A Motor Clutch Model Predicts and Suggests Mechanisms of Cellular Morphological Response to Cyclic Force

Benjamin Scandling, Jia Gou, Jessica Thomas, Jacqueline Xuan, Chuan Xue, and Keith Gooch

Corresponding author(s): Keith Gooch, The Ohio State University

Review Timeline:

| Event                       | Date       |
|-----------------------------|------------|
| Submission Date             | 2020-01-31 |
| Editorial Decision          | 2020-03-06 |
| Revision Received           | 2021-10-25 |
| Accepted                    | 2021-11-10 |

Editor-in-Chief: Matthew Welch

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)
1st Editorial Decision

March 6, 2020

RE: Manuscript #E20-01-0087

TITLE: A Motor Clutch Model Predicts and Suggests Mechanisms of Cellular Morphological Response to Cyclic Force

Monitoring Editor (Remarks to Author):

Dear authors, both reviewers and I were impressed with the results; the main problem is messy presentation and connection between experiment and theory. Please take reviewers’ comments very seriously and rewrite the paper thoroughly. I will send it for the second look to both reviewers.

Sincerely,

Alexander Mogilner
Monitoring Editor
Molecular Biology of the Cell

------------------------------------------------------------------------

Dear Dr. Gooch,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor’s decision letter above and the reviewer comments below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor’s and reviewers’ comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter"). Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

Authors are allowed 180 days to submit a revision. If this time period is inadequate, please contact us at mboc@ascb.org.

Revised manuscripts are assigned to the original Monitoring Editor whenever possible. However, special circumstances may preclude this. Also, revised manuscripts are often sent out for re-review, usually to the original reviewers when possible. The Monitoring Editor may solicit additional reviews if it is deemed necessary to render a completely informed decision.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised manuscript, and figures, use this link: Link Not Available

Please contact us with any questions at mboc@ascb.org.

Thank you for submitting your manuscript to Molecular Biology of the Cell. We look forward to receiving your revised paper.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

------------------------------------------------------------------------

Reviewer #1 (Remarks to the Author):

Dear authors, both reviewers and I were impressed with the results; the main problem is messy presentation and connection between experiment and theory. Please take reviewers’ comments very seriously and rewrite the paper thoroughly. I will send it for the second look to both reviewers.
Understanding cell alignment under cyclic loading has remained enigmatic. These results show an important new theoretical prediction of how forces generated in the cell can interface with forces in the environment to result in specific cell and F-actin alignment, both parallel to and perpendicular to the direction of loading. For example, the ability to now theoretically explain the odd 70 degree orientation observed by many groups, a finding replicated here, is a great achievement, given that cyclic stretching is an important part of the physiology of heart and lung. The authors not only find a biophysical theory that finally explains this effect, they are also able to explain it in non-mathematical terms as well in terms of detachment events during loading-unloading cycles and the time delay before clutch reattachment. While this is all positive, the manuscript as written was difficult to follow in many respects, most notably in aligning the experiments with the specific simulations that corresponded to them. It seems as if there are a lot of simulated scenarios that are not investigated experimentally, although they may be explaining previously published observations that have lacked theoretical explanation. If this is the case, it needs to be made more explicit in the figures. One option would be show, or at least summarize this published work to allow the reader to make a direct comparison between experiment and simulation. As is stands, the experiments appear in only one figure (3), and the simulations in subsequent figures. So a better connection between the experiments, both those presented here and those previously published, and the simulations that inform the conclusions is critical. Thus, I am supportive of publication in principle, but recommend major revisions to achieve a clearer presentation of the connection between experiment and theory.

