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Intercellular model predicts mechanisms of inflammation-fibrosis coupling after myocardial infarction

Mukti Chowkwale, Merry L Lindsey, and Jeffrey J Saucerman
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The following individual(s) involved in review of this submission have agreed to reveal their identity: Ronald Vagnozzi (Referee #1)

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Dear Dr Saucerman,

Re: JP-RP-2022-283346 "Intercellular model predicts mechanisms of inflammation-fibrosis coupling after myocardial infarction" by Mukti Chowkwale, Merry L Lindsey, and Jeffrey J Saucerman

Thank you for submitting your manuscript to The Journal of Physiology. It has been assessed by a Reviewing Editor and by 2 expert Referees and I am pleased to tell you that it is considered to be acceptable for publication following satisfactory revision.

Please advise your co-authors of this decision as soon as possible.

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Yours sincerely,

Bjorn Knollmann
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EDITOR COMMENTS

Reviewing Editor:

Both reviewers commented on an outstanding and potentially impactful study. Major comments to be addressed in the revision include:

- please provide more detail on the experimental data and equations used for model building

- please clarify controversial outcomes and experiment-model disagreements; please add discussion on how your model might not capture chronic post-MI inflammation, and the differential role of tissue-resident macrophages vs. monocyte derived macrophages

- please analyze the simplified fibroblast model to determine the TGFb concentration at which the transcritical bifurcation occurs, and potentially revise the argument of proliferation being the main driver of the ultrasensitive switch

Senior Editor:

I concur with the reviewing editor.

REFEREE COMMENTS

Referee #1:

Here Chowkwale et al present a computational framework and validation to model cellular dynamics and immune-fibroblast interactions in the setting of myocardial infarction (MI). The authors use published experimental data in mouse models to build predictive algorithms for a number of key biological parameters of cardiac wound healing (cell content, cytokine secretion, phagocytosis, etc), and then validate their model through comparison with additional published data. They go on to probe this computation model and reveal interesting interactions between cardiac infarct size, neutrophil content, and collagen deposition through an IL-1beta dependent mechanism.

Overall this is an excellent paper and will likely benefit the field in my opinion. The study is well-written and well executed. I have very few concerns to raise, mostly some suggestions for the authors to expand upon:

1. The strength and validity of this computation model depends on the accuracy of past literature used. The authors provide a very useful set of tables defining their chosen parameters from published studies. These tables would be even more informative if including some description of how key parameters were measured in the initial studies. For example, monocyte dynamics could have been measured by flow cytometry or histology; likewise cytokine/chemokine levels or collagen content might have been assessed differently between these publications. Were paper-specific methods considered in how these parameters were defined, and would that impact the model dynamics?

2. It is intriguing that this model predicts an almost complete loss of macrophages from the post-MI heart by day 21 (Fig 2A). While monocyte-macrophage content certainly peaks in the early phases of MI in mice, there are also numerous studies showing persistent functions and content of cardiac macrophages in the chronically infarcted heart, and during heart failure. The authors should comment on this, and specifically how their model might differ in predictive power between early post-MI wound healing (within the first ~7 days) versus chronic post-MI inflammation. Likewise, a differential role of tissue-resident macrophages as opposed to monocyte derived macrophages might not be fully reflected in this model, based on the studies/parameters selected. The authors should also comment on this in discussion.

Referee #2:

The authors present a new computational model of wound healing post-MI that incorporates known intercellular interactions derived from a large-scale literature review. The model was tuned and suitably validated against published datasets from mice, then used to probe factors that drive and resolve inflammation. Key results include highlighting (i) a coupling between inflammation and fibrosis (ii) ultrasensitivity of collagen deposition to infarct size. Causal sub-features in the model were identified (a positive feedback loop between neutrophils and IL-1b; a transcritical bifurcation in fibroblasts with respect to the TGFb concentration) to provide mechanistic understanding. Overall this is a well-executed study with original and significant results. A few clarifications or minor additions could yet improve the manuscript:

1) It is not mentioned how inhibition (seen in Fig 1) is modeled - please clarify.

2) What are the units of MatColl in Eq 5? It appears to have been normalized rather than a concentration - but, if so, how?

