Generation and Establishment of Human *Ex Vivo* Lung Cultures

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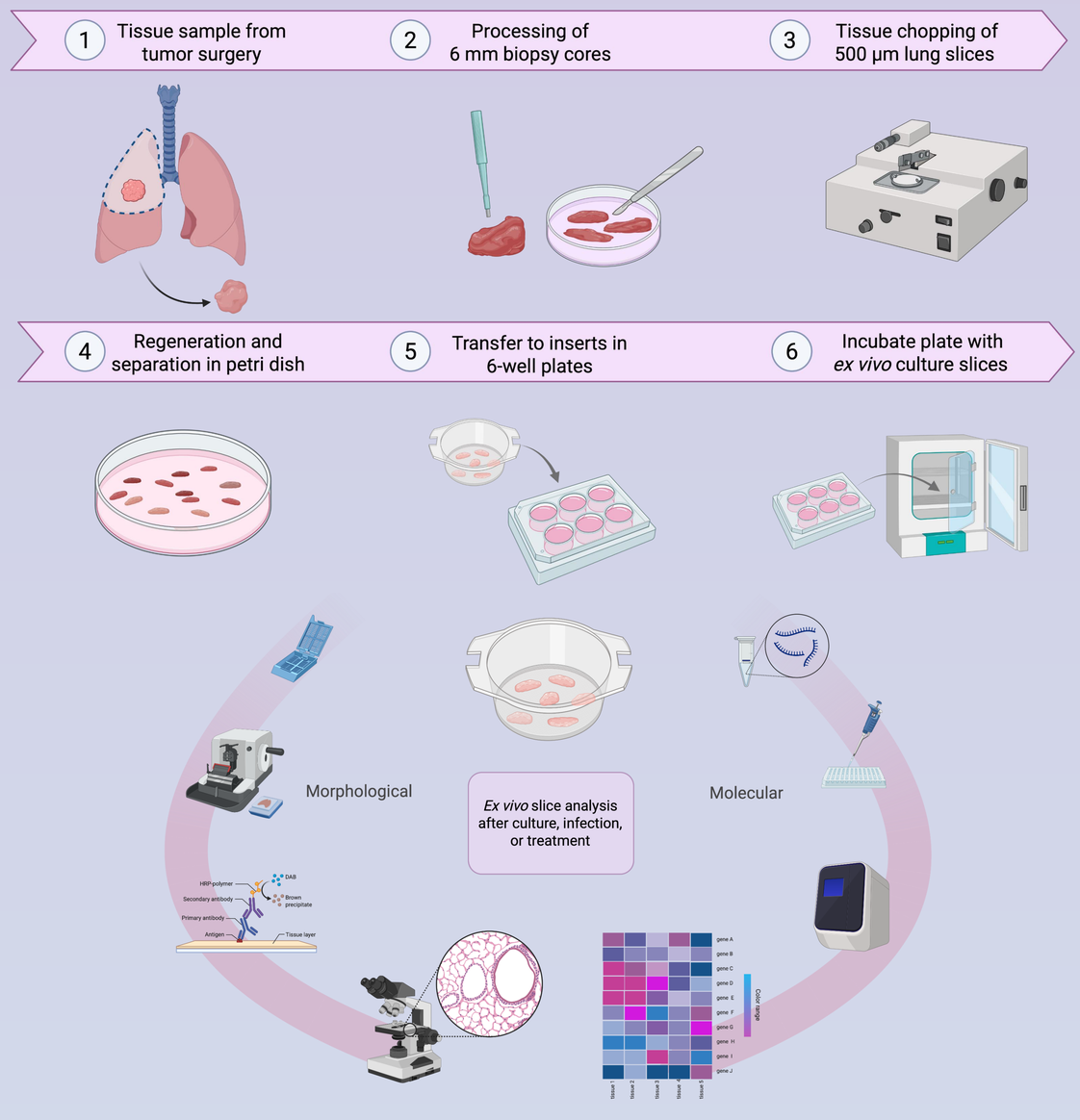
BACKGROUND AND AIM

Ex vivo organ slice cultures generated from surgically resected human tissues represent physiologically relevant short-term *in vitro* models and valuable alternatives to animal experiments in accordance with the 3R principles. Compared with conventional 2D cell culture systems and organoid models, *ex vivo* organ cultures preserve key features of the tissue of origin, including native cellular heterogeneity, spatial organization, extracellular matrix components, and the structural architecture required for organ-specific responses. This makes them particularly suitable for studying biological processes in a human tissue context while maintaining a defined and experimentally accessible culture system.

