Live 3D imaging and mapping of shear stresses within tissues using incompressible elastic beads

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First decision letter

MS ID#: DEVELOP/2021/199765

MS TITLE: Live 3D imaging and mapping of shear stresses within tissues using incompressible elastic beads

AUTHORS: Alexandre Souchaud, Arthur Boutillon, Gaëlle Charron, Atef Asnacios, Camille Noûs, Nicolas B David, François Graner, and François Gallet

I am very sorry and apologize for the very long time before being able to come back to you. I have now received all the referees reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

The overall evaluation is clearly positive and we would like to publish a revised manuscript in Development, provided that the referees' comments can be satisfactorily addressed. Please attend to all of the reviewers' comments in your revised manuscript and detail them in your point-by-point response. If you do not agree with any of their criticisms or suggestions explain clearly why this is so.

We are aware that you may currently be unable to access the lab to undertake experimental revisions. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Reviewer 1

Advance summary and potential significance to field

In this work, the authors develop and characterise a new method to infer mechanical stresses in biological tissues, using incompressible elastic beads.
This is important, as measuring absolute quantities of mechanical stresses remain difficult, and is an outstanding frontier of quantitative biophysics. The paper is well-written and reviews well the different other techniques present in the literature. The author first validate their techniques before applying them to two classical model systems in vitro and in vivo. Overall, this is a potentially interesting paper, and the range of approaches/model systems is commendable. The modelling is sound and well-articulated with the rest of the paper. However, there are a number of significant issues (see below) that would need to be addressed before publications, in particular related to data presentations, statistics number of repetitions and relationship to other models in the literature.

Comments for the author

Major points
1. The “Macroscopic rheometry” part was not very clear: I didn’t understand why the authors were performing the measurements during the gelification procedure (at high temperatures of 60 or 80 degrees very different from the ones that will be used during the actual biological measurements). There is also no associated main or supplementary figures that this cites, so it’s hard to exactly follow and evaluate the exact rheological experiments.

2. The findings on dominantly orthoradial directions of stresses in aggregate, and a trend in the absolute stresses are interesting, but they are not very discussed in relationship with the literature and past modelling approach. Delarue et al Interface Focus 2014 for instance discuss extensively the anisotropy of cellular shape in aggregates, and explains them via a theory of aggregate mechanics with anisotropic stresses, which would seem to be quite related to the direct experimental measurements of stresses here?

3. In the “In situ calibration in aggregates” section similarly, it would be nice to see more “raw” data: the authors show one image of the deformation, and then straight away the measurements of stress vs strain. But it would be really nice I think to have an intermediary figure show the temporal evolution of the different quantities (especially to follow better the color codes and differences between yellow and red/blue crosses, which could be made more intuitive i think directly in the figure.

4. In the Zebrafish part, again the authors show large-scale pictures, and schematics, but no “raw” data (both in Fig. 6-7, and in Supplementary). i guess the beads are not measured from the brightfield pictures of Fig 6a,c? It would be important to add the “mesoscale” view of these experiments, to show how much of the tissue is imaged, where the beads are exactly in xyz, how do they evolve in time for the ones that the authors have been able to follow in time (if not in the main figures, then at least as supplementary figures and movies), otherwise it’s hard to evaluate some aspects of this.

5. I appreciate that the authors are careful with their statements, and clearly say that Fig. 7 is only one sample. However, i find that presenting as an entire figure a single event is problematic, as the authors say themselves that the author 6 cases that they followed did not show significant changes. Of course it could be the one event was at a “privileged” location where stresses does vary but one could also imagine that it is a very abnormal/special event, so that i don’t think one can use it to say “its occurrence demonstrates that the technique enables to follow the time evolution of the shear stress tensor during the prechordal plate migration”. In my opinion, if the authors do not see this again upon repeating the experiment, this would be better suited for a supplementary item (the Discussion talks about “events” with plural). Could the authors also conjecture on why this event might be special? is it in a special location in the fish? does it sit for instance between different populations (the authors mention prechordal plate and notochord for instance?)

