Membrane shrinkage and cortex remodelling are predicted to work in harmony to retract blebs

Thomas E. Woolley, Eamonn A. Gaffney and Alain Goriely

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Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History
RSOS-150101.R0 (Original submission)

Review form: Reviewer 1

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Is it clear how to access all supporting data?
Yes, except for my comments about the numerical solution.

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No
Recommendation?
Major revision is needed (please make suggestions in comments)

Comments to the Author(s)
The authors present the fourth in a series of papers on cell blebbing. Here they describe possible phenomenological mechanisms for retraction of a bleb, and identify that, if the governing mechanisms are membrane and cortex shrinking with the membrane and cortex connected by proteins, then both the membrane and cortex need to shrink at roughly the same rate to enable retraction. Given the arclengths of the membrane and cortex, and regions where they are connected by adhesion proteins, force balance arguments lead to numerical solutions for the shape of the cell. The mathematical modelling is consistent with the hypotheses. The results are interesting but I am concerned that the hypotheses may be unrealistic and the paper needs to be revised to address this (see point 2 below).

Main comments:

1. Although much of the derivation of the model has been published before, there does needs to be some discussion of the numerical methods used and, crucially, evidence that the numerical solutions are accurate should be given.

2. Imposing phenomenological rules for the shrinking of the membrane and cortex in the unstressed reference configuration ignores forces on the membrane and cortex in the current configuration that may well control shrinkage. In addition, Figure 1 suggests that there is localised thickening of the cortex (highly polymerised actin) within the bleb as it retracts. What justification is there for neglecting these mechanisms?

3. Please discuss the biological arguments for possible mechanisms for membrane growth and retraction.

Minor comments:

Ref. 19 is incomplete.

Several initial phrases need to be separated from their main clauses by commas for the sake of clarity, e.g. in the second sentence of the Abstract.

Q in (2.7) should be Q_s.

At the end of line 46 on page 5, add 'in (2.5)' for clarity.

In (2.9). replace 'E(\sigma)^2' by 'E^2(\sigma)'.

On line 11 of page 6 and elsewhere, the notation for units in '100/3/\mu m' is better written as '{\mu m}^{-1}.'

Page 6, line 15: 'because' is redundant or text is missing.

Page 6, line 29: 'As demonstrated IN [19]'.

Page 3, line 21: 'solid mechanicS'.


Review form: Reviewer 2

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Is it clear how to access all supporting data?
Ref. 19 is not available

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No

Recommendation?
Accept with minor revision (please list in comments)

Comments to the Author(s)
This study extends prior work by the authors on a mechanical model of cell blebbing. The model describes interaction between the cell membrane, modeled as a thin shell, and the underlying actin cortex. The model predicts that combined cortical shrinkage and membrane shrinkage can together describe bleb retraction.

Prior work by the authors (ref. 19) is referred to frequently yet is not available for reference, and is presumably under review elsewhere. I cannot therefore independently assess the novelty of the present work in relation to material contained in ref. 19. Subject to this caveat, however, the work as a whole appears original and interesting. My few comments below relate primarily to presentation.

I notice the work is listed as a review, yet focuses primarily on the authors’ own model. I am therefore judging it as a normal article. The unnecessary web link at the bottom of each printed page meant that I could not clearly read some equations and text.

The paper’s title is long and contains the phrase “and suggests that bleb induced blebbing may occur.” However this is not supported by direct evidence, beyond a throwaway phrase at the end of the discussion. The first half of the title is sufficient (but change “is” to “are”).

It is disappointing that there is limited direct experimental data provided in support of the model’s predictions. Measurements of pressure for example would be key in testing the predictions in Fig 8a. To avoid the risk of overselling the results, please avoid the term “phenotypes” (page 6), as it suggests the model somehow directly accounts for gene expression.

The model equations 2.1-2.10 could usefully be described in a little more detail, highlighting force and moment balances for example. It is curious to use the label y (described as a “vertical” coordinate) in an axisymmetric problem when it represents a radial distance from the axis.

In Table 1, please distinguish variables from parameters, as it is helpful for the reader to see the number of unknown parameters that must be determined. eta_1, eta_2 and delta are missing
from the list, and from Table 2. I don’t understand why the key equations in parts b and c of the appendix do not appear in the main text - they are central to the model.

