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

Pooja Desai1, Khalid Amin2, Devin Sheehan1, Nicholas Castro1, David Allen3, Holger Behrsing1

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

ABSTRACT

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 (HUPLS) 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 HUPLS allows for a more realistic interpretation of tissue responses to exposures that evoke complex pulmonary responses. Reports of multi-week culture longevity and complex responses have projected PCLS as a candidate model to evaluate key endpoints associated with severe lung disease.

However, with fewer throughput and the common issue of limited donor tissue upon study begin, PCLS are difficult to employ in a screening setting. In an attempt to increase throughput, our group has redesigned the slicing mechanism of the Krumdieck slicer to improve slicing efficiency and increase the maximum number of slices produced per slicing event. A redesign of the plunger shape allows better slicing and an improved arm assembly allows for the reduction of slicing time. Higher-throughput PCLS cryopreservation techniques may provide a method by which to begin banking tissues of different disease states and enabling larger, more comprehensive studies with greater throughput to evaluate inhaled materials or other potential pulmonary toxicants.

MATERIALS & METHODS

RESULTS

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 (HUPLS) 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 HUPLS allows for a more realistic interpretation of tissue responses to exposures that evoke complex pulmonary responses. Reports of multi-week culture longevity and complex responses have projected PCLS as a candidate model to evaluate key endpoints associated with severe lung disease.

However, with fewer throughput and the common issue of limited donor tissue upon study begin, PCLS are difficult to employ in a screening setting. In an attempt to increase throughput, our group has redesigned the slicing mechanism of the Krumdieck slicer to improve slicing efficiency and increase the maximum number of slices produced per slicing event. A redesign of the plunger shape allows better slicing and an improved arm assembly allows for the reduction of slicing time. Higher-throughput PCLS cryopreservation techniques may provide a method by which to begin banking tissues of different disease states and enabling larger, more comprehensive studies with greater throughput to evaluate inhaled materials or other potential pulmonary toxicants.

MATERIALS & METHODS

RESULTS

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 (HUPLS) 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 HUPLS allows for a more realistic interpretation of tissue responses to exposures that evoke complex pulmonary responses. Reports of multi-week culture longevity and complex responses have projected PCLS as a candidate model to evaluate key endpoints associated with severe lung disease.

However, with fewer throughput and the common issue of limited donor tissue upon study begin, PCLS are difficult to employ in a screening setting. In an attempt to increase throughput, our group has redesigned the slicing mechanism of the Krumdieck slicer to improve slicing efficiency and increase the maximum number of slices produced per slicing event. A redesign of the plunger shape allows better slicing and an improved arm assembly allows for the reduction of slicing time. Higher-throughput PCLS cryopreservation techniques may provide a method by which to begin banking tissues of different disease states and enabling larger, more comprehensive studies with greater throughput to evaluate inhaled materials or other potential pulmonary toxicants.

MATERIALS & METHODS

RESULTS

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 (HUPLS) 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 HUPLS allows for a more realistic interpretation of tissue responses to exposures that evoke complex pulmonary responses. Reports of multi-week culture longevity and complex responses have projected PCLS as a candidate model to evaluate key endpoints associated with severe lung disease.

However, with fewer throughput and the common issue of limited donor tissue upon study begin, PCLS are difficult to employ in a screening setting. In an attempt to increase throughput, our group has redesigned the slicing mechanism of the Krumdieck slicer to improve slicing efficiency and increase the maximum number of slices produced per slicing event. A redesign of the plunger shape allows better slicing and an improved arm assembly allows for the reduction of slicing time. Higher-throughput PCLS cryopreservation techniques may provide a method by which to begin banking tissues of different disease states and enabling larger, more comprehensive studies with greater throughput to evaluate inhaled materials or other potential pulmonary toxicants.

MATERIALS & METHODS

RESULTS

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 (HUPLS) 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 HUPLS allows for a more realistic interpretation of tissue responses to exposures that evoke complex pulmonary responses. Reports of multi-week culture longevity and complex responses have projected PCLS as a candidate model to evaluate key endpoints associated with severe lung disease.

