PONE-D-15-17915

A 3D-Printed Oxygen Control Insert for a 24-Well Plate

PLOS ONE

Dear Arum Han and reviewers,

Thank you for reviewing our manuscript submission to PLOS ONE. We have made several improvements and clarifications resulting what we hope is a stronger manuscript ready for publication.

In addition to this rebuttal letter a clean revised manuscript is submitted as well as a marked-up copy with tracked changes.

Yours sincerely,

David Eddington, Ph.D. University of Illinois at Chicago

Authors' Rebuttal to Reviewers:

Reviewer #1:

It is a different version of design to control oxygen in cell culture, among some recent efforts. One concern is that the group has published quite a number of papers on oxygen control devices, although it is now through 3d printing. It is important to highlight in front the key improvements.

- **Authors' rebuttal:** An overview of improvements from our previous PDMS cast devices starts on line 51 of the original manuscript, now line 64 of the revised manuscript.

Also, it is important to compare with some other methods related to 3d printing such as that done recently by Kamei K. et al. (Biomed Microdevices. 2015 Apr;17(2):36).

- **Authors' rebuttal:** The work by Kamei et al. was cited along with several other papers that used 3D printed parts as molds for PDMS devices on line 33 of the original manuscript, now line 46 of the revised manuscript.

"This is the first 3D-printed device that can be functionalized to control oxygen in cell culture" is a statement which is too strong.

- **Authors' rebuttal:** The wording was changed to "This is first 3D-printed device incorporating gas permeable membranes to facilitate oxygen control in cell culture."

Reviewer #2:

Currently, little consideration is given to mimicking physiological oxygen concentrations in vitro during cell experiments. Oftentimes cells are cultured at atmospheric oxygen conditions which

are higher than what is found in many tissues, including tumor environments. Some systems have been developed to control the oxygen environment in vitro, but these can be cumbersome or costly to implement. 3D printing has emerged as a rapid prototyping method to fabricate devices with high control over structure and therefore is an attractive method to design a microfluidic device capable of controlling oxygen concentrations in culture. Brennan et al. report on a 3D printed control device capable of adjusting oxygen concentrations in a 24-well plate. Although the concept is interesting, the data is not sufficient and additional experiments are needed to improve the manuscript.

Page 3 - In addition to presenting gap distance below insert and well bottom, it would be helpful if the authors included volume of media this space would hold.

- **Authors' rebuttal:** A sentence was added including the approximate volume of media in the gap. See line 87 of the revised manuscript.

Page 4 – Why was 6 hrs chosen for cell culture? It would seem that the authors could demonstrate a more robust change in expression over the concentrations tested if the experiment was extended to a longer time point, especially considering only the 0% group showed a significant difference in VEGFA expression.

- **Authors' rebuttal:** VEGFA is under the transcriptional activity of HIF-1alpha. The time course of HIF-1alpha protein levels in A549 cells demonstrates maximal protein levels at 4 h and a steep decrease in expression by 16 h. Based on the known time course, we looked at a time point slightly delayed from the 4 h peak to account for transcription of the downstream target gene of interest (VEGFA). At longer time points, HIF protein expression is actually suppressed, and many transcripts are not increased with extended duration. This additional information was added along with a citation starting at line 182.

The authors specify concentrations of 21%, 10%, 5%, and 0% were evaluated, however in their Figure 2b, the concentrations appear closer to 13% (not 10%) and 7% (not 5%). Variation of 3% can be considerable in physiological environments and it is unclear if the authors recognize this variation. It is also amplified by the lack of error bars for each group.

- **Authors' rebuttal:** The data in the original submission was analyzed with a two point calibration. This method correlates intensities to oxygen concentration assuming a linear Stern-Volmer relationship. While standard practice, the two-point calibration suffers from increasing inaccuracy as points are measured further away from the calibration points. Characterization data was re-taken with 5 calibration points and analyzed with a two-site Stern-Volmer model to increase accuracy of analyzed data. A new plot replaces figure 2 and includes error bars.

Is sterilization an issue for this device, considering it will be in close contact with biological components?

- **Authors' rebuttal:** Sterilization was preformed by spraying the device with 70% ethanol and leaving it in a biosaftey hood with UV lights overnight. We did not observe any contamination. This information was added to the manuscript. See lines 131, and 141.