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Graft-Host Coupling Changes Can Lead to Engraftment Arrhythmia: A Computational Study

Chelsea E Gibbs, Silvia Marchianó, Kelly Zhang, Xiulan Yang, Charles E Murry, and Patrick M Boyle
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The referees have opted to remain anonymous.

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Dear Dr Boyle,

Re: JP-RP-2023-284244 "Graft-Host Coupling Changes Can Lead to Engraftment Arrhythmia: A Computational Study" by Chelsea E Gibbs, Silvia Marchianó, Kelly Zhang, Xiulan Yang, Charles E Murry, and Patrick M Boyle

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The referee reports are copied at the end of this email.

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We look forward to receiving your revised submission.

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Yours sincerely,

Peter Kohl
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-You must start the Methods section with a paragraph headed Ethical Approval. A detailed explanation of journal policy and regulations on animal experimentation is given in Principles and standards for reporting animal experiments in The Journal of Physiology and Experimental Physiology by David Grundy J Physiol, 593: 2547-2549. doi:10.1113/JP270818.). A checklist outlining these requirements and detailing the information that must be provided in the paper can be found at: https://physoc.onlinelibrary.wiley.com/hub/animal-experiments. Authors should confirm in their Methods section that their experiments were carried out according to the guidelines laid down by their institution's animal welfare committee, and conform to the principles and regulations as described in the Editorial by Grundy (2015). The Methods section must contain details of the anaesthetic regime: anaesthetic used, dose and route of administration and method of killing the experimental animals.

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-Exact p values must be stated. Authors must not use 'greater than' or 'less than'. Exact p values must be stated to three significant figures even when 'no statistical significance' is claimed.

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EDITOR COMMENTS

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Senior Editor:

Please add a statement explaining ethical review of animal-derived samples used in this investigation.

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The authors performed a computational study investigating the effect of graft-host connectivity and graft conductivity on graft-initiated arrhythmias in human pluripotent stem cell-derived cardiomyocytes grafts in the infarcted ventricle. The study is well presented, and the aims are clear. The results are interesting and provide novel insight into the effect of scar and graft-host connectivity on arrhythmias, with potentially important clinical implications. However, there are a few points that the authors should clarify:

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4. Do graft myocytes undergo any physiological changes when they become more mature? If not, the authors could provide a reference for that. If not, this should also be listed as a limitation of the study, as the only temporal factor included in the study is increased graft-host connectivity.

5. The manuscript should undergo careful proof-reading.

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1. Title: leasd should be lead. This is not correct at line 1 but it is correct in the title page.

2. Line 51: unclear what the authors mean by 'spatial coordinates'. Maybe say location, and add 'location especially relative to scar'.

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4. Line 78: remove comma after hPSC-CM

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6. Figure 1A, left: it might be useful to add a zoomed in area of the graft region

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Although we know why reduced coupling leads to focal arrhythmia experimentally and computationally (Circ Res. 2017 Dec 8;121(12):1379-1391.), to the best of my knowledge, no one applied it to EA.

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*In Fig7A, there is large variability from model 1 to model 5. Do you know why?

*Which NHP did you use? Macaque?

*"Leasd" in the title is misspelled.

_____

END OF COMMENTS

Confidential Review **06-Jan-2023**

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SENIOR EDITOR: *Please add a statement explaining ethical review of animal-derived samples used in this investigation.*

We thank the editors for their comments. A statement explaining the ethical review of animals has been added.

We thank both referees for their questions, comments, and suggestions. The manuscript text and figures have been revised accordingly. All significant changes to the manuscript text are shown in blue.

REFEREE #1: The authors performed a computational study investigating the effect of graft-host connectivity and graft conductivity on graft-initiated arrhythmias in human pluripotent stem cell-derived cardiomyocytes grafts in the infarcted ventricle. The study is well presented, and the aims are clear. The results are interesting and provide novel insight into the effect of scar and graft-host connectivity on arrhythmias, with potentially important clinical implications. However, there are a few points that the authors should clarify:

1. The resting membrane potential the authors obtained (~ -72 mV) is not consistent with the measured values stated at line 182 (-68 mV, -57 mV). This should either be addressed, or at least added as a limitation to the study, as the rest membrane potential might have important effects on propagation.

