Increased Throughput and Cryopreservation of Precision-cut Lung Slices Extend the Utility of Human-relevant, 3-Dimensional Pulmonary Test Systems

Pooja Desai[1], Khalid Amin[2], Devin Sheehan[1], Nicholas Castro[1], David Allen[3], Holger Behrsing[1]

[1]Institute for In Vitro Sciences, Inc., Gaithersburg, MD; [2]University of Minnesota, Minneapolis, MN, [3]Integrated Laboratory Systems, Inc., Morrisville, NC

ABSTRACT

Human-relevant, in vitro/ex vivo assays are considered an ethical and economically viable manner by which to screen the thousands of chemicals requiring hazard assessment. Of the 3-dimensional models, human precision-cut lung slices (PCLS) are often considered the most physiologically relevant pulmonary test system, but lower throughput and difficulties in cryopreservation have hampered PCLS use.

We have modified a tissue slicer to accommodate 3 tissue cores for simultaneous slicing. Increased slice production was quantified using agarose and tissue cores in the slicer. To evaluate cryopreservation of PCLS, we have tested 5 cryopreservation formulations using PCLS (frozen on the day of slicing, or after overnight culture). Thawed slice viability in each of the groups was assessed with the WST-8 viability assay, prior to fixation and histological evaluation.

The slicer modification resulted in 2.8-fold and 2.4-fold more slices from agarose cores, and lung cores, respectively. Cryopreservation efforts indicated freezing after slicing yields better average viability (48-73% of fresh, non-frozen control) than culturing overnight and freezing (13-54% of control) when assessing health over 4 days, post-thaw. Cryopreservation buffers containing University of Wisconsin preservation solution preserved viability the best (54%-90% of non-frozen control). Histological findings concurred with WST-8 viability results and indicated the retention of healthy lung tissue features (>75% of normal), post-thaw.

The increased PCLS production indicates larger (or multiple) studies can be initiated from one donor lung. The promising cryopreservation results suggest slices can be banked and utilized at a later date, potentially even allowing the same donor's tissue to be used repeatedly.

INTRODUCTION

One of the most physiologically-relevant non-animal models of the lower lung is precision-cut lung slices (PCLS), offering native lung architecture, including small airways and respiratory parenchyma. These substructures within the human PCLS (HuPCLS) reflect the presence of the many cell types in the human lung, not present in other non-animal models used for toxicity studies. The complement of these cell types in HuPCLS allows for a more realistic interpretation of tissue response to exposures that evoke complex pulmonary responses. Reports of multi-week culture longevity and complex responses has positioned PCLS as a candidate model to evaluate key events associated with severe lung disease.

However, with lower throughput and the common issue of limited donor tissue upon study begin, PCLS are difficult to employ in a screening setting. Despite the availability of precision-cut slicers for several decades, tissue slicing remains a rate limiting bottleneck for study design and size. Further, should the rate of slicing be increased, excess PCLS must be utilized immediately as reliable preservation techniques have not yet been reported. Clearly, increased PCLS production, coupled with reliable preservation techniques would position PCLS as a pulmonary test system with increased utility and accessibility for researchers.

Here, we describe modifications that address both the rate of slice creation and also cryopreservation of PCLS tissue. A redesign of the tissue "core sleeve" triples the number of cores passing over the slicing mechanism of the Krumdieck MD4000 slicer. Also, a comparison of multiple cryopreservation buffer performance was evaluated when thawing and maintaining PCLS for 4-days. These initial results suggest an increased scale of PCLS are possible and that researchers may be able to bank frozen PCLS and allow returning to the same donor tissue on multiple occasions.

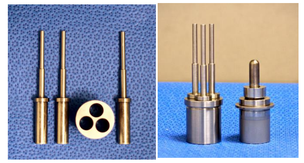
MATERIALS & METHODS

Standard (Single) Tissue Core Slicing

1. Inflate lung tissue and section periphery
2. Create tissue cores
3. Slice cores with slicer
4. Collect slices and place into roller vials
5. Culture slices using roller apparatus, submerged, or at ALI

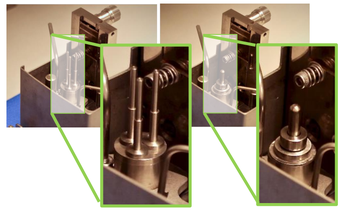
Higher-throughput Slicing

1. A custom designed modified "core sleeve" allows 3 cores to be loaded simultaneously
2. A comparison between the stock unit and multicore sleeve were made



Multicore sleeve with compatible plungers

Multi-core comparison with stock setup



Multi-core enabled slicer (left) run against stock, single core slicer (right)

PCLS Cryopreservation

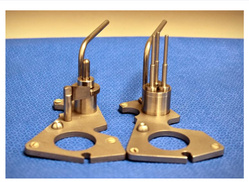
Cryopreservation Method, Variables Tested, & Viability Assessment:

1. Fresh-cut PCLS vs acclimated PCLS: a comparison of slices cultured for 21 days (negative control; N.C.), frozen (Mr. Frosty™) immediately vs slices cultured overnight (in E-199 acclimation medium) and then frozen was made.
2. Five cryopreservation buffers were tested (4 PCLS/buffer-condition), each containing a different combination of ingredients that preserve cells/tissue or are used during freezing procedures
3. N.C. PCLS or thawed PCLS were tested for viability using the WST-8 viability assay. on each of 4 consecutive days.
4. On day 4, PCLS were fixed, processed for H&E staining and assessed histologically for tissue health.

