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INTRODUCTION

hPCLS after native lung architecture including small airways and respiratory paranchyma, making it one of the most physiologically-relevant non-animal models of the lower lung. Substructures within the hPCLS reflect the presence of the many cell types in the human lung, not present in other non-animal models used for toxicology studies. The complexity of these cell types in hPCLS allows for a more realistic interpretation of complex pulmonary responses to various exposures. Reports of multi-cellular lung models and complex responses has prompted hPCLS as a promising test system with increased utility and accessibility for researchers. Here, we evaluated the performance of short- and long-term cryopreserved hPCLS for a period of 4 weeks in culture. The responses of the cryopreserved hPCLS is cytokinal and inflammatory stimuli were evaluated in comparison to that of the fresh hPCLS from the same donor. The initial results suggest that researchers may be able to bank frozen hPCLS and return to the same donor tissue on multiple occasions.

METHODS

Treatments: Control (Veh-Cont), 0.1% Triton X-100, 0.5% DMSO
Endpoints: Viability, Cytotoxicity, Tissue response

Figure 1. Treatment and endpoint assessment. The fresh or cryopreserved (7 weeks) Cryo 1 or Cryo 2 (Cryo 2) slices were evaluated at standard culture conditions for up to 4 days and then maintained in culture (MEMB/12 media) for 4 weeks. All endpoints were assessed at baseline and then at 4 days and 20 days. At the end of each treatment, remnant slices were used to assess endpoint parameters: collagen hydroxyproline (COL) and MMP-9.

Figure 2. Biomarker expression in fresh and cryopreserved hPCLS. The hPCLS were assessed for multiple cytokines and chemokines using a cryo buffered ELISA kit (Cytometric Bead Array (CBA)). The hPCLS from the two different donors were compared for each time point.

Figure 3. Lipopolysaccharide (LPS)-induced response in cryopreserved hPCLS. The hPCLS were pre-cultured and then treated with LPS (100 ng/mL) for 48 hours. The hPCLS were then harvested and evaluated for cytokine production. The hPCLS from the two different donors were compared for each time point.

Table 1. hPCLS histology scores. The hPCLS were stained with H&E, toluidine blue, and immunohistochemistry slides for Collagen (COL) for each time point. The H&E/ Collagen scores were assessed for each time point.

CONCLUSIONS

The cryopreserved hPCLS are an accessible, human-relevant, pulmonary tissue system suitable for testing inhalated materials, evaluating key events in COPD, and the evaluation of therapeutics ‘on-demand’.

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