Additional comments:
1) "Many cells in the body experience cyclic mechanical loading and cyclic stretch can impact cellular processes and morphology." I think this is widely recognized, but it would still be good to put references in here. A lot of this work has been published in biophysics and bioengineering journals, and this study is being considered for a cell biology audience, so appropriate referencing would help.
2) The subheadings 1.1, 1.2, etc. do not seem necessary.
3) "The model consists of an F-actin filament, molecular clutches." I think it would be more appropriate to say "F-actin filament bundle" since I think that is what the authors mean.
4) Fig. 3. Need to show direction of stretch, presumably it is horizontal. Why in Fig. 3D is perpendicular elongation >1? Does the cell shown have perpendicular elongation less than 1?
5) Rather than "p-value < 0.0125" recommend "p < 0.0125" throughout.
6) Fig. 4. Title: make clear this is simulated actin filament reorientation.
7) Fig. 4. Instead of "Actin filament reorientation" suggest "Actin fiber reorientation," (or bundle) since individual filaments are not being tracked but actin fibers are. Use "fiber" instead of "filament" wherever it appears.
8) Fig. 7. Which scenario is correct? Can the authors sum the unbinding events together to see where optimal angle is? It seems some annotation could be added to the figure to help the reader understand how the results inform our understanding.
9) More information on statistics is required, especially exact sample size of the data set.

Reviewer #2 (Remarks to the Author):

Please see the attachment.
We appreciate the constructive comments from both reviewers and the editor. Due to unusual circumstances, we required much more time than typical to complete these revisions. We requested and received from the journal additional time to complete these revisions. We apologize that this delay likely creates additional work for the reviews since they now must evaluate our revisions more than a year after reading the original submission. We have attempted to facilitate this process by clearly presenting and explaining our revisions here. One tool that we used in this is process different fonts. Specifically,

**Bold** = reviewers’ comments.
Normal = our response
*Italicized* = text from the manuscript.
*Italicized and underlined* = new text in the manuscript.

Reviewer #1:

**Summary**

Understanding cell alignment under cyclic loading has remained enigmatic. These results show an important new theoretical prediction of how forces generated in the cell can interface with forces in the environment to result in specific cell and F-actin alignment, both parallel to and perpendicular to the direction of loading. For example, the ability to now theoretically explain the odd 70 degree orientation observed by many groups, a finding replicated here, is great achievement, given that cyclic stretching is an important part of the physiology of heart and lung. The authors not only find a biophysical theory that finally explains this effect, they are also able to explain it in non-mathematical terms as well in terms of detachment events during loading-unloading cycles and the time delay before clutch reattachment. While this is all positive, the manuscript as written was difficult to follow in many respects, most notably in aligning the experiments with the specific simulations that corresponded to them. It seems as if there are a lot of simulated scenarios that are not investigated experimentally, although they may be explaining previously published observations that have lacked theoretical explanation. If this is the case, it needs to be made more explicit in the figures. One option would be show, or at least summarize this published work to allow the reader to make a direct comparison between experiment and simulation. As is stands, the experiments appear in only one figure (3), and the simulations in subsequent figures. So a better connection between the experiments, both those presented here and those previously published, and the simulations that inform the conclusions is critical. Thus, I am supportive of publication in principle, but recommend major revisions to achieve a clearer presentation of the connection between experiment and theory.

We appreciate your positive assessment of the significance of our work and concrete suggestions on how to improve its presentation.

We have made significant modifications to the manuscript to improve the connection between experimental and simulation results. We now provide a more extensive summary of previous in vitro experimental observations both in the introduction and during the discussion of our simulation results. Specifically:

- Previous and new simulation predictions are compared to nine different types of experimental observations as summarized in a new table (Table 1).
- Previous simulation results with different types of cyclic stretch are compared to published in vitro results in a new figure (Figure 5).
- New simulation results are presented and compare to published in vitro results
Actin bundle reorientation as a function of cyclic stretch frequency (new Figure 7 and new section 3.5)

Actin bundle reorientation as a function of motor clutch model myosin motor function (new Figure 8 and new section 3.7)

Rewritten sections 3.3-3.5, 3.6, and 3.8 emphasize the connection between experimental and simulation results.