RESULTS

Higher-throughput, Multi-core Slicing

- Modifications to the slicer swing-arm assembly and core sleeve allows three tissue cores to be loaded at once.
- A redesign of the plunger shape allows better access and lessens the chances of incidental contact with the sterile slicing buffer.
- Visual confirmation of the swing-arm action over the blade assembly confirms all three tissue cores clear the cutting stage and blade assembly.



swing-arm assembly

1. After 15' of slicing, agarose and lung tissue slices were counted.
2. Increased rate of slicing:

   Agarose cores = 2.8-fold,
   Lung tissue = 2.4-fold
   (**Figure 1a & 1b**)

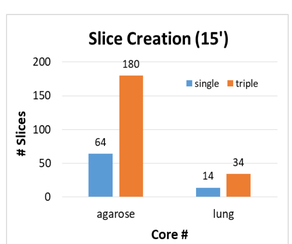
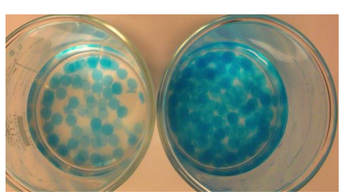


Figure 1a



Stock, single-core slices **Multi-core slices**

Figure 1b

RESULTS cont.

PCLS Cryopreservation

Frozen PCLS were thawed and allowed to recover in acclimation medium overnight.

WST-8 viability readings from fresh cultured and thawed PCLS were taken for 4 days.

WST-8 Results:

- Daily readings demonstrated sustained viability over 4 days (Figure 2a & 2b)
- Freezing on the day of slicing (Day 0) sustained slices better.
- Group 2 (Day 0 freezing) demonstrated the greatest viability, similar to N.C. on day 4 (Figure 2c, 2d, & 2e)

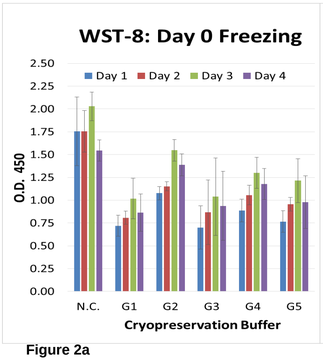


Figure 2a

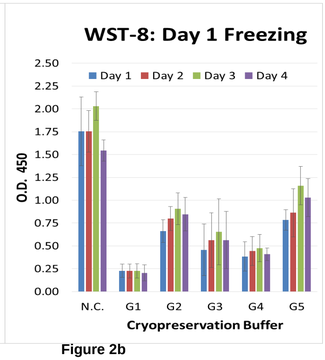


Figure 2b

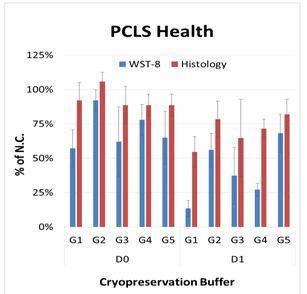


Figure 2c

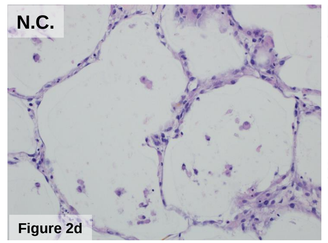


Figure 2d

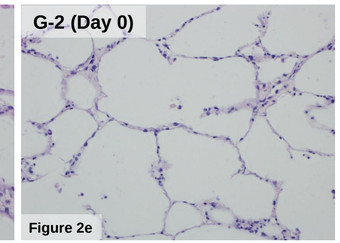


Figure 2e

Histology Results:

Histological evaluation confirmed Group 2 (Day 0 freezing) yielded the best tissue.

Day 0 (Group 2) tissue appears as healthy as the N.C. group (**Figure 2b, 2c, 2d**)

CONCLUSIONS & FUTURE DIRECTIONS

- The modification to the Krumdieck slicer swing-arm successfully increased the rate of agarose (ideal scenario) and also agarose-inflated lung tissue slice creation. It is estimated that rate of single-core slicing can be increased by over 2-fold using the multi-core sleeve design.
- The increased rate of slice production will result in substantially more PCLS, lowering the cost of this highly relevant human lung test system and enabling larger, more comprehensive studies with greater throughput to evaluate inhaled materials or other potential pulmonary toxicants.
- The potential to cryopreserve PCLS (especially with greatly increased production) provides a means to store these very valuable tissues and provides a means to maximally utilize the donated human lung tissue and provide a means to retest the same tissue at a later date.
- Effective PCLS cryopreservation techniques may provide a method by which to begin banking tissues of different disease states and/or genetic predispositions so that targeted research can be conducted on population subsets or other select groups.

REFERENCES

- Behrsing, H. P., et al. (2013). "In vitro exposure of precision-cut lung slices to 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole lysylamide dihydrochloride (NSC 710305, Phortress) increases inflammatory cytokine content and tissue damage." Toxicol Sci 131(2): 470-479.
- Tyson, C. A., K. Amin, et al. (2005). "Comparison of BCNU and SarCNU Toxicity in Long-Term Cultures of Precision-cut Lung Slices." The Toxicologist 84: 240.

ACKNOWLEDGMENTS

- This project was funded in whole or in part with federal funds from the National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN273201500010C.