Extraction of Substances from Organoid Lumen by Micromanipulation

Background

Organoid technology allows for the creation of 3D microtissues and organs in vitro, serving as models for human counterparts. These mimic organ-specific metabolic dynamics and are used for analyzing chronic diseases caused by drug-induced injuries or metabolic abnormalities. However, as current methods for analyzing metabolites are limited and results lack information about “cell polarity”, such as what organoid structures are functional, inaccurate assessments are common, especially for single organoids. To address this, we aimed to directly collect and analyze substances localized within organoid lumens.

Experiment Overview

The experiment utilized liver organoids developed by Takebe et al., which reflect the metabolic dynamics of the liver[1]. These organoids exhibit Cholyl-lysyl-fluorescein (CLF) transport activity as a cholestatic model, allowing CLF uptake into the lumens (Fig. 1). Following the method of Shinozawa et al., CLF was introduced into the lumens of the organoids[2]. Subsequently, using the NSK Manipulation System, we extracted CLF from the lumens of the CLF-incorporated liver organoids and achieved epithelial perforation of the organoids using the NSK piezoelectric actuator(Fig. 2).

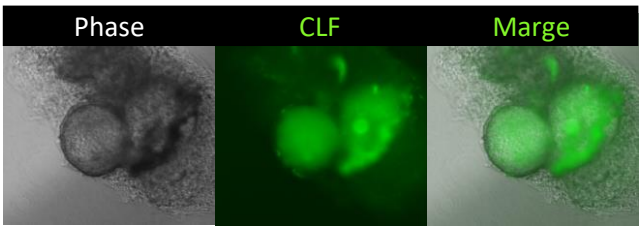


Fig. 1 Incorporation of CLF into organoids

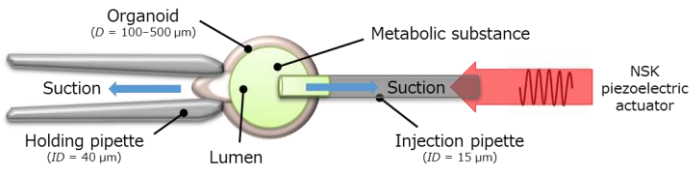
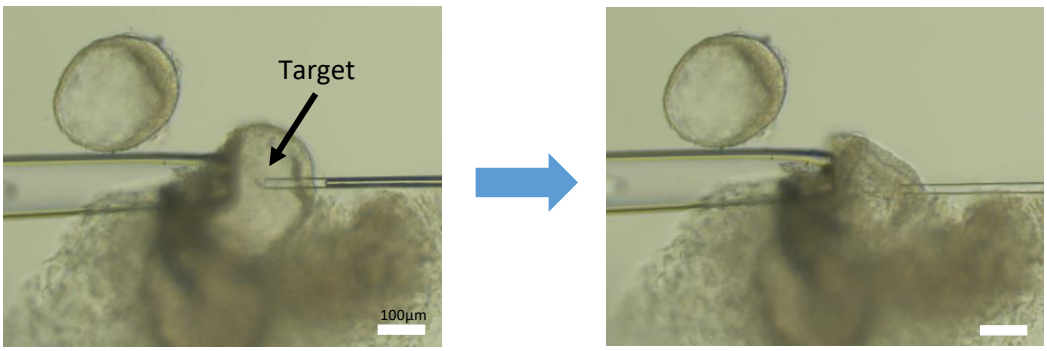


Fig. 2 Perforation and extraction of substance from organoid lumen

Results

1. Extraction of CLF from the organoid

The NSK Manipulation System was used to perform epithelial perforation, capillary insertion, and suction operations on the organoid. As a result, we observed a decrease in the volume of the target organoid during suction operations (Fig. 3).



Before extraction operation

After extraction operation

Fig. 3 Appearance of organoid before and after extraction of luminal substance

Ref. [1] : T. Takebe et al., “Vascularized and functional human liver from an iPSC-derived organ bud transplant”, Nature, vol.499(7459), Jul 2013, 481-484, DOI: 10.1038/nature12271
Ref. [2] : T. Shinozawa et al., “High-Fidelity Drug-Induced Liver Injury Screen Using Human Pluripotent Stem Cell–Derived Organoids”, Gastroenterology, vol.160, Feb 2021, DOI: https://doi.org/10.1053/j.gastro.2020.10.002

2. Analysis of fluorescence intensity before and after CLF extraction

To assess the success of the CLF extraction, we calculated the corrected total cell fluorescence (CTCF) values per unit area from fluorescence imaging of the extracted and non-extracted regions and compared the changes in fluorescence intensity. As a result, we confirmed a significant decrease in fluorescence intensity in the extraction region (Fig. 4). Additionally, we obtained fluorescence images of the interior of the capillary post-extraction, confirming the fluorescence of the solution within the capillary (Fig. 5).