Bottom of page 11: “bleb expansion greatly reduces … pressure” - the qualifier “greatly” is used here on the basis of a single example. Is this a robust statement?

Decision letter (RSOS-150101)

13-Apr-2015

Dear Dr Woolley:

Manuscript ID RSOS-150101 entitled “Membrane shrinkage and cortex remodelling is predicted to work in harmony to retract blebs and suggests that bleb induced blebbing may occur” which you submitted to Royal Society Open Science, has been reviewed. The comments from reviewers are included at the bottom of this letter.

In view of the criticisms of the reviewers, the manuscript has been rejected in its current form. However, a new manuscript may be submitted which takes into consideration these comments.

Please note that resubmitting your manuscript does not guarantee eventual acceptance, and that your resubmission will be subject to peer review before a decision is made.

You will be unable to make your revisions on the originally submitted version of your manuscript. Instead, revise your manuscript and upload the files via your author centre.

Once you have revised your manuscript, go to https://mc.manuscriptcentral.com/rsos and login to your Author Center. Click on "Manuscripts with Decisions," and then click on "Create a Resubmission" located next to the manuscript number. Then, follow the steps for resubmitting your manuscript.

Your resubmitted manuscript should be submitted by 11-Oct-2015. If you are unable to submit by this date please contact the Editorial Office.

I look forward to a resubmission.

Sincerely,

Emilie Aime
Senior Publishing Editor, Royal Society Open Science
openscience@royalsociety.org

Associate Editor Comments to Author:
Associate Editor: 1
Comments to the Author:
Please revise the paper as recommended by the two reviewers, and resubmit it together with a full description of all changes made.

Reviewers' Comments to Author:
Reviewer: 1

Comments to the Author(s)
This study extends prior work by the authors on a mechanical model of cell blebbing. The model describes interaction between the cell membrane, modeled as a thin shell, and the underlying
actin cortex. The model predicts that combined cortical shrinkage and membrane shrinkage can
together describe bleb retraction.

Prior work by the authors (ref. 19) is referred to frequently yet is not available for reference, and
is presumably under review elsewhere. I cannot therefore independently assess the novelty of
the present work in relation to material contained in ref. 19. Subject to this caveat, however, the
work as a whole appears original and interesting. My few comments below relate primarily to
presentation.

I notice the work is listed as a review, yet focuses primarily on the authors’ own model. I am
therefore judging it as a normal article. The unnecessary web link at the bottom of each printed
page meant that I could not clearly read some equations and text.

The paper’s title is long and contains the phrase “and suggests that bleb induced blebbing may
occur.” However this is not supported by direct evidence, beyond a throwaway phrase at the
end of the discussion. The first half of the title is sufficient (but change “is” to “are”).

It is disappointing that there is limited direct experimental data provided in support of the
model’s predictions. Measurements of pressure for example would be key in testing the
predictions in Fig 8a. To avoid the risk of overselling the results, please avoid the term
“phenotypes” (page 6), as it suggests the model somehow directly accounts for gene expression.

The model equations 2.1-2.10 could usefully be described in a little more detail, highlighting force
and moment balances for example. It is curious to use the label y (described as a “vertical”
coordinate) in an axisymmetric problem when it represents a radial distance from the axis.

In Table 1, please distinguish variables from parameters, as it is helpful for the reader to see the
number of unknown parameters that must be determined. eta_1, eta_2 and delta are missing
from the list, and from Table 2. I don’t understand why the key equations in parts b and c of the
appendix do not appear in the main text - they are central to the model.

Bottom of page 11: “bleb expansion greatly reduces … pressure” - the qualifier “greatly” is used
here on the basis of a single example. Is this a robust statement?

Reviewer: 2

Comments to the Author(s)
The authors present the fourth in a series of papers on cell blebbing. Here they describe possible
phenomenological mechanisms for retraction of a bleb, and identify that, if the governing
mechanisms are membrane and cortex shrinking with the membrane and cortex connected by
proteins, then both the membrane and cortex need to shrink at roughly the same rate to enable
retraction. Given the arclengths of the membrane and cortex, and regions where they are
connected by adhesion proteins, force balance arguments lead to numerical solutions for the
shape of the cell. The mathematical modelling is consistent with the hypotheses. The results are
interesting but I am concerned that the hypotheses may be unrealistic and the paper needs to be
revised to address this (see point 2 below).