We appreciate the referee's comment regarding our models resting membrane potential. In modifying the Kernik model, we aimed to make the fewest adjustments possible to reduce the likelihood of model derangement. Nevertheless, the point regarding resting potential is well taken, and text regarding this limitation has been added accordingly (lines 521-526).

2. The authors should perform simulations with slow but conducting scar because that might affect the conclusion of the study, since graft conductivity and scar presence seem to have quite a significant effect on graft-initiated activation.

We thank the referee for this excellent suggestion. In response, we ran a completely new set of experiments in models with slow-conducting scar (electrical properties based on prior simulation studies, as now described on lines 261-264 in Methods). Unlike non-conductive scar, which creates a barrier to intercellular propagation, slow-conducting scar acts as a pure electrotonic sink, absorbing excitatory current that would have otherwise contributed to membrane depolarization in simulated graft myocytes. This reduced the overall number of graft host excitations in our model compared to non-conductive scar. This point is illustrated in the additions we made to Figs. 7 and 8 as well as in text on lines 372-378 and lines 403-408.

3. The authors should also mention in the limitations that they did not consider border zone tissue around the scar and that 3D modelling, while more expensive, might show different vulnerable windows and change the results significantly.

The referee brings up an important point. We chose to use 2D models instead of 3D because histology images allowed us to include the exact locations of graft, scar, and host myocardium in our models. Inter-slice spacing (~3 mm) in standard histology preparations is too coarse to create reasonable 3D models by aligning and stacking slices (i.e., granularity of spatial features would be much coarser in the Z direction vs. the XY plane, creating a "staircasing" effect). Thus, to use 3D models, we would have had to use synthetic approaches to generate patterns of graft and/or scar. Regarding border zone, the author is correct that we do not modify the underlying ionic currents in host myocardium directly adjacent to scar. However, we would highlight out that our models do have an explicit high-resolution representation of the patchy intermingling of myocardium and infarct in these areas, with a resolution far greater than what could be observed as "gray zone," for example via LGE-MRI. We have added a condensed version of these interesting points to our discussion section on limitations (lines 504-515).

4. Do graft myocytes undergo any physiological changes when they become more mature? If not, the authors could provide a reference for that. If not, this should also be listed as a limitation of the study, as the only temporal factor included in the study is increased graft-host connectivity.

This is a crucial point. It has been shown that as hPSC-CM mature they undergo myofibril alignment and t-tubule formation. Moreover, the cells become electrically quiescent due to increase I_{K1} expression and down-regulation of I_f and I_{CaT}. In our study, because we used computational models in which we can exercise fine-grain control over our parameters, we deliberately explored models in which variables like graft-to-host electrical coupling (p_C) were the *only* differences, ruling out other potential confounding factors like changes due to intrinsic cell-scale maturation that could potentially explain EA propensity. Additionally, since the exact changes in EP properties of engrafted cells over time are not yet fully characterized *in vitro*, it would be hard to properly calibrate models attempting to probe their importance to EA at this time. We have added text to our Discussion to reflect these aspects of our study (lines 497-503).

5. The manuscript should undergo careful proof-reading.

The submitted manuscript has undergone careful proof-reading by all authors. We thank the referee for pointing out several typos or other errors under Minor Points, which were very helpful guidance in this regard.

Minor points:

The authors should provide how many nodes and elements each model had (for instance at line 130 where they state the mesh resolution).

We appreciate the referee for pointing this out. A new table (Table 1) was added to clarify this point.

