Dear editor,

We received two referee reports concerning our manuscript #LE19173, “Mechanical plasticity of cell membranes enhances epithelial wound closure.” In their reviews, the referees asked about the conceptual novelty of the manuscript, given that we model and interpret experimental data from reference [3]. In addition, the referees had questions about the applicability of our computational model for wound healing, for example, why did we not consider chemoattractants, cell-cell frictional interactions, and the extracellular matrix, why did we include inertia in the equations of motion, and what is the justification for our mechanical model of cell membranes. We have addressed each of the referees’ comments below and made changes to the manuscript where appropriate. We believe that our manuscript has improved after responding to the referees’ comments. We thank the reviewers for their insights and for helping us to improve the manuscript. We now seek to transfer our manuscript from *Physical Review Letters* to *Physical Review Research* and believe that it is now ready for publication in *Physical Review Research*.

Sincerely,

Andrew T. Ton, Arthur K. MacKeith, Mark D. Shattuck, and Corey S. O’Hern

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Report of Referee A -- LE19173/Ton  
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Major comments:

*- The manuscript is written rather technically, and the results of a rather complex model are contained to area and cell shape dynamics. Can the authors predict more from their new model, such as stress patterns around the healed wound that could be tested by laser ablation experiments? What is the conceptual novelty of this work and their model compared to literature?*

Yes, the deformable particle model can predict stress patterns around the wound as a function of time during wound healing. (See for example our prior work in *Physical Review Materials* 5 (2021) 055605.) Because we compared to *in vivo* samples in this work, we focused on measurements of cell motion and shape dynamics, which are readily obtained from the experimental images. In future studies, we can also compare our computational studies of wound healing to *in vitro* assays, where traction force microscopy measurements of stress patterns can be performed.

The conceptual novelty of this work is our emphasis on the contribution of cell shape changes to wound healing. In general, there are multiple ways to close wounds. Our hypothesis is that differences in cell shape plasticity can affect wound healing times and properties of the tissue following wound healing. Existing computational models are unable to describe the cell shape changes that occur during Drosophila embryo wound healing. In particular, existing models neglect the contribution of cell shape plasticity to wound healing.

The theory and model in Ref. [3] predict that increasing the cell intercalation rate enhances wound healing speeds in model wounds. However, this prediction contradicts the fact that Drosophila embryo wounds have less intercalation than those in the larval wing disc epithelium, yet faster wound healing speeds. We analyze the contribution from cell shape change plasticity to wound closure to resolve this contradiction. Our deformable particle model shows that greater cell shape plasticity gives rise to faster wound healing speeds without cell intercalation. The novelty of our work is in explaining this contradiction related to reduced intercalation yet faster wound closure speeds and in advancing a novel mechanism of wound healing that has been neglected in existing models.

In the revised manuscript, at the end of Paragraph 3, we added a statement that more clearly distinguishes between the deformable particle model predictions and the existing model described in Ref. [3]. In addition, we added a sentence at the beginning of Paragraph 4 that clarifies that our hypothesis tests the effect of cell shape plasticity on wound closure rate and cell shape deformation. In Paragraph 5, we also added two sentences to clarify the strengths of the deformable particle model relative to other models in the literature. We also include new references in Paragraph 5 that demonstrate the broader applicability and impact of the deformable particle model in describing cell mechanics and dynamics.

*- It is unclear what the properties of the model tissue are, what kind of material can it describe, are there distinct phases in the parameter space? How does it compare to other established models and  
what are its advantages that could not be done in other models?*

The deformable particle (DP) model has been studied extensively over the past five years in both two (2D) (see *Physical Review Materials* 5 (2021) 055605) and three dimensions 3D (see *Soft Matter* 17 (2021) 9901) and has been employed to model flows and jamming of emulsion droplets (*Soft Matter* 17 (2022) 8071), development of flower microstructure (*J. R. Soc. Interface* 19 (2022) 20220602), and invasion of cancer cells into adipose tissue. It has been shown that static packings of deformable particles compressed above jamming onset with shape parameters and cell-cell adhesive strength in the range that we study are solid-like with non-zero bulk and shear moduli. As the shape parameter and adhesive strength decrease, the tissues modeled using the deformable particle model will transition from solid- to fluid-like, with zero tissue-scale elastic moduli.