Main comments:

1. Although much of the derivation of the model has been published before, there does needs to
be some discussion of the numerical methods used and, crucially, evidence that the numerical
solutions are accurate should be given.

2. Imposing phenomenological rules for the shrinking of the membrane and cortex in the
unstressed reference configuration ignores forces on the membrane and cortex in the current
configuration that may well control shrinkage. In addition, Figure 1 suggests that there is
localised thickening of the cortex (highly polymerised actin) within the bleb as it retracts. What justification is there for neglecting these mechanisms?

3. Please discuss the biological arguments for possible mechanisms for membrane growth and retraction.

Minor comments:

Ref. 19 is incomplete.

Several initial phrases need to be separated from their main clauses by commas for the sake of clarity, e.g. in the second sentence of the Abstract.

Q in (2.7) should be $Q_s$.

At the end of line 46 on page 5, add 'in (2.5)' for clarity.

In (2.9), replace 'E(\sigma)^2' by 'E^2(\sigma)'.

On line 11 of page 6 and elsewhere, the notation for units in '100/3/\mu m' is better written as '\mu m^{-1}.'

Page 6, line 15: 'because' is redundant or text is missing.

Page 6, line 29: 'As demonstrated IN [19].'

Page 3, line 21: 'solid mechanic$\$'.

Author's Response to Decision Letter for (RSOS-150101)

See Appendix A.

RSOS-150184.R1 (Revision)

Review form: Reviewer 1

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Is it clear how to access all supporting data?
No supporting data provided

Do you have any ethical concerns with this paper?
No
Have you any concerns about statistical analyses in this paper?  
No

Recommendation?  
Accept with minor revision (please list in comments)

Comments to the Author(s)  
The paper is improved and I recommend it for publication.
Page 3, line 20: remove "as"
Page 4: (2.2) is not a purely geometrical relationship
Page 10, line 4: remove "are"
Page 11, line 51: add "is" after "process"
Page 14, line 19: add "are" before "stretched"

Review form: Reviewer 2

Is the manuscript scientifically sound in its present form?  
Yes

Are the interpretations and conclusions justified by the results?  
Yes

Is the language acceptable?  
Yes

Is it clear how to access all supporting data?  
N/A

Do you have any ethical concerns with this paper?  
No

Have you any concerns about statistical analyses in this paper?  
No

Recommendation?  
Accept with minor revision (please list in comments)

Comments to the Author(s)  
I am content with the responses and changes to the paper, except that it is important to include some appropriate text explicitly dealing with the issue that Figure 1 really does seem to show localized thickening of the cortex but this is absent from the model.

Decision letter (RSOS-150184)

26-Jun-2015

Dear Dr Woolley,

The Subject Editor assigned to your paper ("Membrane shrinkage and cortex remodelling is predicted to work in harmony to retract blebs") has now received comments from reviewers. We would like you to revise your paper in accordance with the referee and Subject Editor suggestions which can be found below (not including confidential reports to the Editor). Please note this decision does not guarantee eventual acceptance.
Please submit a copy of your revised paper within three weeks (i.e. by the 19-Jul-2015). If we do not hear from you within this time then it will be assumed that the paper has been withdrawn. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office in advance. We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Editors, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers.

To revise your manuscript, log into http://mc.manuscriptcentral.com/rsos and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision. Revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you must respond to the comments made by the referees and upload a file "Response to Referees" in "Section 6 - File Upload". Please use this to document how you have responded to each of the comments, and the adjustments you have made. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response.

In addition to addressing all of the reviewers' and editor's comments please also ensure that your revised manuscript contains the following sections before the reference list:

- Ethics statement
  If your study uses humans or animals please include details of the ethical approval received, including the name of the committee that granted approval. For human studies please also detail whether informed consent was obtained. For field studies on animals please include details of all permissions, licences and/or approvals granted to carry out the fieldwork.

- Data accessibility
  It is a condition of publication that all supporting data are made available either as supplementary information or preferably in a suitable permanent repository. The data accessibility section should state where the article's supporting data can be accessed. This section should also include details, where possible of where to access other relevant research materials such as statistical tools, protocols, software etc can be accessed. If the data has been deposited in an external repository this section should list the database, accession number and link to the DOI for all data from the article that has been made publicly available. Data sets that have been deposited in an external repository and have a DOI should also be appropriately cited in the manuscript and included in the reference list.

- Competing interests
  Please declare any financial or non-financial competing interests, or state that you have no competing interests.

