point response to all referees' comments and a clear description of the changes and additions that you have made (we suggest highlighting the changes in the main text).

Link Not Available

We hope that you find the referees' comments helpful when preparing a revised manuscript. If you have any questions, please feel free to contact me at materials@nature.com.

With kind regards,

Amos

Dr Amos Matsiko
Associate Editor
Nature Materials

REFEREE REPORTS

Reviewer #1 (Remarks to the Author):

The authors describe the mechanical response of an early embryonic tissue to magnetic bead pulling. To make these measurements the authors embed a 40 um diameter bead in the early zebrafish blastoderm, midway between the yolk cap and the enveloping layer. This region of the embryo undergoes rapid rounds of cell division to convert a small number of early large blastula cells a much larger number of small cells that will follow the movements of epiboly and later drive axis formation. At these early stages cells in this region are loosely organized and are thought to be only marginally

cohesive. The authors find stage-specific increases in elastic stiffness and changes in viscosity that can be related elements in a creep model of the tissue rheology. These descriptions parallel other reports that tissues exhibit dynamic changes in their mechanical properties during early development. The author next turn to disrupting Rho-family GTPase signaling pathways and their downstream actomyosin effectors. Surprisingly, the authors find that inhibiting RhoA signaling with a dominant negative construct increases the elastic response to bead pulling. They suggest that Rho-inhibition activates the Rac1 signaling system to increase protrusions by cells serving to increase elastic cohesion of these blastula tissues. They further suggest that increased Rac1 activity serves to reduce cell rearrangement under loads imposed by the magnetic bead and that the plastic response of the tissue is due to resulting cell-cell rearrangements. The hypothesis is an interesting one and suggests that the mechanical properties of the tissues at these most early stages before cells form a cohesive mass are determined by the rate and density of cell-cell protrusions. Analogs to this type of behavior might be seen in soft-granular matter when strain energy storage in adhesions between grains exceeds the strain energy stored in grain deformation. The physical material properties of tissues in this regime would thus be dependent on the mechanics of adhesions rather than the mechanics of the cell cortex. However, there are several major issues with the biophysical measurements and limitations to the cell biological approaches used by the authors.

The major question is whether the viscoelastic model represents the tissue mechanical properties or the coupling of the bead to the tissue. The authors are clearly measuring response of the bead to magnetic force but I am not convinced this reflects only the mechanical properties of the deep cell layer. Instead I suspect they are also measuring the compliance and friction of the bead's connection to the surrounding cells. Furthermore, the rearrangements that the authors' suggest are the source of plastic deformation appear to be rearrangements between the bead and the cells not between cells.

Furthermore, the small molecule inhibitors, knock-down, and overexpression constructs are not limited in effecting only the deep cells. How do the authors know their observations are not confounded by compaction of deep cells due to changes in external tissues such as the enveloping layer and the yolk syncytial layer?

The authors need to confirm the effect of cell biological factors. There is no support that alterations in E-cad do not alter Rac1 or that ROCK inhibitor alters Rac1 as suggested. Furthermore, none of the approaches seek to reverse or 'rescue' the impact of the perturbations. How do we know that these are not off target effects due to the large amounts of mRNA, MOs, inhibitors needed to impact adhesion and cytoskeleton of early cleaving embryos? Do the authors have positive controls for these inhibitors?

It is not clear to me how an AFM measurement of whole embryo embryo compliance, including vitelline membrane, EVL, DEL, YSL, and yolk cell are related to the microrheology of the DEL. I would suspect that the vitelline membrane and yolk cell are considerably stiffer and more elastic than the DEL and would tend to dominate the mechanical measurements yielded by the AFM. Changes in the mechanics of the whole embryo, e.g. stiffening over time, may simply correlate with changes in DEL mechanics.

Figures showing cell outlines and rearrangements are unclear. The images look as if they were collected in DIC. Given the eminence of the Adams group in imaging and the advanced adoption of light-sheet microscopy for this project I am greatly disappointed in the quality of the images and the reliance on 2D sections to show what is really a 3D environment around the bead. Bead-tissue coordination (e.g. contact between the bead and surround cells) and cell rearrangements in this blastula tissue should be shown in 3D rather than 2D.

Reviewer #2 (Remarks to the Author):

The authors present rheological analysis of zebrafish embryonic tissues. To this end the authors develop a novel magnetic tweezer instrument that they combine with ligtsheet microscopy to study tissue deformation in response to precisely controlled external force pulse. One novel feature of the described setup is the ability to apply force in all three spatial directions. The authors determine several material properties of embryonic tissue by fitting their data to a simple spring-dashpot model and study temporal evolution of those parameters during the course of development. They find that those properties undergo a fairly dramatic change concomitantly with the onset of a morphogenetic change ("high to sphere-shape transformation"). To interpret the results of the rheological measurements in terms of changes in cellular behavior, the authors quantify the extent to which cells rearrange in response to applied force during the different phases of the inflicted deformation. Additionally, the authors perform several genetic and pharmacological perturbations to see how those affect the measured material properties. Finally, the authors quantify changes in cell motility during the course of development.
I believe that, overall, the paper presents a significant technical innovation and biological insight to be ultimately of interest to biology/biophysics-oriented Nature Materials readership. However, I believe several issues will have to be resolved for this work to warrant publication.