Line 169: 'island-by-island-basis' is unclear at this point. It becomes clearer when looking at Figure 6 where all models are shown, and the reader can clearly appreciate that each model has many islands. This is not clear in Figure 1C, as the model the authors show does not have many islands. Also, the authors might consider changing the blue-green color combination, as the colors can be difficult to distinguish at times.

We thank the referee for bringing this to our attention. To better emphasize this concept of multiple islands, we have outlined each in orange in Figure 1C to increase contrast. We have chosen to keep the blue-green colour combination because we believe it provides a good contrast that is also colourblind friendly (especially important for the senior author, who is extremely colourblind).

Line 178: can the authors please specify what species the Kernik model was originally developed for? Human, NPH.

We thank the referee for this question. The Kernik model was originally developed as a model of human induced pluripotent stem cell-derived cardiomyocytes at the cellular scale. This information is provided on line 213.

Line 180-181: the authors should provide some references for experimental data supporting their (1) claim about faster intrinsic rate. […] Line 196: is 1.9 Hz frequency consistent with any experimental data? The authors should provide a reference for this.

We thank the referee for this suggestion. Currently, we are unaware of published *in vitro* data that document a faster intrinsic beating rate in hPSC-CM. However, since the rates of tachycardias observed in animals during EA can be as fast as ~5 Hz, we believe it is justified to increase the beating rate of the cell-scale model and impose a less negative MDP. As expressed on lines 215-216, the rationale for these changes was to create a more accurate representation of what is occurring *in vivo*.

Line 199: the authors should specify how the cell models were initialised before running the simulations at the tissue scale. Were the modified Kernik and the Ten Tusscher model ran for a number of beats to reach a near steady state?

The referee's point is well taken. In the interest of model reproducibility, we used identical initial conditions to published values for both ionic models (including in the Kernik model, with modifications to key conductance parameters as described elsewhere). No additional beats were run to reach a steady state for either model. This key information is provided on lines 235-236 of our revised manuscript.

Line 230: just for clarification, the authors should specify that 'maturity' refers to the percentage of host-graft connectivity.

We thank the referee for this point of clarification. We have replaced the word "maturity" on line 270 with p_C (graft-host connectedness level).

Line 299: the orange dots in Figure 6 are very difficult to see. Maybe an arrow would be more visible?

We thank the referee for bringing this to our attention. We have changed the marker to a grey asterisk and increased the size.

Figure 7: what does the color on the dots mean? If it does not have any meaning, then the dots should

all be the same color. A similar figure should also be shown for Model 5, as that also seemed to be an interesting case.

We thank the referee for this feedback. All points in figure 7 are now yellow to improve clarity and a column showing Model 5 was added.

Additional Minor Points

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- *Line 51: unclear what the authors mean by 'spatial coordinates'. Maybe say location, and add 'location especially relative to scar'.*
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We thank the referee for pointing out these issues. They have all been corrected or clarified as suggested.

REFEREE #2: Gibbs et al. studied how graft-host coupling affects engraftment arrhythmia using computer models. The models are based on experimentally obtained histology images. Although we know why reduced coupling leads to focal arrhythmia experimentally and computationally (Circ Res. 2017 Dec 8;121(12):1379-1391.), to the best of my knowledge, no one applied it to EA.

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of the stochastic approach is not provided. Line 165 "We examined N levels of graft connectedness" : What are N levels? and so on and so forth...

We appreciate the referee's comments and concern regarding our methods. The first address the concern on line 159, we added a new component to Figure 3 to help visually clarify what is being changed when we modify graft-host coupling. Elsewhere, throughout the Methods section (with particular focus on the examples highlighted by the referee) we have revised for clarity and we have included as much detail as possible, towards the goal of making our work reproducible.