Major comments:

1. "Many cells in the body experience cyclic mechanical loading and cyclic stretch can impact cellular processes and morphology." I think this is widely recognized, but it would still be good to put references in here. A lot of this work has been published in biophysics and bioengineering journals, and this study is being considered for a cell biology audience, so appropriate referencing would help.

We have expanded the content of this sentence and provided references. Specifically, “Many tissues and organs in the body experience cyclic mechanical loading including the cardiovascular systems with the beating of the heart and subsequent pulse wave through the vasculature, the lungs with breathing, the digestive system with peristalsis, and the muscular skeletal system with locomotion. The cyclic mechanical loading and resulting cyclic stretch are thought to impact the structure and functions of these tissues as well as the associated cells in vivo, as summarized in various review articles (Gupta and Grande-Allen, 2006; Birukov, 2009; Morita et al., 2013; Qiu et al., 2014; Hye-Sun Yu et al., 2016).”

Also to make this work more accessible to a cell biology audience, we also provided additional explanations of the mechanical stretching device used in our experiments in a new figure (Figure 2) and types of stretch used (Figure 3B).

2. The subheadings 1.1, 1.2, etc. do not seem necessary.
Agreed. We have removed these subheadings.

3. "The model consists of an F-actin filament, molecular clutches." I think it would be more appropriate to say "F-actin filament bundle" since I think that is what the authors mean.
We have changed the text here and elsewhere to “F-actin bundle” or “actin bundle”.

4. Fig. 3. Need to show direction of stretch, presumably it is horizontal. Why in Fig. 3D is perpendicular elongation >1? Does the cell shown have perpendicular elongation less than 1?

In Figure 4 (previously Fig. 3), the direction of applied stretch is now shown in the figure and experimental images (Figure 4A and B) have been rotated to match the direction used elsewhere in the manuscript. An elongation value greater than 1 means the cell is longer in its direction perpendicular to stretch than it is in its parallel direction. The representative image (Figure 4B Perpendicular Elongation) now clearly shows the case of a cell with an elongation value greater than 1.

5. Rather than "p-value < 0.0125" recommend "p < 0.0125" throughout.
Changed here and throughout the manuscript.

6. Fig. 4. Title: make clear this is simulated actin filament reorientation.
Done. The title of the figure (now Figure 6) has been changed to:
“Figure 6: Simulated actin bundle reorientation as a function of cyclic stretch type, amplitude and, substrate stiffness.”

7. Fig. 4. Instead of "Actin filament reorientation" suggest "Actin fiber reorientation," (or bundle. since individual filaments are not being tracked but actin fibers are. Use "fiber" instead of "filament" wherever it appears.
We now use the term “actin bundle” here and throughout the manuscript.

8. Fig. 7. Which scenario is correct? Can the authors sum the unbinding events together to see where optimal angle is? It seems some annotation could be added to the figure to help the reader understand how the results inform our understanding.
Presentation of the proposed mechanism was clearly lacking. We have rewritten section 3.8 to explain the mechanism better. In short, the presented perpendicular and parallel scenarios are both correct. Our mechanism states that by analyzing when clutches unbind in relation to the cyclic stretch cycle (whether most clutches unbind during stretch or relaxing), one can predict whether an actin bundle will reorient more towards the direction perpendicular to the direction of stretch or the direction parallel to stretch. The third scenario (right column) shows that bundles can align both parallel and perpendicular to the direction of applied stretch as predicted by analysis of unbinding events) dependent on the bundles’ initial orientation.

9. More information on statistics is required, especially exact sample size of the data set.
Experimental sample size information has been added to Figure 4 legend (n = 50 - 100 cells for each stretch condition) and in section 2.1, “50 – 100 cells were analyzed for each condition and results are presented as means”. Simulation sample size information has been added to Figure 6 legend (n = 10 for all simulations) and in section 2.2.3, “10 simulations were run for each condition and results are presented as mean orientations”.