- Authors’ contributions
  All submissions, other than those with a single author, must include an Authors’ Contributions section which individually lists the specific contribution of each author. The list of Authors should meet all of the following criteria; 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published.

All contributors who do not meet all of these criteria should be included in the acknowledgements.
We suggest the following format: AB carried out the molecular lab work, participated in data analysis, carried out sequence alignments, participated in the design of the study and drafted the manuscript; CD carried out the statistical analyses; EF collected field data; GH conceived of the study, designed the study, coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

• Acknowledgements
Please acknowledge anyone who contributed to the study but did not meet the authorship criteria.

• Funding statement
Please list the source of funding for each author.

Once again, thank you for submitting your manuscript to Royal Society Open Science and I look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Yours sincerely,
Emilie Aime
Senior Publishing Editor, Royal Society Open Science
openscience@royalsociety.org

Comments to Author:
Associate Editor's comments:
Associate Editor
Comments to the Author:
Both reviewers are happy with the paper apart from some minor comments (a short list of typos from one and a request from the other to explain the cortex thickening shown in Fig 1). My recommendation is that the paper should be accepted subject to these changes being made.

Reviewers' Comments to Author:
Reviewer: 1

Comments to the Author(s)
The paper is improved and I recommend it for publication.
Page 3, line 20: remove "as"
Page 4: (2.2) is not a purely geometrical relationship
Page 10, line 4: remove "are"
Page 11, line 51: add "is" after "process"
Page 14, line 19: add "are" before "stretched"

Reviewer: 2

Comments to the Author(s)
I am content with the responses and changes to the paper, except that it is important to include some appropriate text explicitly dealing with the issue that Figure 1 really does seem to show localized thickening of the cortex but this is absent from the model.

Author's Response to Decision Letter for (RSOS-150184)
See Appendix B.
Membrane shrinkage and cortex remodelling are predicted to work in harmony to retract blebs (and suggests that bleb induced blebbing may occur).

Thomas E. Woolley, Eamonn A. Gaffney, Alain Goriely
University of Oxford, Andrew Wiles Building, Radcliffe Observatory Quarter, Woodstock Road, Oxford, OX2 6GG.

May 4, 2015

1 Reviewer 1

Prior work by the authors (ref. 19) is referred to frequently yet is not available for reference, and is presumably under review elsewhere. I cannot therefore independently assess the novelty of the present work in relation to material contained in ref. 19. Subject to this caveat, however, the work as a whole appears original and interesting. My few comments below relate primarily to presentation.

We thank the reviewer and editor for their time and comments and hope that the accompanying changes meet with their satisfaction. In addition to the changes discussed we have used this revision to make small changes aimed at improving style and presentation. Reference 19 has just been accepted for publication in the Journal of Theoretical Biology. The citation has been updated to reflect this, however, only the DOI is available as it an “online first” article. In terms of novelty, the work in this paper and reference 19 are completely separate. The previous paper deals with the production of blebs with the correct expansion features, as such it does not consider a reforming cortex and, consequently, does not seek to understand retraction in any way.

Please note that due to changes in the article some of the reference numbers have altered. In particular, reference 19 is now reference 20.

1. I notice the work is listed as a review, yet focuses primarily on the authors own model. I am therefore judging it as a normal article. The unnecessary web link at the bottom of each printed page meant that I could not clearly read some equations and text.

The reviewer is quite correct, this work should have been listed as a normal article. We apologise for the web link obscuring some of the text. However, this appears once the tex file has been compiled through the journal’s submission servers, thus, we do not believe we are able to alter this.

2. The papers title is long and contains the phrase “and suggests that bleb induced blebbing may occur.” However this is not supported by direct evidence, beyond a throwaway phrase at the end of the discussion. The first half of the title is sufficient (but change “is” to “are”).
The suggested changes have been incorporated into the manuscript. The title is now “Membrane shrinkage and cortex remodelling are predicted to work in harmony to retract blebs”.

3. **It is disappointing that there is limited direct experimental data provided in support of the models predictions. Measurements of pressure for example would be key in testing the predictions in Fig 8a.**

This is true and we hope that this and our previous work provides impetus for experimental groups to try and experimentally evaluate the intracellular pressure of blebbing cells. One particular avenue we are exploring is in collaboration with the University of Reading and University College London. Currently, the only paper we know of that tries to measure the pressure difference in blebbing cells is referenced in the paper: J. Dai and M. P. Sheetz. Membrane tether formation from blebbing cells. Biophys. J., 77(6):3363–3370, 1999. It is from this paper that we generate the value of $\Delta P$ that is used throughout the manuscript.