3) In the highlighted experimental/model disagreements (lines 260-265), the choice of some of the examples are not clear to me e.g. the effects of IL-1b and TGFb inhibition on collagen do appear to agree qualitatively between simulation/experiment (Fig 3C).

4) In Fig 3 and related analysis, is it appropriate to consider decreased MMP9 after MMP9 inhibition, decreased TNFa after TNFa inhibition and decreased IL-1 after IL-1 receptor KO as predictions/validations? It seems that these are effectively the model inputs, so a justification would be needed.

5) The transcritical bifurcation in the simplified fibroblast model is a very interesting result with potentially important physiological consequences. Have the authors considered finding the steady state and TGFb concentration at which the bifurcation occurs analytically, i.e. in terms of the parameters lambda, d, Fmax and infarct size? It is not quite at TGFb=0, as perhaps implied in lines 371/372, and this relatively short calculation could provide more rigor to the argument of proliferation being the main driver of the ultrasensitive switch (without first changing the model structure as in Figs 7E,F). It would also show how altered parameters can shift the critical TGFb concentration needed to trigger the switch (and resulting severity) - relevant to the discussion of potential therapeutic strategies.

6) For consistency, consider changing the rate parameter subscripts in Eqs 4-6 to match those in Table 4 (or vice versa).

7) In Fig 6B,D, a more descriptive y-axis label would be helpful, i.e. what does "area under the curve" represent physiologically?

END OF COMMENTS

Confidential Review **19-May-2022**

We thank the reviewers and editors for highlighting the manuscript's strengths and for providing constructive criticism, which has improved the clarity and quality of our study. In this revision, we have made the suggested revisions. We believe that these additions have further enhanced the quality of our study. Detailed responses are provided below in red.

EDITOR COMMENTS

Reviewing Editor:
Both reviewers commented on an outstanding and potentially impactful study. Major comments to be addressed in the revision include:
We appreciate the positive comments of the Reviewing Editor and Senior Editor.

- please provide more detail on the experimental data and equations used for model building
As described in response to Referee #1 Comment 1, we updated the text and Tables to provide more details.

- please clarify controversial outcomes and experiment-model disagreements; please add discussion on how your model might not capture chronic post-MI inflammation, and the differential role of tissue-resident macrophages vs. monocyte derived macrophages
As described in response to Referee #1 Comment 2 and Referee Comment 3, we have clarified and better contextualize the few instances of experiment-model disagreements.

- please analyze the simplified fibroblast model to determine the TGFb concentration at which the transcritical bifurcation occurs, and potentially revise the argument of proliferation being the main driver of the ultrasensitive switch
As described in response to Referee #2 Comment 5, we performed additional analysis of the transcritical bifurcation, which identifies the critical TGFb concentration and further supports the role of proliferation.

Senior Editor:
I concur with the reviewing editor.

REFEREE COMMENTS

Referee #1:

Here Chowkwale et al present a computational framework and validation to model cellular dynamics and immune-fibroblast interactions in the setting of myocardial infarction (MI). The authors use published experimental data in mouse models to build predictive algorithms for a number of key biological parameters of cardiac wound healing (cell content, cytokine secretion, phagocytosis, etc), and then validate their model through comparison with additional published data. They go on to probe this computation model and reveal interesting interactions between cardiac infarct size, neutrophil content, and collagen deposition through an IL-1beta dependent mechanism.

Overall this is an excellent paper and will likely benefit the field in my opinion. The study is well-

written and well executed. I have very few concerns to raise, mostly some suggestions for the authors to expand upon:
We thank the reviewer for their accurate summary of the manuscript's contributions and positive comments on the writing and rigor of the study.

1. The strength and validity of this computation model depends on the accuracy of past literature used. The authors provide a very useful set of tables defining their chosen parameters from published studies. These tables would be even more informative if including some description of how key parameters were measured in the initial studies. For example, monocyte dynamics could have been measured by flow cytometry or histology; likewise cytokine/chemokine levels or collagen content might have been assessed differently between these publications. Were paper-specific methods considered in how these parameters were defined, and would that impact the model dynamics?
We appreciate the reviewer's suggestion to include descriptions of how key parameters were measured. We updated **Tables 3 and 4** to describe these methods per parameter. A few examples from Table 4 are shown below.