Reviewer #2: Summary
In the manuscript entitled “A Motor Clutch Model Predicts and Suggests Mechanisms of Cellular Morphological Response to Cyclic Force” authors have used stochastic motor-clutch model developed by Chan and Odde (2008) and modified it to confined and unconfined uniaxial stretch systems of the substrate. They use these models along with other parameters such as substrate stiffness, frequency and percent of stretch to explain the reorientation and alignment of cells and actin filaments as a result of substrate stretch. This model provides insights into changes in actin filament length and orientation as well as cellular morphology in response to cyclic stretch.
The language of the manuscript has many grammatical errors and requires proofreading. In addition, authors have not used case sensitive variables for denoting different parameters. This manuscript requires major revision before I can recommend its publication.

Major comments:
1. Please rephrase the sentence- “a computational simulation was developed by building on a computational model of the actin-myosin-integrin motor-clutch system developed by others”

Section 2.2.3 has been revised significantly. During this revision, the above sentence was change to…
To consider the impact of cyclic stretch, we adapted the original Chan and Odde motor clutch model described above (Chan and Odde, 2008).

2. Figure 1A (caption)- grammatical error- “Fibers may lengthen or shorten based on their orientation in respect to the direction of applied stretch”. Replace “in” with “with”.
Done.

3. “Only cells below passage 8 were used to avoid senescence”- Rephrase the sentence and explain the terms as it may not be clear to general readers.
Changed to:
“Lonza (Lonza, Morristown, NJ) human umbilical vein endothelial cells (HUVEC) were cultured with EGM-2 Bullet Kit media (Lonza, Morristown, NJ) and maintained below passage 8 to ensure constant cellular proliferation.”

4. “The stiffness of this substrate is estimated to be on order of ~1-2 MPa”- grammatical error. Replace “on order of” by “in the order of”.
“On order of” is incorrect. We intended “On the order of”, which we believe is the American English version of the British English term “in the order of”. To avoid confusion, we reworded the sentence. Specifically, ...

“The stiffness of this substrate is estimated to be about 1 MPa.”

5. “The casting was the removed from the mold and autoclaved.”- Replace “the” by “then”.
Done.

6. Please explain and add reference for “The Bonferroni correction”.
We now explain the Bonferroni correction in the text more completely. Specifically,
“Independent simultaneous t-tests were performed to determine statistical significance of each stretch condition compared to the no stretch control condition. To control for multiple comparison associated Type I error, the Bonferroni correction (Miller, 1981) was utilized to determine a new statistical significance level. Specifically, the initial significance level of 0.05 was reduced to 0.0125.”

7. Please use the same symbols for variables in text, figure captions and tables.
Done.

8. Position of clutch has been denoted by \( x_i \) in the text, whereas authors have denoted it by \( x_{\text{clutch}(i)} \) in the figure.
It is now designated consistently.

9. “reverse rate constant (\( K_{off}^* \))”- please use lower case “k” for koff wherever it has been used in the article, as authors have denoted it by koff in the figure. Also, does reverse rate constant means detachment rate of clutches from Actin filament? If so, then it is advised to authors to use the term “detachment rate” instead of “reverse rate constant”, as authors have done in the text initially. Using different terms for a single parameter might confuse the readers.
We changed rate constants to lower case $k$.

“Clutch binding and unbinding occurs stochastically with a binding rate constant ($k_{\text{on}}$) and a detachment rate constant ($k_{\text{off}}$).”

10. In section 2.2.3 (Modifications to model cyclic stretch), it would be better if authors provide a schematic of two of their computational models—confined and unconfined cyclic uniaxial stretch to mimic different experimental systems.

Two new representations of cyclic stretch have been added, Figures 2 and 3B. Figure 2 is a schematic of the experimental set-up and details how cultured cells are exposed to cyclic stretch. Figure 3B has been added to detail the 3 distinct types of stretch that are utilized in simulations.

