STRONG CHILDREN'S RESEARCH CENTER

Summer Research Scholar

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ABSTRACT

Title: Investigating Cell Viability in Precision Cut Lung Slices, an *Ex Vivo* Respiratory Model

Background: Respiratory infection and disease are among the leading cause of death worldwide, ranking as the third, fourth, and sixth leading causes of mortality.[1] Traditional cell culture and organoid models fail to accurately replicate the human lung, especially given the complex cell-to-cell interactions which occur throughout an individual's lifetime. Precision Cut Lung Slice (PCLS) modeling is an *ex vivo* technique that involves slicing small, uniform sections of agarose-inflated lung tissue originating from human or animal donors. This method closely mimics *in vivo* lung physiology by preserving the natural 3D architecture of the donor's lung, providing a more accurate representation of the lung compared to lung-like structures grown from cells. Consequently, PCLS enhances the translational research potential in lung disease pathology, enabling researchers to evaluate new drug therapies and more thoroughly examine tissue samples with specific respiratory diseases.[2] To further advance the understanding and application of PCLS, the Pryhuber Lab is investigating the PCLS model's cell viability under a wide variety of protocol conditions.
For this study, two types of tissues are being examined. The first type comprises of pre-freeze PCLS prepared after lung inflation with agarose and prior to cryopreservation. The second type involves post-freeze PCLS, where the lung tissue is first inflated with agarose and cryopreserved in blocks, then thawed and sectioned into PCLS as needed. We will also be using DMEM as media with and without Phenol Red (PR) to culture the tissue and monitor for contamination.

Objective 1: To determine cell viability capacity in the pre-freeze vs. post-freeze PCLS model, evaluating slice timing, cryofreeze method, and cutting technique. **Objective 2:** To assess the reliability of fluorescent imaging microscopes (EVOS-5000 and Keyence BZ-X810) in determining cell viability with a Live/Dead stain.

Results: Throughout the summer, a total of five experiments were conducted, each building progressively on the previous one. Using multifluorescent imaging and WST assay analysis, the data indicated a lack of cell viability in the post-freeze model. Various factors, such as culture time, slicing technique, timing of slicing, and cryopreservation methods did not significantly alter the post-freeze cell viability as assessed by the WST assay. Generally, the post-freeze PCLS exhibited cell viability levels like those of the positive control groups (media without tissue or media with heat-killed tissue), showing little to no mitochondrial activity. The heat kill tissue was determined as a good positive control when conveying WST activity levels similar to the blank media. Conversely, the pre-freeze PCLS demonstrated higher levels of activity compared to the media blank post-freeze PCLS, further supporting existing knowledge about the PCLS model.
There remains an unexplained discrepancy in the reliability of fluorescent imaging, where the ethidium channel signal is relatively weak compared to the calcein channel. We hypothesize that the tissue has a higher level of structural integrity than originally thought, preventing the ethidium stain from passing the cell membrane and binding to DNA for staining.

Conclusion: In terms of cell viability, the results of the WST assay analysis further support previous research about PCLS cell viability--pre-freeze PCLS is a more viable model. Although there was no change in cell viability for post-freeze tissue based on the post-freeze PCLS protocol, there is still a wider

variety of factors left to investigate. We know that agarose-inflation and vibratome slicing pre-freeze, with a thaw and culture time of about 5 days has led to relatively high levels of cell viability. However, due to the complexity of PCLS, it would be important to hone-in on the preparation of the pre-freeze PCLS model itself in the future. In this way, the research purpose is to optimize results and develop a standardized, reliable PCLS preparation model. Additionally, performing a plain media wash between the culture and the WST assay ensures that phenol red in the culture media does not significantly interfere with the WST assay. Consequently, media with phenol red can and should be used in future experiments to monitor for contamination without worry of interfering in a WST assay. Meanwhile, for imaging, this experiment brought to light new concerns about the reliability of the Live/Dead stain, specifically for the Keyence BZ-X810 Fluorescent Microscope. The PCLS is cut on the horizontal plane, however a scalpel is used to block the lung tissue and can cause excess damage along the tissue's edge. The additional damage could have broken down the cell membrane barriers for ethidium passage; meanwhile, even though the center of the tissue is dead (as seen in WST assay), the cell membrane could remain intact and therefore, prevent a strong ethidium signal. A potential future investigation could focus on examining the integrity of the cell membrane in dead cells of the PCLS tissue.

References

1. World Health Organization. (2020, December 9). *The top 10 causes of death*. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death
2. Liu, G., Betts, C., Cunoosamy, D.M. et al. Use of precision cut lung slices as a translational model for the study of lung biology. Respir Res 20, 162 (2019). https://doi.org/10.1186/s12931-019-1131-x