Compared to other established models for epithelia, e.g. the vertex model, the deformable particle model excels at describing cell shape dynamics where gaps can form between cells, such as in the case of wound healing. In addition, the deformable particle model can describe curved cell shapes and explicitly model cell-cell adhesion. Drawbacks of the vertex model include that it was developed for confluent tissues, that cell shapes in the vertex model are polygonal and described by a relatively small number of vertices, and there are no explicit forces that describe cell-cell adhesion.

In Paragraph 5 of the revised manuscript, we added two sentences to clarify the strengths of the deformable particle model relative to other models in the literature for computational descriptions of wound closure. In the revised manuscript, we also included new references in Paragraph 5 that show the broader applicability and impact of the deformable particle model in describing cell mechanics and dynamics.

*- Connection to the experimental facts seems to be lacking: What is the 'membrane'? In the manuscript, the model of membrane is contrasted to the cytoskeleton and junction models (above Eq. 5) so should I assume it is the actual cell membrane? But the cell membrane, as far as I know, is rather fluid in animal cells, do the authors have any support for ingredients they put into their model? Furthermore, well documented active processes are ignored, such as mechanosensitive response of cortex to stress. Could such active processes produce equivalent outcome as the one proposed in this manuscript? If yes, why do the authors think it is the 'membrane plasticity' that is relevant and different to other possibilities, how could one test this?*

We would like to clarify that 'membrane' in the original manuscript refers to the actual cell membrane. The cell membrane in animal cells is viscoelastic: liquid-like on the longest length and time scales, but it is well-recognized that the cell membrane has solid-like response on short length and time scales (see *Annual Review of Biomedical Engineering* 9 (2007) 1). For example, the cell membrane can transmit forces, and there are energy costs for stretching and bending (see *Methods in Cell Biology* 55 (1997) 157).

In response to the referee's comments, in the revised manuscript we now emphasize that we use membrane plasticity to model the overall phenomenon of cell shape plasticity. Cell shape plasticity can arise from several active cellular processes, such as remodeling of the actin cortex in response to stress and modulation of membrane area through vesicle trafficking and folds such as caveolae. The coarse-grained resolution of our model does not differentiate between these active processes, instead it uses a membrane plasticity parameter to describe their effects. In future work, experimental studies can be carried out to test the separate contributions of these active processes to the cell shape plasticity, for example, by using Nystatin to inhibit caveolar endocytosis and measuring the resulting cell shape plasticity.

In Paragraph 7 of the revised manuscript, we elaborate on our discussion of Equation 5 to emphasize that cell shape plasticity can arise from several active cellular processes, such as actin cortex remodeling and membrane area modulation via vesicle trafficking and caveolae. At the beginning of Paragraph 7, we also clarify that cell membranes have solid-like responses and include the two references above to support this statement. We further include a discussion of how we are using membrane plasticity to describe the overall phenomenon of cell shape plasticity. At the end of the manuscript, we propose future experiments that can investigate the separate contributions of actin cortex remodeling and membrane area modulation to cell shape plasticity.

*- Ref. 3 does provide an explanation of the wound healing, in some ways very close to the one proposed in this paper - what is the novelty here, why is the proposed theory in Ref. 3 completely ignored? Is the submitted manuscript an alternative, and if yes, how is it different from the proposed scenario in Ref. 3? Can the authors also explain the MyoII preturbation experiments in Ref. 3? Unless the authors can show that their model explains something that was not explained by previous works I don't think it should be published, except maybe as a technical study of their DP model. In any case I do not think this paper warrants a publication in PRL as it adds at best a little to the past work and lacks broad conceptual novelty.*

The model in Ref. [3] proposes that a reduction in cell line tension can increase cell intercalation rates in the wing disc tissue, which then leads to accelerated wound healing. The authors of Ref. [3] show how Myosin II perturbations of wing disc tissue results in reduced line tension and accelerated wound healing.