4. **To avoid the risk of overselling the results, please avoid the term “phenotypes” (page 6), as it suggests the model somehow directly accounts for gene expression.**

Phenotypes has been changed to morphologies

5. **The model equations 2.1-2.10 could usefully be described in a little more detail, highlighting force and moment balances for example.**

The equation list has been rewritten such that there are explicit labels by each equation. These labels, along with the accompanying detailed discussion of the equations, should help the reader better understand the construction of the system.

6. **It is curious to use the label $y$ (described as a “vertical” coordinate) in an axisymmetric problem when it represents a radial distance from the axis.**

The reviewer is correct that since we dealing with an axisymmetric problem we could have presented the problem as dependent on a radial variable instead of $y$. However, due to this symmetry we only need to consider a single plane of the solid, in which this radial variable and the normal Cartesian variable are identical. Hence, in order to reduce confusion, we reserve names that have rotational connotations to name spherically symmetric bodies, such as the initial cortex, $r_c$. When we are working on the cylindrically symmetric body, we prefer to work with the Cartesian variables instead. The second paragraph of Section 2 has been altered to emphasise this point

“Equation set (2.1) define the axisymmetrical geometry of the shell around the axis of rotational symmetry, here taken to be the z-axis (see Figure 2). Since the two-dimensional shell is axisymmetric we only need to consider a one-dimensional cross-section, at which point the radial coordinates can be related to the standard rectilinear Cartesian coordinates. Specifically, a reference configuration, $(\z, \y)$, corresponding to the unstressed state, is parameterized by its arc length, $\sigma$ and measured from the intercept of the curve with the $z$-axis.”

7. **In Table 1, please distinguish variables from parameters, as it is helpful for the reader to see the number of unknown parameters that must be determined. $\eta_1$, $\eta_2$ and delta are missing from the list, and from Table 2.**
Table 1 has been reorganised such that all of the variables are at the top and all of the parameters, including $\eta_1$ and $\eta_2$, are at the bottom. A small break in the table has been included to delimit the transition. Table 2 is specifically for parameters that are fixed throughout all simulations and are based on data. $\eta_1$ and $\eta_2$ do not have any specified values and, further, we are interested in their ratio, rather than their individual values, thus, we have not added them to Table 2. However, we have added the following sentence to the first paragraph of the results section “The value of the ratio $\eta_1/\eta_2$ can be found in the caption of each simulation.”

8. I don’t understand why the key equations in parts b and c of the appendix do not appear in the main text - they are central to the model.

We have added these sections into paragraph 3 and 8 of Section 2, respectively.

9. Bottom of page 11: “bleb expansion greatly reduces pressure” - the qualifier “greatly” is used here on the basis of a single example. Is this a robust statement?

The large reduction in pressure is seen throughout all our work on blebbing cells. In particular, this feature is observed in all models of reference 16, which deals with three similar, but different models of blebs that capture different levels of detail. To emphasise this point we have added further detail: “Namely, bleb expansion greatly reduces the intracellular pressure and, thus, the expansion force acting on the bleb, which matches the predictions of our previous work [15,16,20].”

2 Reviewer 2

The authors present the fourth in a series of papers on cell blebbing. Here they describe possible phenomenological mechanisms for retraction of a bleb, and identify that, if the governing mechanisms are membrane and cortex shrinking with the membrane and cortex connected by proteins, then both the membrane and cortex need to shrink at roughly the same rate to enable retraction. Given the arclengths of the membrane and cortex, and regions where they are connected by adhesion proteins, force balance arguments lead to numerical solutions for the shape of the cell. The mathematical modelling is consistent with the hypotheses. The results are interesting but I am concerned that the hypotheses may be unrealistic and the paper needs to be revised to address this.

We thank the reviewer for their comments and hope that the accompanying changes meet with their satisfaction.

1. Although much of the derivation of the model has been published before, there does need to be some discussion of the numerical methods used and, crucially, evidence that the numerical solutions are accurate should be given.