Minor points:
1. “Coating the PDMS with cell adhesion proteins is also possible in principle. “:
Is there a reference for this where this is done, or is this based on the experience/trials of the authors?
2. There are a typo in the Latex compilation (“??”) at the end of the legend of Fig. 4 3. Statistical details are missing in a number of places (for instance are error bars SD or SEM in Fig. 5 and 6)
4. ”possibly to reach a maximum and decrease when approaching the edge of the aggregate." seem too strong given the error bars, and should be removed in my opinion.

Reviewer 1

Advance summary and potential significance to field

This manuscript describes a new experimental method to measure mechanical stresses in biological tissues using non-compressible PDMS sensors. The work is convincing and the combination of compressions with beads measurements is a nice validation method. Combining in vitro and in vivo experiments demonstrates the experimental feasibility of the method (i.e. embedding the beads in different tissues). In future works, it would be interesting to compare measurements from compressible beads versus PDMS beads.

Comments for the author

I have found no major issues and believe the work deserves publication after the following questions have been addressed.

Line 117: Could the authors detail more why it is easier to use elastic beads than liquid beads?
Line 138: In general, could the authors discuss more the interest of using incompressible beads versus compressible beads?
Line 186: Could the authors discuss why it is more interesting to have monodisperse beads than polydisperse?
Line 371: Did the authors quantify experimentally deviations from the ellipsoid shape and would these deviations be of some interest (spectral analysis in space and time for example for studying tissue fluctuations)?
Line 421: Could the authors state more precisely what is the success rate of beads injection within the prechordal plate?
Line 609 : I do not understand the meaning of the value of gamma_c as this depends on the specific adhesions at the surface between the beads and the tissue, which is unknown. Could you discuss in more detail the possibility of coating the PDMS beads with specific adhesion proteins?
Line 651: There is a ref to an equation or Figure missing in the Figure legend.
Line 660: Do the authors believe that embedding beads of different radius in the same compression experiment would help determining gamma_c, which seems to be unknown in reality. Could the remodeling of adhesions at the interface between the bead and the tissue during the rapid compression make vary gamma_c and explain the deviation between the global stress and local stress measured in the yellow points corresponding to the timepoints just after compression ?

Figure 5: It seems quite unintuitive that the stress would be positive in the zz component as we would expect a negative component due to the spreading. Intuitively, I would have expected to see an eigenvector of the stress along the r vector in 3D spherical coordinates with a negative eigenvalue corresponding to compression. Could the authors compute the projection of the stress along the 3D radial axis? Again, for beads near the aggregate surface, I would expect to have a compression along the axis normal to the aggregate surface.

Line 722: Could the authors comment more about that? Is it due to plasticity events such as cell rearrangements?

Line 933: I do not understand how the z axis is defined here, is it locally normal to the embryo surface or is it the absolute z axis corresponding to the optical axis?

Line 1010: Did you correct for the psf in the z axis? Did you try image deconvolution in z?

Line 1025: Is there a generic physical explanation for such variation?

First revision

Author response to reviewers' comments

Dear Editor,

Please find attached a revised version of our manuscript « Live 3D imaging and mapping of shear stresses within tissues using incompressible elastic beads », by Souchaud et al., for resubmission in Development.

We would like to sincerely thank both reviewers for their positive opinion and for their critical reading of the manuscript. As listed below in the detailed answers, we have taken all their remarks into account, and we have modified accordingly the manuscript and the Supporting Information, in order to clarify some ambiguous points, and to add some information helpful for a better understanding.

Our main modifications are:
- Adding supplementary figures S2, S3, S4, S6.
- Adding explanations and discussions (in red).
- Adding two references: Towolfe 2004, Schneider 2011 (see line 157)

We hope that this significantly improved version now meets the requirements for a final acceptance in Development.

Best regards, F Gallet

Answer to Referee 1

Major points
1. The “Macroscopic rheometry” part was not very clear: I didn’t understand why the authors were performing the measurements during the gelification procedure (at high temperatures of 60 or 80 degrees very different from the ones that will be used during the actual biological measurements). There is also no associated main or supplementary figures that this cites, so it’s hard to exactly follow and evaluate the exact rheological experiments.

We added some explanations in this section, that hopefully clarify the protocol: the shear modulus of the bulk gel μ_b is measured on the plateau at the end of the polymerizing process, and it is compared to the final shear modulus of the sensors prepared in the same manner, i.e. after baking them at T=80°C during 3 h. In both cases the plateau value is the reference value. We also added a supplementary figure (S2) showing the evolution of G’ versus time during the gelification process for a bulk sample. Once the plateau is reached, the elastomer is fully polymerized, its shear modulus remains constant with time and does not depend on the operating temperature.