We offer an alternative model for accelerated wound healing in wing disc tissue. We propose that greater cell shape plasticity can also lead to accelerated wound healing. The authors of Ref. [3] note that during wound healing, the Drosophila embryo wounds feature much fewer intercalations than in the wing disc wounds. The model of Ref. [3] would then predict that the embryo heals more slowly than the wing disc. Instead, we show that the Drosophila embryo wounds have a healing rate nearly ten times that of the wing disc. The novelty of our work is that we explain this contradiction, and we put forward a novel mechanism of wound healing that has been neglected in current theoretical models.

In the revised manuscript, we expanded the discussion in Paragraph 14 to better describe the contrast between the wound healing model in the present work and that in Ref. [3]. In particular, Ref. [3] cannot explain the difference in wound closure rates between embryo and wing disc wounds. We emphasize that our deformable particle model describes a mechanism of wound healing based on cell shape plasticity, which explains the differences between Drosophila embryo and wing disc wound healing.

Minor comments:  
  
*- In the model, why do the authors choose to introduce the plasticity through rest length? What is the biological process that would affect it? Did the authors consider instead adding plasticity to equilibrium bending angle? Would this be redundant to the rest length approach used in the paper?*

Cell shape plasticity can occur from multiple sources, and the referee has noted that active actin cortex remodeling is one source. In addition to cortex remodeling, there are also membrane reservoirs called caveolae (see Ref. [30]) and vesicle trafficking processes using exocytosis and endocytosis (see Ref. [26]).

These processes, which are referred to in the literature as 'mechanoprotection', are used to modulate the available cell membrane surface area in response to stress. In our model, a natural way to incorporate changes in cell membrane surface is to add plasticity through the rest length.

We incorporate cell shape plasticity through rest-length remodeling to capture the overall process of mechanoprotection that we observe in the experimental wound assays. The referee suggests using bending angle plasticity, which is one component of mechanoprotection that emphasizes actin cortex remodeling as opposed to membrane reservoirs. We use rest length plasticity due to the observation that the cell membrane perimeter undergoes fluctuations during wound healing. In systems where the cell membrane perimeter does not fluctuate significantly, it would be interesting to investigate cell shape plasticity via changes in the equilibrium bending angle. We also note that because curvature depends on both membrane lengths and angles, there is some overlap between rest length remodeling and bending angle remodeling.

In the revised manuscript, we added text to Paragraph 7 to emphasize that a natural way to incorporate cell membrane surface changes is to add plasticity through the rest length. We note that we listed “unfolding and trafficking” as processes that contribute to remodeling in the original manuscript. We now emphasize that these processes are well-studied biological processes by changing the phrasing to “membrane folding and unfolding via caveolae and vesicle trafficking via endocytosis and exocytosis”. In addition, we include a new section in the supplemental material describing our decision to use rest-length remodeling rather than bending angle plasticity to control cell shape plasticity.*- Why is friction between cells neglected? Epithelial cells are tightly connected with various junctions, I would expect that dynamics of removing and adding these junctions would produce high effective friction.*

A novel aspect of this manuscript is that we developed a 'frictionless' model for attractive interactions between adjacent, smooth cell surfaces, whereas previous deformable particle models have used bumpy surfaces to implement attractive cell-cell interactions. We use this 'frictionless' model to remove the high friction caused by the interdigitating circular bumps on the deformable particle surfaces. However, there is still an effective friction between cells due to the attractive interactions between line segments on neighboring cells.

We have revised the manuscript to better emphasize the nature of the cell-cell interactions and reduce confusion between the smooth sliding cell-cell interactions and the velocity-dependent friction due to damping. To do this, we changed the phrasing in Paragraph 6 and in Section S2 from 'frictionless sliding adhesion' to 'smooth sliding adhesion'. We also added a sentence at the beginning of Section S2 to more clearly differentiate between bumpy (non-smooth) and smooth adhesions between particles.