Parameter	**Description**	**Value**	**Unit**	**Measurement techniques**	**Citation**
TNFα parameters					
$k_{TNFα,CM}$	Secretion rate by cardiomyocytes	5e-7	pg/ml/cell/hour	ELISA	(Horton et al., 2006)
$k_{TNFα,N}$	Secretion rate by neutrophils	1.2e-3	pg/ml/cell/hour	Immunofluorescence	(Finsterbusch et al., 2014)
$k_{TNFα,Mo}$	Secretion rate by monocytes	1.58e-4	pg/ml/cell/hour	ELISA	(Matic & Simon, 1991)
$k_{TNFα,Mϕ}$	Secretion rate by macrophages	3.15e-4	pg/ml/cell/hour	ELISA	(Minshawi et al., 2019)
$k_{TNFα,F}$	Secretion rate by fibroblasts	9.5e-5	pg/ml/cell/hour	ELISA	(Del Re et al., 2010)
$k_{TNFα,deg}$	Degradation rate	0.4786	1/hour	Ribonuclease protection assay and curve fitting	(Deten & Zimmer, 2002)

The rigor and reproducibility quality of the methods for each individual article reviewed were considered while deriving the raw parameter values from literature. The articles selected for inclusion were also selected based on whether absolute value outputs of the assays (e.g. protein concentrations in ELISAs) were available. For instances when absolute values were not available, assays that provided relative values (e.g. relative expression in Western blots) were selected to guide parameter estimation. The variations in methods across the articles selected did not impact the model dynamics as the literature-derived parameters were optimized to fit experimental output time courses.

2. It is intriguing that this model predicts an almost complete loss of macrophages from the post-MI heart by day 21 (Fig 2A). While monocyte-macrophage content certainly peaks in the early phases of MI in mice, there are also numerous studies showing persistent functions and content of cardiac macrophages in the chronically infarcted heart, and during heart failure. The authors should comment on this, and specifically how their model might differ in predictive power between early post-MI wound healing (within the first ~7 days) versus chronic post-MI

inflammation. Likewise, a differential role of tissue-resident macrophages as opposed to monocyte derived macrophages might not be fully reflected in this model, based on the studies/parameters selected. The authors should also comment on this in discussion.
The reviewer makes an important point about highlighting one potential limitation of the model. In response to this comment, we added a paragraph in the discussion to reflect upon macrophage numbers:
Lines 472-480: "Our model predicts dynamics for acute, transient inflammation post myocardial infarction. In the presence of chronic inflammation due to conditions such as cardiometabolic defects, aging, or co-medications, there is a dysregulation of inflammation resolution (Halade & Lee, 2022; Kolpakov et al., 2020). This model could be further extended to predict intercellular dynamics in the presence of chronic post-MI inflammation. Moreover, the model does not include tissue-resident macrophages (Dick et al., 2019; Jia et al., 2022; Nahrendorf et al., 2007). Tissue-resident macrophages are lost post-MI, but they recover to pre-infarct levels by around 4 weeks after infarction (Dick et al., 2019). Sufficient data was not available to model the dynamics of tissue-resident macrophages. However, the model accurately simulates the behavior of monocyte-derived macrophages post myocardial infarction."

Referee #2:

The authors present a new computational model of wound healing post-MI that incorporates known intercellular interactions derived from a large-scale literature review. The model was tuned and suitably validated against published datasets from mice, then used to probe factors that drive and resolve inflammation. Key results include highlighting (i) a coupling between inflammation and fibrosis (ii) ultrasensitivity of collagen deposition to infarct size. Causal sub-features in the model were identified (a positive feedback loop between neutrophils and IL-1b; a transcritical bifurcation in fibroblasts with respect to the TGFb concentration) to provide mechanistic understanding. Overall this is a well-executed study with original and significant results. A few clarifications or minor additions could yet improve the manuscript:
We appreciate the reviewer's detailed description of the new contributions and positive comments on the manuscript.

1) It is not mentioned how inhibition (seen in Fig 1) is modeled - please clarify.
We have updated the text to illustrate an example of how inhibition is modeled.
Lines 149-151: "In Eq. 5, the terms $\frac{TGF\beta}{TGF\beta+K_{TGF\beta}}$ and $\frac{MMP9}{MMP9+K_{MMP9}}$ show activation of the dynamics by TGFβ and MMP-9, while the term $\left(\frac{1}{MatColl+1}\right)$ models inhibition by mature collagen."