Minor comments:
**In Fig7A, there is large variability from model 1 to model 5. Do you know why?*

We thank the referee for this observation and question. As explained in Methods, the models came from two different animals and the short axis slices were taken from different parts of the heart (i.e., some were more apical while others were more basal). As such, some of the variability is attributable to differences in MI location and size, how many cells engrafted in the heart of each animal, details of cardiac anatomy, and locations of the slices with respect to cell injection sites. In Model 5 it notably appears that the upper left graft seemed to be localized around a vein. We have added a condensed version of this interesting discussion to our revised manuscript (lines 432-435).

**Which NHP did you use? Macaque?*

We thank the referee for this question. The images used in this study came from Macaques. Clarification was added on line 133.

**"Leasd" in the title is misspelled.*

We thank the referee for bringing this to our attention. It has been corrected.

Dear Dr Boyle,

Re: JP-RP-2023-284244R1 "Graft-Host Coupling Changes Can Lead to Engraftment Arrhythmia: A Computational Study" by Chelsea E Gibbs, Silvia Marchianó, Kelly Zhang, Xiulan Yang, Charles E Murry, and Patrick M Boyle

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 we are pleased to tell you that it is acceptable for publication following satisfactory minor revision.

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

The referee reports are copied at the end of this email.

Please address all the points raised and incorporate all requested revisions or explain in your Response to Referees why a change has not been made. We hope you will find the comments helpful and that you will be able to return your revised manuscript within 4 weeks. If you require longer than this, please contact journal staff: jp@physoc.org.

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We look forward to receiving your revised submission.

If you have any queries, please reply to this email and we will be pleased to advise.

Yours sincerely,

Peter Kohl
Senior Editor
The Journal of Physiology

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-Papers must comply with the Statistics Policy https://jp.msubmit.net/cgi-bin/main.plex?form_type=display_requirements#statistics

In summary:

-If n {less than or equal to} 30, all data points must be plotted in the figure in a way that reveals their range and distribution. A bar graph with data points overlaid, a box and whisker plot or a violin plot (preferably with data points included) are acceptable formats.

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-'n' clearly defined (e.g. x cells from y slices in z animals) in the Methods. Authors should be mindful of pseudoreplication.

-All relevant 'n' values must be clearly stated in the main text, figures and tables, and the Statistical Summary Document (required upon revision)

-The most appropriate summary statistic (e.g. mean or median and standard deviation) must be used. Standard Error of the Mean (SEM) alone is not permitted.

-Exact p values must be stated. Authors must not use 'greater than' or 'less than'. Exact p values must be stated to three significant figures even when 'no statistical significance' is claimed.

-Statistics Summary Document completed appropriately upon revision

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EDITOR COMMENTS

Reviewing Editor:

Congratulations on a nice paper. Please note that statistics information needs to be revised to comply with the JP policy.

Standard Deviation (SD) must be used instead of Standard Error of the Mean (SEM). Please state precise P values.

REFEREE COMMENTS

Referee #1:

I have no additional comments.

Referee #2:

All of my comments have been addressed appropriately. I have no additional comments.

END OF COMMENTS

1st Confidential Review **14-Mar-2023**

Reviewing Editor: Congratulations on a nice paper. Please note that statistics information needs to be revised to comply with the JP policy. Standard Deviation (SD) must be used instead of Standard Error of the Mean (SEM). Please state precise P values.

We thank the editor for bring this to our attention. We have updated the figure legend on line 240 to show mean (SD) and added exact p values to figure 4B. We have also provided a new statistical summary table using SD.

REFEREE COMMENTS

We thank both referees for their time.

Referee #1:

I have no additional comments.

Referee #2:

All of my comments have been addressed appropriately. I have no additional comments.

Dear Dr Boyle,

Re: JP-RP-2023-284244R2 "Graft-Host Coupling Changes Can Lead to Engraftment Arrhythmia: A Computational Study" by Chelsea E Gibbs, Silvia Marchianó, Kelly Zhang, Xiulan Yang, Charles E Murry, and Patrick M Boyle

Congratulations and many thanks for submitting your work to JP!

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

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Reviewing Editor:

Thank you!

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Congratulations and many thanks for submitting your work to JP!