11. “To do model confined cyclic stretch”—grammatical error. Replace “To do model” with “To model”.
Done.

12. “amplitude (A) and period (P) of movement can varied to explore a wide range of cyclic stretching regimes”—grammatical error. Replace “movement can varied” by “movement can be varied”.
Error was corrected.

13. “By recording the motion of fiduciary markers placed on our in vitro PDMS deformable substate,”—spelling mistake
Done.

14. It is observed that perpendicular alignment of filaments is favored on substrate with low stiffness, where as it is favored for higher stretch frequency. This implies that substrate stiffness has a negative effect, whereas stretch frequency has a positive effect on perpendicular alignment of filaments. So, which of the factors is the better regular of perpendicular alignment of filaments? It would be better if authors provide an explanation for this observation.

In the manuscript we now discuss the effects of both stiffness and frequency on simulated bundle reorientation, in addition to amplitude effects that were presented in the original manuscript. While both factors have a clear impact on bundle reorientation, the impacts are different in nature. Specifically, substrate stiffness largely impacts final bundle orientation, while stretch frequency impacts only the rate of reorientation.

Frequency effects: (from section 3.5)
“Under simulation conditions most comparable to their experimental conditions (simple elongation, 10% stretch, and high substrate stiffness (10 kPa), 24 hours of stretching), we observed that increasing stretch frequency decreased the characteristic time required for reorientation (Figure 6A). However, stretch frequency has no impact on final bundle orientation in simulations...”

Stiffness effects: (from section 3.6)
“While holding all other parameters constant, decreasing substrate stiffness by one order of magnitude (from 10 to 1 kPa) diminishes the perpendicular reorientation response to simple elongation (Figure 6)
and purely uniaxial cyclic stretch (Supplemental Figure 3). That is, at a given stretch amplitude, actin bundles aligned closer to the perpendicular direction with the higher substrate stiffness (Figure 6B vs. 6C) and a smaller stretch amplitude was required to approach maximal alignment with higher stiffness.

“However, decreasing substrate stiffness further (from 1 to 0.1 kPa) results in a more complex behavior (Figure 6D). At the lowest stretch investigated (0.1% stretch), there was negligible reorientation of actin bundles. With increasing levels of stretch, a greater fraction of the actin bundles aligned parallel to the applied stretch until the highest stretch where all bundles are aligned nearly parallel to the stretch.”

15. In the footnote of Figure 6, (D) should be written before the full stop. Also, it is advised to authors to mention the label of substrate as well. In addition, the “stretching” and “relaxing” of substrate should be mentioned as “stretching substrate” and “relaxing substrate” in the figure (written along with blue arrows).
Done.

16. In section 3.4, “experimental values and be considered as useful for a generic cell”—grammatical error. Replace “be” by “were”.
Corrected.

17. Sentence “there is the opportunity in future work to alter parameters to improve the agreement between our model predictions and experimental results.” Should be modified to “there is an opportunity in future to improve the agreement between our model predictions and experimental results by altering parameters”
We have changed this sentence in the text as suggested by the reviewer.

18. “we determined the Poisson’s ratio to be 0.52, similar to that reported for silicone, which we approximated as 0.5 in simulations of unconfined uniaxial tension.” Poisson’s ratio for which quantity was 0.52, reference for silicone missing and how it led to equation 7 is completely unclear.
Reference has been added, and the inclusion of Poisson’s ratio has been more clearly explained: “The compression ratio was calculated as a function of stretch applied in the parallel (x-) direction and the estimated Poisson’s ratio of the modeled substrate. Our in vitro experimental system applied stretch in the x-direction and the substrate was not confined in the y-direction. By recording the motion of fiduciary markers placed on our in vitro PDMS deformable substrate, we determined the Poisson’s ratio to be 0.52, similar to that reported for elastomers (specifically silicone rubbers) (O’Hara, 1983), which we approximated as 0.5 in simulations of simple elongation.”