The whole simulation can be envisioned simply as an iterated root finding problem. Specifically, once the simulations are initialised, the boundary value problem is solved repeatedly with various different values of $\Delta P$ until the difference between the volume of the output solution and the initial volume constant is below some given tolerance.
Both the root finding and the boundary value problem were solved using algorithms from MATLAB R2013a, namely BVP5C and fsolve. The algorithms were used for their flexibility and ability to automatically control errors.

Due to the linearisation of the boundaries at $\sigma = 0$ and $\pi \rho$ (discussed in the appendix), we can only be accurate to within an order of $\epsilon^2 = 10^{-6}$ at these points, as such, we fixed the error tolerances in the codes to be $10^{-6}$. The maximum error in each simulation of the expansion and contraction simulation is illustrated in Figure 1 and demonstrates that this bound is attained after each iteration.

![Figure 1: During the expansion and contraction phases of the simulated bleb the maximum residual error over all variables never grows above $10^{-6}$ in magnitude.](image)

Although the reviewer is quite right to check our error terms, we do not believe that adding in a number of figures similar to Figure 1, would be of benefit. We have added the following paragraph which does evidence the fact that we have checked that the error terms stay small throughout the simulation. However, if the reviewer disagrees we are happy to put the figures in. The additional paragraph is as follows and occurs at the start of the results section.

"Before we discuss the results of bleb retraction, we first, briefly, address the matter of simulation work flow and, in particular, bleb production. The whole simulation can be envisioned as an iterated root finding problem. Specifically, upon initialisation, system (2.1)-(2.5) is solved as a boundary value problem (boundary constraints..."
are discussed in Appendix (a)) for various different values of $\Delta P$. The value of $\Delta P$ is iterated until the difference between the volume of the output solution and the initial volume constant is below a given tolerance. Once this tolerance is achieved the reference configuration and cortex are updated, if needed. Since each iteration only modifies the solution a small amount we would expect the next solution state to be approximately similar to the previous solution state. Thus the updated reference configuration and cortex curves are fed back into the root finding algorithm, along with the previous solution state. The algorithm uses the previous solution as an initialisation state, around which to search for the new solution. Throughout the simulation it was ensured that the numerical errors were never greater than the errors included due to the linearisation of the boundary points (data not shown).

2. **Imposing phenomenological rules for the shrinking of the membrane and cortex in the unstressed reference configuration ignores forces on the membrane and cortex in the current configuration that may well control shrinkage.** In addition, Figure 1 suggests that there is localised thickening of the cortex (highly polymerised actin) within the bleb as it retracts. **What justification is there for neglecting these mechanisms?**

   One should note that the system is extensively overdamped. The force balance per se does not dictate its evolution in that the system relaxes to its equilibrium very rapidly. Thus the forces in the current configuration will be compensated on very fast timescales by the relaxation of the cortex and membrane to quasi-static equilibrium. Such processes cannot be directly driving bleb retraction due to the mismatch of timescales. Instead it is more appropriate that the evolution of the properties of the cortex and membrane guide the retraction.

   Further the impact of the localised thickening of the cortex in Fig 1 is unclear as, for instance, it is not clear if it is accompanied with enhanced myosin aggregation and thus greater contractile forces. Without further information we have presented the simplest case of no change.

   These justifications suggest that a phenomenological model is justified and secondly that a more detailed model cannot be supported from the available data and understanding. Hence our choice of modelling framework, including the representation of retraction.

   The following paragraph has been added to the Bleb retraction sections

   “As the bleb is retracted the system is effectively always in quasi-steady state as the system is overdamped. Hence retraction is driven by remodelling timescales. Equally, the impact of any heterogeneity in the thickness of the cortex is unclear, because we cannot be sure that it is accompanied with enhanced myosin aggregation and, thus, greater contractile forces, or if a thicker cortex would be harder to manipulate and, thus, slower to retract. Without further information we have presented the simplest case of constant heterogeneous structures.”

3. **Please discuss the biological arguments for possible mechanisms for membrane growth and retraction.**

   Currently, it is not known, in the case of blebbing, how extra membrane can be produced and removed as quickly as observed. It has been hypothesised that extra membrane stems from high levels of wrinkling in the cellular surface. Alternatively,
it has been suggested that endo- and exo-cytosis processes could account for the membrane activity through localised recruitment. In both cases, the growth of the membrane can be modelled as an increase reference configuration, which is our approach.