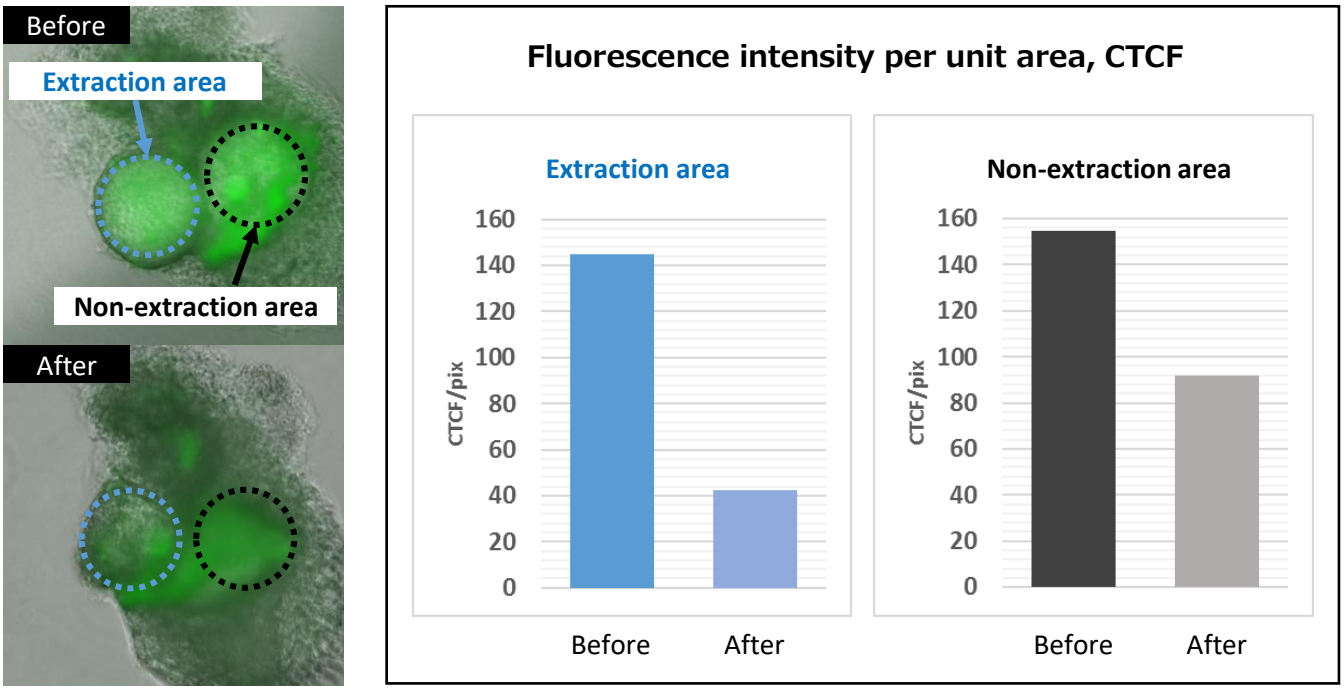


Fig. 4 Comparison of fluorescence intensity before and after CLF extraction

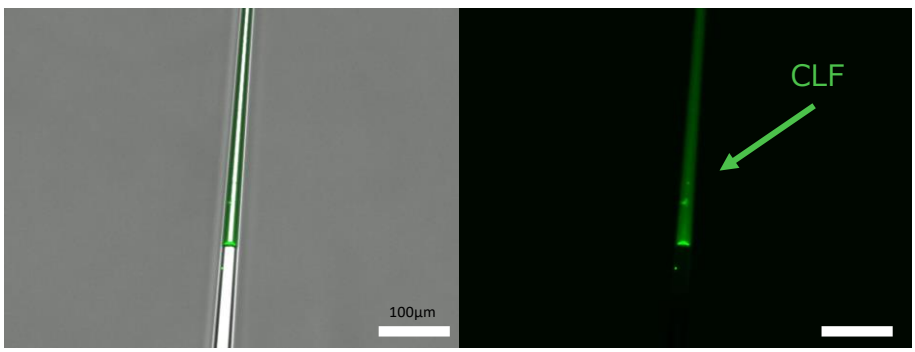


Fig. 5 Fluorescence images of capillary after CLF extraction

Conclusion

The results obtained confirm that CLF incorporated into the lumens of organoids can be directly recovered using the substance extraction functions of the NSK Manipulation System. Utilizing this method is expected to promote new insights into the internal structure and function of three-dimensional cultured tissues.

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