*- Why do the authors simulate an epithelial tissue using inertial dynamics (with rather high m= 1 if I understand the parameters correctly)? It is well accepted that any inertial effects are negligible on scales relevant in this problem (and the authors can convince themselves of this with a simple estimate using water density for cells, water viscosity to obtain a lower limit for dissipative stresses, typical values for velocities and distances they use in the manuscript). Is it clear that inertia does not affect the dynamics reported in the simulations?*

Inertial effects are indeed negligible on the scale of cell motion. In the previous simulations, we included inertia, but for the parameters we selected, the equations of motion were overdamped. However, in response to the referee’s comment, we modified Equation S5 to remove the inertial term and repeated all simulations of the deformable particle model in the completely overdamped limit. Using the new results from the completely overdamped simulations, we updated Figures 3 and 4 in the revised manuscript. The overall change between the old and new results is that the wound closure speed is slightly slower for all parameter values. We observed the same trends and draw the same conclusions as for the data from the original set of simulations. Additionally, we modified Equations 5 and 10 in the revised manuscript so that they are written in the completely overdamped limit.

*Fig. 1. a) and 4. a) should be on log-normal scale so that the fitted/proposed exponential decay can be clearly seen.*

We thank the referee for this suggestion, however, we consider it best to use a linear-linear scale in Figs. 1 (a) and 4 (a), because the log-linear scale compresses the fast phase of wound closure at short times. To improve visual clarity in the revised manuscript, we replotted Figs. 1 (a) and 4 (a) so that they are larger and the fitted exponential decay can be seen more clearly.

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Report of Referee B -- LE19173/Ton  
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*The paper by A. Ton et al. deals with epithelial wound healing. It aims to analyze experimental results of wounds made on the Drosophila larval wing and on Drosophila embryonic ectoderm. The authors propose a deformable particle model that complements the classical vertex model with the aim of justifying an interpretation of the results of the simulations by plastic deformation of the cells at the wound boundary.  
  
I have several comments. First, it is sometimes difficult to understand that the experimental data have been produced by the authors of Ref. 3 who also use a vertex-based model to interpret of  
their own data. So, we can ask what is really new here and if the physical interpretations of both groups are different, this needs to be discussed. In biophysics, many interpretations can be given to the same experimental results, this is true, but it becomes more difficult to be the second group and then publish in PRL.*

The model proposed in Ref. [3] hypothesizes that a reduction in line tension increases cell intercalation rates in the wing disc tissue, which leads to accelerated wound healing. The authors of Ref. [3] explain how Myosin II perturbations in wing disc tissue results in reduced line tension, accelerating wound healing.

We offer an alternative model for accelerated wound healing in wing disc tissue. We propose that greater cell shape plasticity can also lead to accelerated wound healing. The authors of Ref. [3] note that during wound healing, the Drosophila embryo wounds feature much fewer intercalations than in the wing disc wounds. The model of Ref. [3] would then predict that the embryo heals more slowly than the wing disc. Instead, we show that the Drosophila embryo wounds have a healing rate nearly ten times that of the wing disc. The novelty of our work is that we explain this contradiction, and we put forward a novel mechanism of wound healing that has been neglected in current theoretical models.

In the revised manuscript, we have clarified in the Figure 1 caption and in Paragraph 3 that the experimental data is produced by experiments performed by the authors of Ref. [3]. In Paragraph 3, we clarify that the authors of Ref. [3] use a vertex-based model to interpret their data. We also expanded the discussion at the end of Paragraph 14 to better describe the contrast between the wound healing model in the present work and that in Ref. [3]. In particular, Ref. [3] cannot explain the difference in healing rates between embryo and wing disc wounds. We emphasize that our model describes a mechanism where wound healing depends sensitively on cell shape plasticity, which explains the differences between Drosophila embryo and wing disc wound healing.

*As a biophysicist who has worked on wound healing, I know that chemoattractants play a critical role in the process of re-epithelialization. It is even more important when the scar is not carefully made. So, I am surprised that this effect is not introduced. Perhaps the reason is that it is rather difficult to introduce chemicals into the vertex simulations. However, and especially for live embryos, I am sure that chemoattactants play a role.*

We appreciate the referee's comment on the importance of biochemical contributions to wound healing, and agree that chemoattractants are indeed important for re-epithelialization. The scope of our work is to understand the biomechanical behaviors governing wound healing. For this reason, the level of detail in our computational model does not explicitly include chemical gradients, but rather their effects on the mechanics of cells and tissues through cell-cell adhesion and purse-string activity. As such, we consider chemoattractants and other chemical gradients a deeper level of modeling that we are not aiming to describe in the present work.