2) What are the units of MatColl in Eq 5? It appears to have been normalized rather than a concentration - but, if so, how?
MatColl is in units of pg/ml as shown in Table 4. We provide units for all parameters and variables in Tables 3-5.

3) In the highlighted experimental/model disagreements (lines 260-265), the choice of some of the examples are not clear to me e.g. the effects of IL-1b and TGFb inhibition on collagen do appear to agree qualitatively between simulation/experiment (Fig 3C).

We thank the reviewer for pointing out this discrepancy, and have removed the example from the manuscript.

4) In Fig 3 and related analysis, is it appropriate to consider decreased MMP9 after MMP9 inhibition, decreased TNFa after TNFa inhibition and decreased IL-1 after IL-1 receptor KO as predictions/validations? It seems that these are effectively the model inputs, so a justification would be needed.
The comparisons “decreased MMP-9 after MMP-9 inhibition” and “decreased TNFa after TNFa inhibition” were included to mimic structure of the experimental studies, but the reviewer makes a good point. Hence, we removed the two from the validation study and changed Figure 3 to reflect that. However, IL-1 receptor KO (not a model input) limits the downstream effects of IL-1, but does not have a direct effect on IL-1 in the system. Thus, that validation relationship tested emergent behavior, and not direct effects.

5) The transcritical bifurcation in the simplified fibroblast model is a very interesting result with potentially important physiological consequences. Have the authors considered finding the steady state and TGFb concentration at which the bifurcation occurs analytically, i.e. in terms of the parameters lambda, d, Fmax and infarct size? It is not quite at TGFb=0, as perhaps implied in lines 371/372, and this relatively short calculation could provide more rigor to the argument of proliferation being the main driver of the ultrasensitive switch (without first changing the model structure as in Figs 7E,F). It would also show how altered parameters can shift the critical TGFb concentration needed to trigger the switch (and resulting severity) - relevant to the discussion of potential therapeutic strategies.
The reviewer is correct. Based on this suggestion, we solved the transcritical bifurcation analytically and it was found to be at TGFβ = 0.0717 pg/ml. We updated section 4 in the methods (Reduced fibroblast model) to include parameter values used to calculate the transcritical bifurcation, and we included another section titled “Analyses of reduced fibroblast model” to discuss the steady state and bifurcation analysis.

6) For consistency, consider changing the rate parameter subscripts in Eqs 4-6 to match those in Table 4 (or vice versa).
We appreciate the reviewer’s suggestion and updated Eqs 4-6 to match the values in Table 4.

7) In Fig 6B,D, a more descriptive y-axis label would be helpful, i.e. what does "area under the curve" represent physiologically?
To reflect the reviewer’s comment, we updated the manuscript to explain the physiological representation of “area under the curve”.
Lines 378-379: “The relative area under the curve represents a cumulative sum of the secreted factors in simulated time course.”

END OF COMMENTS

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Roberts, R., DeMello, V., & Sobel, B. E. (1976). Deleterious effects of methylprednisolone in patients with myocardial infarction. *Circulation*, *53*(3 Suppl), I204-206.

Dear Dr Saucerman,

Re: JP-RP-2022-283346R1 "Intercellular model predicts mechanisms of inflammation-fibrosis coupling after myocardial infarction" by Mukti Chowkwale, Merry L Lindsey, and Jeffrey J Saucerman

I am pleased to tell you that your paper has been accepted for publication in The Journal of Physiology.

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Bjorn Knollmann
Senior Editor
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EDITOR COMMENTS

Reviewing Editor:

Reviewers both deem the revision satisfactory. I concur. Congratulations on very nice work!

Senior Editor:

Excellent work, congratulations! Please make sure that all data are included in the main manuscript without supplemental material. There are still references to supplemental figures in the text.

Please update your article file (in Word) to include the following:

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REFEREE COMMENTS

Referee #1:

Authors have addressed all of my concerns, very nice study!

Referee #2:

The revisions clarify and improve this excellent study. All comments have been addressed.

1st Confidential Review **08-Jul-2022**