19. There is typo in equation 8.
The error in equation 8 has been corrected.

20. Authors mention that “data suggest that cell shape is not altered by cyclic stretch as cellular aspect ratio (ratio of major axis length to minor axis length) is unchanged, with the exception of the 4% amplitude case (Figure 3E).” How do authors explain this?
There is a small, but statistically significant difference between the 4% case and all other cases. The extent of elongation by endothelial cells is influenced by their confluence (percent of the available area on the culture flask covered by the cells). When a relatively small fraction of the surface is covered by the endothelial cells, the cells are more elongated. As the cells proliferate and cover a greater fraction of
the available area, they adopt their classic cobblestone morphology with less elongation. The observation that the aspect ratio for the 4% amplitude is slightly greater than other cases suggests that these cells are slightly less confluent than other cultures. We do not assign any biological significance to this small difference.

21. Authors should re-write the conclusions section by first summarizing their major findings from computational model.

The conclusions section was re-written to summarize the major findings from the computational model. Specifically,

Endothelial cells subjected to cyclic stretch alter their cell and actin alignment, but not their aspect ratio, which is consistent with a fiber rotation mediated model of alignment. A computational simulation of the motor clutch system that allows for fiber rotation and accounts for cyclic stretch of the substrate was developed. The major findings from the computational model are 1) actin bundles align roughly perpendicular to the direction of the applied stretch, ~90° for pure uniaxial stretch and ~56° for simple elongation stretch. In both cases these directions coincide with the direction of minimal strain. 2) Under specific conditions, such as low substrate stiffness, actin bundles are predicted to align parallel to the direction of stretch. 3) Increasing stretch amplitude tends to promote a greater degree of predicted actin bundle alignments while increasing the stretch frequency tends to increase the rate at which fibers reorient. 4) Myosin motor function is critical in the perpendicular reorientation response. All these model predictions are generally in good agreement with the experimental data (Table 1). The model suggests that though a number of factors including stretch amplitude, stretch frequency, substrate stiffness and initial bundle orientation can influence the reorientation of bundles, the impact of all of these factors can largely be understood in light of their impact on cell-substrate detachments events. Conditions that lead to more detachment events occurring when the substrate is stretching than when the substrate is relaxing cause the bundles to orient away from the direction of applied stretch. Conversely, conditions that lead to more detachment events when the substrate is relaxing cause alignment toward the direction of applied stretch.
November 10, 2021

2nd Editorial Decision

RE: Manuscript #E20-01-0087R
TITLE: "A Motor Clutch Model Predicts and Suggests Mechanisms of Cellular Morphological Response to Cyclic Force"

Dear Prof. Gooch:

I am pleased to accept your manuscript for publication in Molecular Biology of the Cell.

Sincerely,
Alexander Mogilner
Monitoring Editor
Molecular Biology of the Cell

------------------------------------------------------------------------

Dear Prof. Gooch:

Congratulations on the acceptance of your manuscript. A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC. Within approximately four weeks you will receive a PDF page proof of your article.

Would you like to see an image related to your accepted manuscript on the cover of MBoC? Please contact the MBoC Editorial Office at mboc@ascb.org to learn how to submit an image.

Authors of Articles and Brief Communications are encouraged to create a short video abstract to accompany their article when it is published. These video abstracts, known as Science Sketches, are up to 2 minutes long and will be published on YouTube and then embedded in the article abstract. Science Sketch Editors on the MBoC Editorial Board will provide guidance as you prepare your video. Information about how to prepare and submit a video abstract is available at www.molbiolcell.org/science-sketches. Please contact mboc@ascb.org if you are interested in creating a Science Sketch.

We are pleased that you chose to publish your work in MBoC.

Sincerely,
Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

------------------------------------------------------------------------

Reviewer #2 (Remarks to the Author):

The authors have successfully addressed all the major and minor comments of the review. I, therefore, recommend the publication of this manuscript in MBoC.