Additionally, chemoattractants, like chemokines, are known to be important for certain types of wound healing, such as adult skin wound healing, especially during the inflammatory response to wounding

(see *International Journal of Molecular Sciences* 19 (2018) 3217). However, Drosophila embryos are known to heal small wounds rapidly with minimal inflammation and no scars. (See Ref. [8].) Likewise, there is no mention of inflammation, chemoattractants, or scarring in the Drosophila wing disc laser ablation study. (See Ref. [3].) The literature suggests that chemoattractants play a more important role in slowly healing wounds, larger wounds, and in adult models (see *Birth Defects Research* 96 (2012) 258). Based on these references, inflammation and the role of chemoattractants in the wounds we are studying have been shown to be suppressed relative to larger adult wounds.

In the revised manuscript, at the beginning of Paragraph 8, we note that we are not modeling explicit chemical signals in our simulations, and instead the present manuscript focuses on the biomechanical behaviors resulting from such signals.   
  
*What about the extracellular matrix, which is the soft substrate of the epithelium?*

In our approach, we consider the coarse-grained effect of the extracellular matrix substrate through the dissipative term in the equations of motion. The effects of the cells’ environment on cell dynamics are commonly modeled using a single friction coefficient (see *PLoS Computational Biology* 13 (2017) e1005569 and *Nature Communications* 8 (2017) 13929). An interesting future study would include the effects of the alignment of fibers in the extracellular matrix on cell motion during wound healing.

In the revised manuscript, in Paragraph 8, we have added the above references as well as a discussion of the decision to model the effects of the intercellular environment using a single friction coefficient, and clarify that our deformable particle simulations are conducted using completely overdamped dynamics according to Equation S5.

*It may be a detail, but I find it surprising that in Figure 2 (d), the cells are not fully connected and have tiny holes. This is clearly unrealistic.*

In the schematic wound in Fig. 2 (d), the holes between cells are caused by the fact that we used a lower initial cell shape parameter in the deformable particle model simulations than the wounds shown in Fig. 4 (c) and (d). In the revised manuscript, we now prepare the wound in Fig. 2 (d) to more closely match the initial cell shape parameter used in Fig. 4 (c) and (d) to mimic the wound healing experiments in Ref. [3]. Changing the initial cell shape parameter used in Fig. 2 (d) has resulted in fully confluent cell monolayers similar to the starting configurations in Fig. 4, which appear more realistic per the referee’s expectation.

*The paper is poorly written and is very technical for PRL.*

We take advantage of the larger allowed word count in *Physical Review Research* to expand the text to improve the manuscript and address the comments of the referees. A summary of the most important changes is included below:

In the revised manuscript, the text has been expanded in Paragraphs 3-8 and 14-15. We also added a Supplement Section S7, and made changes to Supplement Section 2, Equations 5 and 10, and Equation S5. We replotted Figures 1a and 4a for visual clarity, replotted Figure 2d with updated initial conditions, and repeated all simulations with the updated completely overdamped equations of motion. We clarified multiple points in the manuscript. For example, we clarified our hypothesis that cell shape plasticity affects the wound healing rate and cell shape deformation. We added a discussion that clearly distinguishes between our deformable particle model predictions and the existing model of wound closure in Ref. [3]. We also further distinguish the deformable particle model from other models in the literature, and expand on the deformable particle model’s broad applicability and impact. We elaborate on the modeling decisions behind Equation 5 and the description of the membrane with a solid-like response. We also more clearly state the biological motivation behind Equation 5 in terms of active remodeling processes. The revised manuscript also includes a discussion of our decision to model the dissipative effects of the cellular environment using a single friction coefficient. Supplement section S7 discusses rest length remodeling as opposed to bending angle plasticity. We also expand the future work discussion to propose investigations of the separate contributions of actin cortex remodeling and membrane area modulation to cell shape plasticity.