2. The findings on dominantly orthoradial directions of stresses in aggregate, and a trend in the absolute stresses are interesting, but they are not very discussed
in relationship with the literature and past modelling approach. Delarue et al, Interface Focus 2014 for instance discuss extensively the anisotropy of cellular shape in aggregates, and explains them via a theory of aggregate mechanics with anisotropic stresses, which would seem to be quite related to the direct experimental measurements of stresses here?

The discussion about this point has been reshaped, and a detailed comparison with the work by Delarue et al. has been added (see Section: discussion).

3. In the “In situ calibration in aggregates” section similarly, it would be nice to see more “raw” data: the authors show one image of the deformation, and then straight away the measurements of stress vs strain. But it would be really nice I think to have an intermediary figure show the temporal evolution of the different quantities (especially to follow better the color codes and differences between yellow and red/blue crosses, which could be made more intuitive i think directly in the figure.

Following the reviewer suggestion, we have added a supplementary figure (S3) showing raw data of the evolution of the aggregate stress and sensor deformations versus time, for the same data set as in figure 4c, using the same color code. Indeed, it enlightens the two different relaxation regimes between the short (t<30s) and longer (t>30s) time scales and helps to understand the stress-deformation relationship shown in fig 4c. We have added the corresponding explanations in the section: in situ calibration of aggregates (see lines 737- 750).

4. In the Zebrafish part, again the authors show large-scale pictures, and schematics, but no “raw” data (both in Fig. 6-7, and in Supplementary). i guess the beads are not measured from the brightfield pictures of Fig 6a,c? It would be important to add the “mesoscale” view of these experiments, to show how much of the tissue is imaged, where the beads are exactly in xyz, how do they evolve in time for the ones that the authors have been able to follow in time (if not in the main figures, then at least as supplementary figures and movies), otherwise it’s hard to evaluate some aspects of this.
The new Figure S4 shows several bright field and fluorescence images of a sensor inside the PPl, both at the whole PPl scale, and zoomed on the deformed sensor. (see supp. info. and line 948)

5. I appreciate that the authors are careful with their statements, and clearly say that Fig. 7 is only one sample. However, i find that presenting as an entire figure a single event is problematic, as the authors say themselves that the other 6 cases that they followed did not show significant changes. Of course it could be the one event was at a “privileged” location where stresses does vary, but one could also imagine that it is a very abnormal/special event, so that i don’t think one can use it to say “its occurrence demonstrates that the technique enables to follow the time evolution of the shear stress tensor during the prechordal plate migration”. In my opinion, if the authors do not see this again upon repeating the experiment, this would be better suited for a supplementary item (the Discussion talks about “events” with plural). Could the authors also conjecture on why this event might be special? is it in a special location in the fish? does it sit for instance between different populations (the authors mention prechordal plate and notochord for instance?)
In the revised version, we argue that this single event has at least a physical meaning and cannot be an experimental artifact related to image analysis. Indeed, new Fig. S6 shows that the volume of the sensor remains constant while its shape changes. This check gives confidence in the measurement of the shear stresses. Of course, no biological interpretation of this event can be proposed at this stage. This observation validates the technique developed here to measure shear stresses in tissues, which is the main purpose of the paper. We brought modifications to clarify this point in the concerned paragraph and also in the conclusion.

Minor points:

1. “Coating the PDMS with cell adhesion proteins is also possible in principle. “: Is there a reference for this where this is done, or is this based on the experience/trials of the authors?
We have added two references describing techniques used to modify PDMS surface and to graft fibronectin on it (see line156). We also attempted to graft cadherins on the sensors, but a fully reliable protocol is not yet available.

2. There are a typo in the Latex compilation (“??”) at the end of the legend of Fig. 4
Corrected

3. Statistical details are missing in a number of places (for instance are error bars SD or SEM in Fig. 5 and 6)
The errors bars on Fig. 5 and 6 represent standard deviations. This has been added in the captions.