In the section describing rheological data (referring to Figure 2b), it is claimed the response to the applied force is biphasic. As far as I can tell from the presented plots, there is little evidence for this conclusion. To examine this further, I would suggest that the authors collapse all their data from Figure 2b onto one master curve by re-scaling the axes. Additionally, I think it would be very helpful to represent bead displacement in a series of logarithmic plots (suitably deferred to the supplement). In particular, I would wish to see the displacement of the bead in the loading phase (the period of time

during which the forces being applied) on a log-log plot. Likewise, the displacement during the unloading phase should be shown as both a log-log plot and a semi-logarithmic plot where only the displacement axis is logarithmic (to better appreciate whether the decay best fits an exponential, a multi-exponential or a power law).
When the authors discuss the characteristic time-scale on which tissue elasticity persists, they compute the ratio of spring elasticity to dashpot friction coefficient in the Kelvin-Voigt element of their simplified model. I do not find this appropriate for the following reason. Suppose one assumes that the dashpot connected in series with the Kelvin-Voigt element has an infinite friction coefficient. In this case, one would observe complete recoil to the initial configuration (zero strain) after arbitrarily long applied deformation. Thus, one should conclude that in the corresponding limit elasticity persists on an infinitely long time-scale. Hence, the time-scale of elastic stress relaxation should not be quantified based exclusively on the parameters of the Kelvin-Voigt element. Put another way, if the time-scale of elastic stress relaxation were 10 seconds as the authors claim, it would be six-fold shorter than the minute-long time during which the force is applied in a typical reported measurement, and only a very small recoil would have been recovered. Thus, I believe that the 10-second estimate of the time-scale on which elasticity persists is incorrect. I would rather suggest fitting the data to the Maxwell model (a single dashpot and a single spring in series) and quantifying the relevant time-scale by taking the ratio of material constants of those two elements. In this case the initial velocity at the onset of deformation would have to be infinite, but this is a rather fair approximation since the velocity at the onset of motion is indeed very much larger than it is on average during the course of the experiment.
In my view, a major issue has to do with the section entitled "stiffness is linked to cell shape and viscosity to cell rearrangements". I find both the measurements and the analysis of the corresponding data unconvincing. In my view, this section should be omitted altogether, or at least deferred to the supplement. The authors performed a very suitable analysis using strain decomposition to quantify cell rearrangements, and apply rigorous statistical analysis to assess significance. Despite this effort, I believe that most of the data in Figure 3 is by far too noisy to serve reasonable evidence for the presented conclusions. Additionally, as far as I understood from the text, the authors only considered deformation in a single plane and did not track cells in 3D. Thus, apparent shrinking of cells in front of the bead may reflect out-of-pane deformation rather than cell rearrangement. I would hold it that rigorously interpreting the data in terms of cellular behaviors would necessarily involve developing a model that describes spatial aspects of the deformation and not merely fitting to a spring-dashpot circuit. Taken together, I find the results of the corresponding section insufficiently rigorous both in terms of experimental reproducibility as well as in terms of the theoretical analysis.
I think that English/presentation of the paper must be re-worked considerably. The text requires major proofreading as there are several incomplete sentences as well as cumbersome wording. Here are a few specific examples:
Third sentence of the second section ending in "and preclude temporal". This sentence looks to be incomplete.
End of fourth paragraph of the third section ending in "the shape of cells previous compressed ahead of the bead " appears awkward. Likewise the sentence "We conclude that the cellular signature of the elastic periods is largely accounted for by cell shape deformations" in the same paragraph.
At one point the authors refer to "cell valency". I believe they actually mean the number of certain contact sites made by a cell with its neighbors. At any rate, I don't believe this is a standard term.
These are just a few examples and I believe the presentation/English needs major re-working.

In summary, I believe the paper makes a meaningful contribution to the field. The major shortcoming is not lack of data but more rigorous and critical assessment and presentation of the already available data.

Reviewer #3 (Remarks to the Author):

In this study, the mechanical properties of mesenchymal cells of the zebrafish blastula are measured 3-4 h after fertilization (during the transition from an ellipsoidal to a spherical shape) with a 41 µm magnetic bead that has been injected into the developing embryo. The magnetic bead displacement in response to a 10 nN force step that lasted for 1 min was consistent with a simple visco-elastic model consisting of a parallel spring (E) and dashpot (eta_1) in series with a second dashpot (eta_2).
The authors report an increase of tissue elasticity (E) and viscosity (eta_2) during the transition period from an ellipsoidal to a spherical shape. The authors argue that the increased tissue elasticity is associated with reduced cell deformations, and the increased viscosity is associated with decreased cell rearrangements. Knock-down of Rac1, Rho-kinase or E-Cadherin was then performed with the intention to alter myosin activity, the ability of the cells to form protrusion, or the make stable cell-cell contacts. The results are difficult to interpret, however, as the mechanical effects appear to strongly depend on the time points of the measurements during the transition phase. The authors also performed experiments with the myosin inhibitor blebbistatin but – strangely – only report morphological changes and not mechanical measurements.
I find it problematic how the cell rearrangements (and cell deformations) are extracted from the deformation field of the tissue around the bead during different phases of the force application protocol. An ill-explained model is then fitted to the deformation field and the velocity field, with the intention to separate cell-scale from tissue-scale deformations. Cell rearrangements (termed “intercalation”) are then estimated from the difference between cell and tissue scale deformations, which seems arbitrary and is not backed up by independent measurements. This procedure is then applied to different time points during the force protocol (early “elastic” phase, late “creep” phase, recovery phase). The resulting data points are noisy, show diverging trends for the different phases, and don’t seem to

explain the increased viscosity during the transition period or the findings from the Rac1, Rho-kinase or E-Cadherin knock-down experiments.
I suggest the authors provide a more direct measure of cell rearrangements. Since they can directly observe the cell contours, this does not seem to pose a problem. The "diffusion coefficient" as extracted from the MSD, however, should be corrected for spurious effects from rotations etc., e.g. by using cell-cell distances instead of absolute positions.