BEC BIOENERGETICS COMMUNICATIONS
Bioenerg Commun 2022.9
https://doi.org/10.26124/bec:2022-0009.r2
Open peer review
Open Access

Open peer review and authors' responses

How to optimize respiratory models for SARS-CoV-2 research

Authors: Posch W, Dichtl S, Zaderer V, Lass-Flörl C, Wilflingseder D

Manuscript submitted 2022-03-27, 2022-08-16 (revision)

Manuscript accepted 2022-08-22

https://doi.org/10.26124/bec:2022-0009

Reviewer 2

Marcus Oliveira

Institute of Medical Biochemistry Leopoldo de Meis, Federal University of Rio de Janeiro, BR

Manuscript reviewed 2022-04-05
https://doi.org/10.26124/bec:2022-0009.r2

*Only major points from review and responses included.

Reviewer 2

On page 3, the authors discuss the drawbacks of lung organoid cultures and the potential limitations due to hypoxia. I think this is one of the key points of the manuscript and should be developed a bit further with proper literature support. A key factor to bear in mind is how the organoid size affects cell metabolism due to limitations of oxygen/nutrient diffusion. In this regard, evidence demonstrates that the growth of non-luminal organoids follows Kleiber´s power law given a minimum threshold of oxygen concentration is provided (https://pubmed.ncbi.nlm.nih.gov/31417119/). This points out that researchers using organoid/ALI culture models should take into account the size of organoids as a potential limiting factor especially when studying cell bioenergetics.

Authors

Thank you for the comment. To address the size of organoids as their limiting factor better and to get more insights on hypoxia as disadvantage in organoid culture, we were rephrasing our statement and included further literature. We additionally tried to point out that the fact organoids suffer from hypoxia, makes them a favored model when it comes to tumor research. The changes in the manuscript were added on page 3 including following references:

1) Zhao Y, Li ZX, Zhu YJ, Fu J, Zhao XF, Zhang YN, et al. Single-Cell Transcriptome Analysis Uncovers Intratumoral Heterogeneity and Underlying Mechanisms for Drug Resistance in Hepatobiliary Tumor Organoids. Adv Sci (Weinh). 2021;8(11):e2003897.
2) Ziolkowska-Suchanek I. Mimicking Tumor Hypoxia in Non-Small Cell Lung Cancer Employing Three-Dimensional In Vitro Models. Cells. 2021;10(1).

Reviewer 2

I think some evidence (or discussion) about the potential metabolic artifacts generated by 3D cultures should be briefly described. For example, are the activities of critical metabolic steps including glycolysis, TCA cycle, oxidative phosphorylation, or even mitochondrial morphology, different from cells in vivo from those grown as organoids/ALI cultures? Is there any evidence from the literature dealing with these issues in a comparative framework?

Authors

Thank you for the comment. By adding different references on page 3 (organoid introduction) we want to show the similarity of the human in vivo system compared to 3D organoids. Although 3D models can generate metabolic artefacts, the similarity to the human system is far better than for example 2D cell models or model organisms. References added to the section are following:

1) Frieboes HB, Zheng X, Sun CH, Tromberg B, Gatenby R, Cristini V. An integrated computational/experimental model of tumor invasion. Cancer Res. 2006;66(3):1597-604.

2) Ghosh S, Spagnoli GC, Martin I, Ploegert S, Demougin P, Heberer M, et al. Three-dimensional culture of melanoma cells profoundly affects gene expression profile: a high density oligonucleotide array study. J Cell Physiol. 2005;204(2):522-31.

3) Mazzoleni G, Di Lorenzo D, Steimberg N. Modelling tissues in 3D: the next future of pharmaco-toxicology and food research? Genes Nutr. 2009;4(1):13-22.

4) Pampaloni F, Reynaud EG, Stelzer EH. The third dimension bridges the gap between cell culture and live tissue. Nat Rev Mol Cell Biol. 2007;8(10):839-45.

5) Semino CE, Merok JR, Crane GG, Panagiotakos G, Zhang S. Functional differentiation of hepatocyte-like spheroid structures from putative liver progenitor cells in three-dimensional peptide scaffolds. Differentiation.2003;71(4-5):262-70.

Reviewer 2

Given the novelty of the described methods, the authors should briefly describe which methodologies can (and which cannot) be applied for the proper assessment of cell bioenergetics. For example, the quantification of mitochondrial membrane potential by live-cell imaging using fluorescent probes seems logical. The same cannot be stated for respirometry as seems quite challenging due to the impossibility to interrogate mitochondrial metabolic states at individual cells within a complex and heterogeneous organoid.

Authors

By trying to answer the addressed concerns, we added a separate abstract to our report. In the section "2.5 Investigation of mitochondrial bioenergetics in 3D models" we want to demonstrate options for bioenergetic-analysis in 3D models. The abstract is supported by literature.