4. “possibly to reach a maximum and decrease when approaching the edge of the aggregate."

seem too strong given the error bars, and should be removed in my opinion.
We agree. The new formulation underlines that the observed maximum might not be significant (see line 823).

Answer to Referee 2
Line 117: Could the authors detail more why it is easier to use elastic beads than liquid beads?
We underline in the revised version (line 105) that the use of liquid droplets remains a tour de force. Elastic beads are easier to use in terms of synthesis, manipulation, and data analysis. In particular, in linear deformations, an elastic sphere transforms into an ellipsoid, which main axes and anisotropy yield exactly the required information to determine the sphere strain deviator and thus the tissue stress deviator (Equation 1).

Line 138: In general, could the authors discuss more the interest of using incompressible beads versus compressible beads?
Incompressible sensors are directly sensitive to the shear stress tensor, i.e. to the stress anisotropy in the tissue, while compressible sensors are mainly sensitive to the local pressure inside the tissue (in principle the full stress tensor can be retrieved, but the accuracy on the shear stress, measured by difference, is poorer than with incompressible sensors). According to the question to be solved, one or the other method can be chosen (see lines 162-166).

Line 186: Could the authors discuss why it is more interesting to have monodisperse beads than polydisperse?
Here the monodisperse distribution is not a requirement, but it allows to identify possible optical artefacts in image analysis: we discarded images of beads with a measured radius out of the expected range (see lines 230-234). As another example, new Fig. S6 shows that the sensor shape may change while its volume is checked to be constant and compatible to what is expected. Also, monodispersity avoids to take into account the dependence of the sensor mechanical calibration with its radius (see equation 8).

Line 371: Did the authors quantify experimentally deviations from the ellipsoid shape and would these deviations be of some interest (spectral analysis in space and time for example for studying tissue fluctuations)?
Indeed it would be quite interesting to detect deviations from the ellipsoïd shape, and thus possible variations of the stress tensor at a scale smaller than the sensor's size, but the accuracy of the image analysis did not allow us to detect such higher order deformations (see line 393).

Line 421: Could the authors state more precisely what is the success rate of beads injection within the prechordal plate?
The success rate of sensor injection inside the PPl is about 50%. This is now stated in the article (line 434).

Line 609 : I do not understand the meaning of the value of gamma_c as this depends on the specific adhesions at the surface between the beads and the tissue, which is unknown.
Indeed gamma_c depends on the properties of the PDMS/tissue interface, but in principle it can be measured by varying the sensor's radius (see answer to line 660)

Could you discuss in more detail the possibility of coating the PDMS beads with specific adhesion proteins?
As mentioned in the answer to Reviewer 1's minor point 1, we have added two references describing techniques used to modify PDMS surface and to graft fibronectin on it (see line 156). We also attempted to graft cadherins on the sensors, but a fully reliable protocol is not yet available.

Line 651: There is a ref to an equation or Figure missing in the Figure legend.
Corrected

Line 660: Do the authors believe that embedding beads of different radius in the same compression experiment would help determining gamma_c, which seems to be unknown in reality.
According to Eq. (10), μ_e depends on gamma_c and on the bead radius a. In principle, it is possible to retrieve gamma_c by varying a, provided that μ_e is independently measured with a good accuracy.

Could the remodeling of adhesions at the interface between the bead and the tissue during the rapid compression make vary gamma_c and explain the deviation between the global stress and local stress measured in the yellow points corresponding to the timepoints just after compression ?
It is likely that during the first 30 seconds after compression, the stress relaxation in the aggregate is dominated by several rapid relaxation processes. Adhesion remodelling may be one of them, together with cytoplasm viscoelasticity or T1 processes. This has been added in the text (lines 743-746).

Figure 5: It seems quite unintuitive that the stress would be positive in the zz component as we would expect a negative component due to the spreading.
Intuitively, I would have expected to see an eigenvector of the stress along the r vector in 3D spherical coordinates with a negative eigenvalue corresponding to compression. Could the authors compute the projection of the stress along the 3D radial axis? Again, for beads near the aggregate surface, I would expect to have a compression along the axis normal to the aggregate surface.

We have added a note in the caption, referring to the Discussion, where it is explained that the systematic apparent elongation of the sensors in the z direction might be an artefact related to the imaging method.