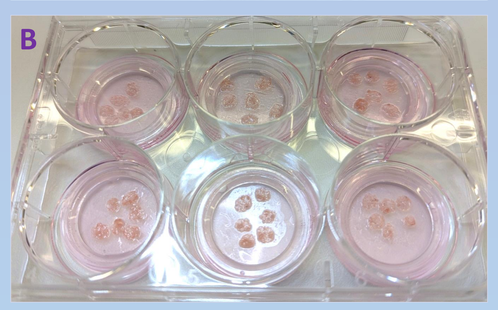
At MUG, an *ex vivo* model based on human precision-cut lung slices (PCLuS) has been established and validated using patient-derived surgical lung tissue. PCLuS enables the investigation of host–pathogen interactions under conditions that more closely reflect the physiological complexity of the human lung. Beyond histological evaluation, the model supports complementary molecular and cellular readouts, including viability assessment, protein quantification, gene expression profiling, and transcriptome-wide analyses. Together, these approaches enable the assessment of tissue integrity as well as culture-, infection-, or treatment-induced molecular responses.

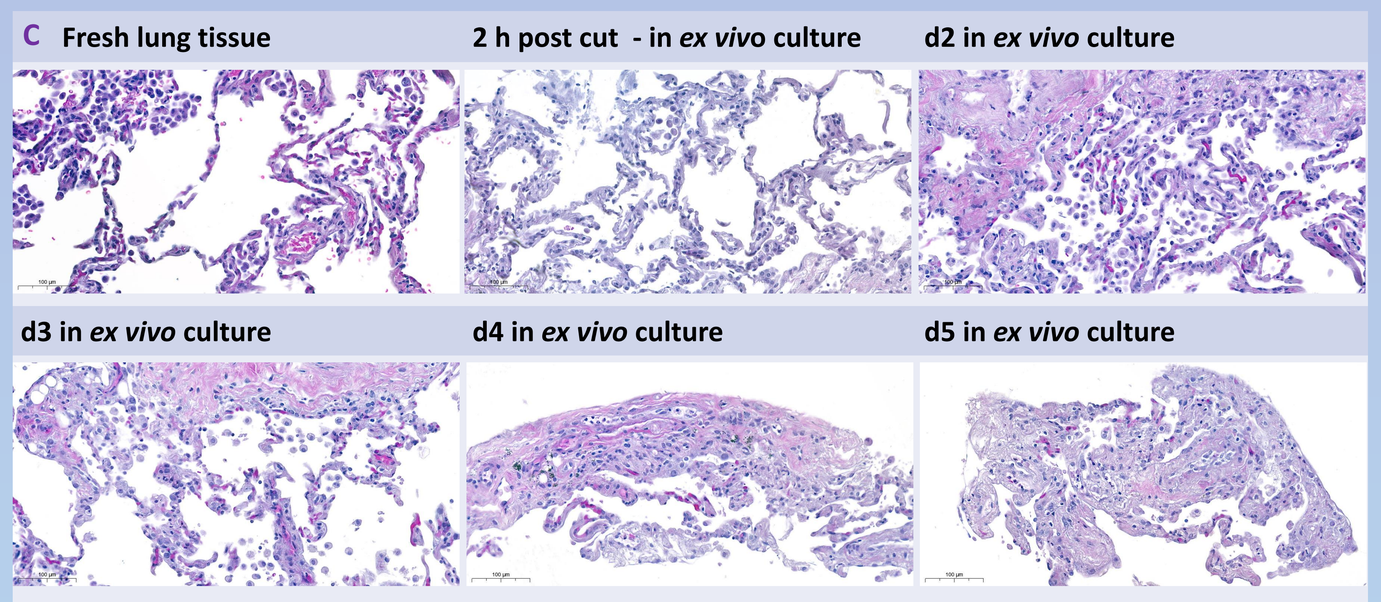
Building on this established platform, the application of human PCLuS is being further explored for antiviral drug testing and therapeutic evaluation. The model provides a human-relevant *ex vivo* environment for assessing antiviral candidates, small molecules, drug efficacy, and potential tissue responses. While limitations such as donor variability, restricted tissue availability, and limited culture duration remain, PCLuS can contribute to bridging the gap between simplified *in vitro* systems and *in vivo* models and support translational infection research.