However, with fewer throughput and the common issue of limited donor tissue upon study begin, PCLS are difficult to employ in a screening setting. In an attempt to increase throughput, our group has redesigned the slicing mechanism of the Krumdieck slicer to improve slicing efficiency and increase the maximum number of slices produced per slicing event. A redesign of the plunger shape allows better slicing and an improved arm assembly allows for the reduction of slicing time. Higher-throughput PCLS cryopreservation techniques may provide a method by which to begin banking tissues of different disease states and enabling larger, more comprehensive studies with greater throughput to evaluate inhaled materials or other potential pulmonary toxicants.

MATERIALS & METHODS

RESULTS

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 (HUPLS) 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 HUPLS allows for a more realistic interpretation of tissue responses to exposures that evoke complex pulmonary responses. Reports of multi-week culture longevity and complex responses have projected PCLS as a candidate model to evaluate key endpoints associated with severe lung disease.

However, with fewer throughput and the common issue of limited donor tissue upon study begin, PCLS are difficult to employ in a screening setting. In an attempt to increase throughput, our group has redesigned the slicing mechanism of the Krumdieck slicer to improve slicing efficiency and increase the maximum number of slices produced per slicing event. A redesign of the plunger shape allows better slicing and an improved arm assembly allows for the reduction of slicing time. Higher-throughput PCLS cryopreservation techniques may provide a method by which to begin banking tissues of different disease states and enabling larger, more comprehensive studies with greater throughput to evaluate inhaled materials or other potential pulmonary toxicants.

MATERIALS & METHODS

RESULTS

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 (HUPLS) 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 HUPLS allows for a more realistic interpretation of tissue responses to exposures that evoke complex pulmonary responses. Reports of multi-week culture longevity and complex responses have projected PCLS as a candidate model to evaluate key endpoints associated with severe lung disease.

However, with fewer throughput and the common issue of limited donor tissue upon study begin, PCLS are difficult to employ in a screening setting. In an attempt to increase throughput, our group has redesigned the slicing mechanism of the Krumdieck slicer to improve slicing efficiency and increase the maximum number of slices produced per slicing event. A redesign of the plunger shape allows better slicing and an improved arm assembly allows for the reduction of slicing time. Higher-throughput PCLS cryopreservation techniques may provide a method by which to begin banking tissues of different disease states and enabling larger, more comprehensive studies with greater throughput to evaluate inhaled materials or other potential pulmonary toxicants.

MATERIALS & METHODS

RESULTS

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 (HUPLS) 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 HUPLS allows for a more realistic interpretation of tissue responses to exposures that evoke complex pulmonary responses. Reports of multi-week culture longevity and complex responses have projected PCLS as a candidate model to evaluate key endpoints associated with severe lung disease.

However, with fewer throughput and the common issue of limited donor tissue upon study begin, PCLS are difficult to employ in a screening setting. In an attempt to increase throughput, our group has redesigned the slicing mechanism of the Krumdieck slicer to improve slicing efficiency and increase the maximum number of slices produced per slicing event. A redesign of the plunger shape allows better slicing and an improved arm assembly allows for the reduction of slicing time. Higher-throughput PCLS cryopreservation techniques may provide a method by which to begin banking tissues of different disease states and enabling larger, more comprehensive studies with greater throughput to evaluate inhaled materials or other potential pulmonary toxicants.

MATERIALS & METHODS

RESULTS

CONCLUSIONS & FUTURE DIRECTIONS

• The modification to the Krumdieck slicer swing-arm assembly successfully increased the rate of agarose (celadon) and also agarose-infused 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 agarose production has resulted in substantially more PCLS, lowering the cost of the 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 relate 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 genetic predispositions so that targeted research can be conducted on population subsets or other select groups.

REFERENCES


• 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. HHSN27220100001C.

ACKNOWLEDGMENTS

• The authors would like to thank the following individuals for their assistance and support: Holger Behrsing, Khalid Amin, Nicholas Castro, Devin Sheehan, and David Allen.