Line 722: Could the authors comment more about that? Is it due to plasticity events such as cell rearrangements?
The discussion concerning the rapid relaxation regime after compression has been deepened in the text of the article (lines 737-750) and is also illustrated in the new Figure S3 in supplementary files. See also comments about line 660.

Line 933: I do not understand how the z axis is defined here, is it locally normal to the embryo surface or is it the absolute z axis corresponding to the optical axis?

We added in the caption of Fig.6 that the axis z is normal to the PPl and is confounded with the optical axis. The PPl being parallel to the surface, the z axis is also locally normal to the embryo surface.

Line 1010: Did you correct for the psf in the z axis? Did you try image deconvolution in z?
We did not apply such corrections to our images. As stated in line 1046, light diffusion by the heterogeneities of the tissue is important and might be the principal limit to the image quality.

Line 1025: Is there a generic physical explanation for such variation?
Following minor comment #4 of the first referee, we point out that the observed maximum of sigma observed in our case along the radial direction might not be significant (line 823).
Before attempting any interpretation of such variations, further experiments are required to comfort this result.

Second decision letter

MS ID#: DEVELOP/2021/199765

MS TITLE: Live 3D imaging and mapping of shear stresses within tissues using incompressible elastic beads

AUTHORS: Alexandre Souchaud, Arthur Boutillon, Gaëlle Charron, Atef Asnacios, Camille Noûs, Nicolas B David, François Graner, and François Gallet

I am really sorry and apologise for the delay. I have now received all the referees reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

The overall evaluation is very positive and we would like to publish your manuscript in Development. I agree with reviewer 1 that Figure 7 ought to be in supplement since this is based on a single occurence. While interesting, this is hardly an important and strong point in the manuscript. Please revise the manuscrit accordingly and I will proceed with formal acceptance thereafter.

Reviewer 1

Advance summary and potential significance to field

The authors have provided additional supplementary view of the bead deformations and additional detail on the experimental procedures + discussion of past findings. Together, this improved and clarified the manuscript.

Comments for the author

My main remaining worry is on my previous comment on Figure 7, which i don't think was addressed. I think the general approach of stating
"We were able to follow the evolution of the stress components for 7 sensors during 15 to 30 min, at different stages of epiboly. They did not show any significant changes, except for one event which we describe now." is a strange one (making an entire figure of the main text on n=1 event not representative of the rest of the data is something i've never seen). I would think main figures should reflect the most frequent observation that the authors made, although there could be brief mention in Supplementary of some fine and rare features of the dataset.

Even if it's a "real" event from the point of view of detection, this could still be something very special and non-physiologically relevant biologically (this was what i meant in my previous review: observing this once doesn't mean you can measure biologically relevant forces that occur in normal gastrulation - since it seems like the other 6 cases had no discernible trend). I would still strongly advise the authors to significantly change this part the paper - either remove a big chunk of it including Fig 7 or add more data.

Reviewer 2

Advance summary and potential significance to field

his manuscript describes a new experimental method to measure mechanical stresses in biological tissues using non-compressible PDMS sensors. The work is convincing and the combination of compressions with beads measurements is a nice validation method. Combining in vitro and in vivo experiments demonstrates the experimental feasibility of the method (i.e. embedding the beads in different tissues).

Comments for the author

I am satisfied with the revisions performed by the authors. In my opinion, the manuscript is suitable for publication. I am looking forward to read the final published version in Development.

Second revision

Author response to reviewers' comments

Following the recommendation of reviewer 1, we have suppressed the whole paragraph "Time Evolution", and the associated figure, from the section : "Stress distribution in the prechordal plate of zebrafish embryos", and we moved them to the Supporting Information.

The rest of the paper was adapted accordingly, in order to remove or modify all mentions of the time evolution of the stress
- abstract (line 30)
- introduction (line 183)
- in the discussion, we modified line 1035 to 1039 + line 1048

Third decision letter

MS ID#: DEVELOP/2021/199765

MS TITLE: Live 3D imaging and mapping of shear stresses within tissues using incompressible elastic beads

AUTHORS: Alexandre Souchaud, Arthur Boutillon, Gaëlle Charron, Atef Asnacios, Camille Noûs, Nicolas B David, François Graner, and François Gallet
ARTICLE TYPE: Techniques and Resources Article

I am very happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.