WORKFLOW



Workflow was created with biorender.com





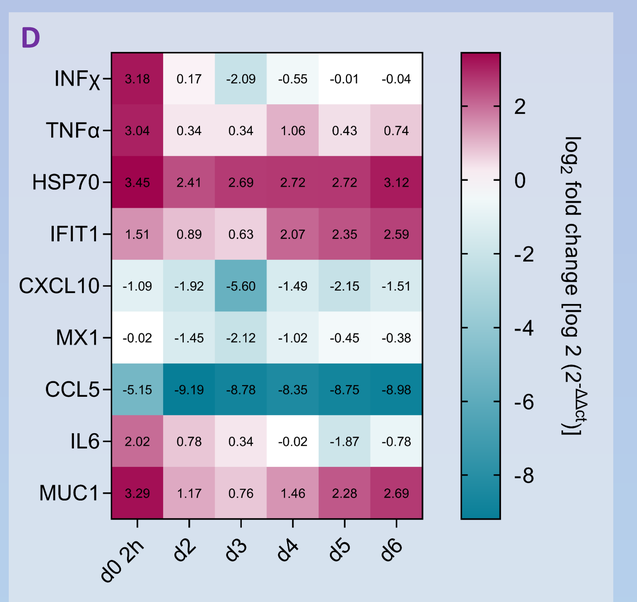


Fig. A: Generation of human *ex vivo* lung slices using the McIlwain Tissue chopper device. **Fig. B:** Generated PCLuS are maintained *ex vivo* in inserts placed in 6 well plates as air liquid interface culture. **Fig. C:** H&E staining from fresh tissue to day 5 of culture. **Fig. D:** Host-response gene expression shown as $\log_2$ fold change relative to fresh tissue analysed with qRT-PCR. Fig. C+D data from one representative donor is shown.

RESULTS

Ex vivo cultured PCLuS preserve alveolar architecture short-term but induce time-dependent tissue stress and transcriptional remodeling

Ex vivo lung slices, or PCLuS were generated from tumor-adjacent, tumor-free human lung tissue of one representative donor to preserve native tissue architecture and cellular heterogeneity (Fig. A, B). H&E staining was used to assess morphology during *ex vivo* culture of lung slices. Tissue morphology was considered acceptable up to day 3, with preservation of infection-relevant lung structures and cell populations, including alveolar regions with alveolar epithelial cells and bronchial epithelium (not shown). From day 4 to day 5, increasing detachment and accumulation of alveolar macrophages were observed, indicating progressive culture-associated changes and reduced morphological stability during prolonged cultivation (Fig. C). qRT-PCR-based host-response profiling from the same donor revealed dynamic transcriptional changes during culture. Gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method and visualized as $\log_2$ fold change relative to fresh tissue (Fig. D). At 2 h after culture initiation, IFNγ, TNFα, IL6, HSP70, and MUC1 were upregulated, consistent with an acute processing- and culture-associated stress response. HSP70 remained elevated throughout culture, while CXCL10, MX1, and CCL5 were reduced over time. Later increases in IFIT1 and MUC1 coincided with declining alveolar integrity. Overall, this representative donor time course indicates that *ex vivo* lung slices preserve key alveolar structures during short-term culture, particularly up to day 2, whereas prolonged cultivation is associated with progressive tissue stress, architectural impairment, and transcriptional remodeling.

CONCLUSION

Infection-relevant lung cell types were preserved in *ex vivo* cultured hPCLuS up to day 3, and the initial processing-induced host response largely normalized after early culture. Thus, day 1 to day 3 represents a suitable window for infection experiments in this *ex vivo* lung slice model.

References:
https://doi.org/10.64898/2026.02.12.705542 and
https://doi.org/10.1016/j.coviro.2025.101462

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