In terms of retraction it is thought that myosin motors contract the cortex, causing it to shrink. To our knowledge there are currently no theories regarding the coupling the cortex contraction to the membrane shrinking. Due to the lack of knowledge at this level, we use a phenomenological description of membrane and cortex shrinking, which captures the idea that the myosin motors are causing the cortex to shrink, but is not based on further dynamics.

The second, third and fifth paragraphs of the introduction have been expanded to include these details,

“ If a cell’s internal pressure is higher than the external pressure the pressure difference induces a flow of the cell’s cytosol driving the membrane away from the cell and into a spherical protrusion, known as a bleb [8]. This localised swelling requires additional membrane to cover the bleb, but it is not currently known how this extra membrane can be produced and removed as quickly as observed. It has been hypothesised that extra membrane stems from high levels of wrinkling in the cellular surface [9]. Alternatively, it has been suggested that endo- and exo-cytosis processes could account for the membrane activity through localised recruitment [10]. In both cases, the growth of the membrane can be modelled as an increase in reference configuration, which is the approach taken here.

After approximately 10-30 seconds the bleb expansion stops and an actin cortex reforms inside the bleb. Over a longer time scale of 1-2 minutes the cell retracts the newly formed cortex within the bleb (which is coupled to the membrane), causing the bleb to shrink back into the cell, and allowing the process to begin again (see Figure 1). It is thought that myosin motors contract the cortex, causing it to shrink [10]. It is this retraction phase that we are interested in modelling in this paper because bleb retraction is required for cells to move efficiently.”

“Although blebbing is an extremely complex behaviour, the structural shape of a cell is thought to depend on three components: a flexible lipid bilayer membrane, a stiff actin cortex and adhesion proteins that couple these two structures [8]. Mathematical modelling offers a framework within which hypotheses can be tested, generating new predictions concerning the underlying mechanisms that control the blebbing expansion and retraction cycle. The hypothesis currently present in the biological literature is that blebs shrink simply due to the retraction of the blebs reformed cortex, implying that the membrane is slave to the dynamics of the cortex [10]. Here, we test the biophysical and mechanical plausibility of experimentally suggested mechanisms that induce bleb retraction. However, an absence of molecular-level knowledge entails that we implement a phenomenological model of membrane and cortex retraction which captures the concept that myosin motors induce cortex shrinkage, but without detailed dynamics.”

4. Ref. 19 is incomplete.

Reference 19 has just been accepted for publication in the Journal of Theoretical Biology. The citation has been updated to reflect this.

5. Several initial phrases need to be separated from their main clauses by commas for the sake of clarity, e.g. in the second sentence of the Abstract.
The article has been proofread again and additional commas have been added for clarity.

6. $Q$ in (2.7) should be $Q_s$. At the end of line 46 on page 5, add ‘in (2.5)’ for clarity. In (2.9), replace $E(\sigma)^2$ by $E^2(\sigma)$. On line 11 of page 6 and elsewhere, the notation for units in ‘100/3/µm’ is better written as $\mu m^{-1}$.

Page 6, line 15: ‘because’ is redundant or text is missing. Page 6, line 29: ‘As demonstrated IN [19]’. Page 3, line 21: ‘solid mechanicS’.

These small errors have all been corrected.
Membrane shrinkage and cortex remodelling are predicted to work in harmony to retract blebs.

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1 Alterations

We thank the reviewer and editor for their time and comments and hope that the accompanying changes meet with their satisfaction.

Reviewer 1 All of their small alterations have been included except for their suggestion that the word “are” should be removed form Page 10, line 4 as we could not see which word they were referring to.

Reviewer 2 In order to discuss the lack of cortex thickening in our model we highlight this process in the introduction with the addition of the line

“It is thought that myosin motors contract the cortex, causing it to shrink and, potentially, thicken.”

as well as the more detailed discussion in Section 2.(a)

“As the bleb is retracted the system is effectively always in quasi-steady state as the system is overdamped. Hence retraction is driven by remodelling timescales. Equally, the impact of any heterogeneity in the thickness of the cortex is unclear, because we cannot be sure that it is accompanied with enhanced myosin aggregation and, thus, greater contractile forces, or if a thicker cortex would be harder to manipulate and, thus, slower to retract. Without further information we have presented the simplest case of constant heterogeneous structures.”

We have also added the extra sections of Competing interests, Authors Contributions, Acknowledgements and Funding statement.