Application Note

How to Grow HUB Derived Upper and Lower Respiratory Lung Organoids in the ClinoStar System

- This application note details a protocol using the ClinoStar and ClinoReactor system to culture upper and lower respiratory lung organoids.
- The ClinoStar culture system enables a dynamic culture that can be maintained in a closed, contamination-free environment for long periods of time.
- Our ClinoStar culture method produce *3D lung organoids without further use of matrices*.

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Introduction

Standardized 3D cell culture has emerged as models for infectious diseases, including for study of SARS-CoV-2 [1-3]. Human airway organoids from broncho-alveolar resections consists of basal cells, functional multi-ciliated cells, mucus-producing secretory cells, and club cells [2]. The current gold standard for modelling the lung epithelium is air-liquid interface (ALI) culture of human bronchial epithelial cells, a system with limited cell expansion and lengthy differentiation protocols [4]. Hence, an easy-to-use lung organoid protocol expands the model systems available in this research field.

Lung organoids in the ClinoReactor

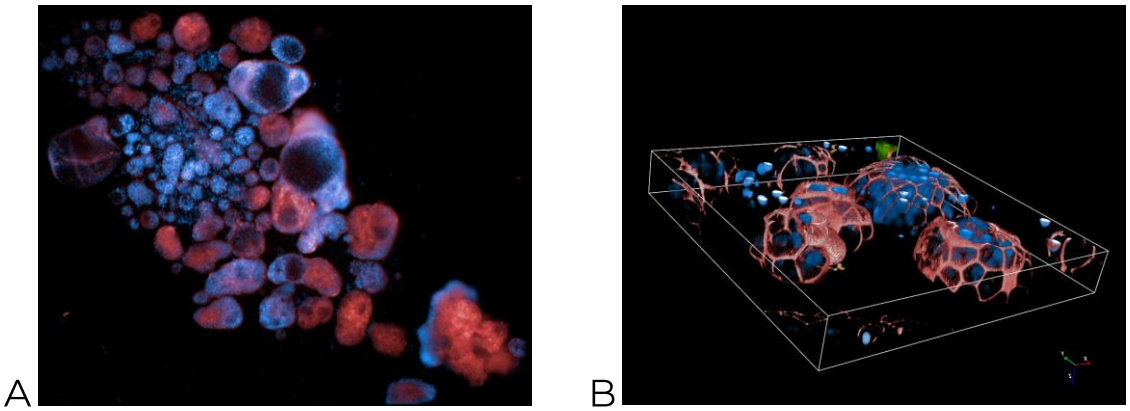

FIGURE 1 Lung organoids cultured in ClinoReactor and stained for Phalloidin (red) and Hoechst (blue). (**A**) Microscoped with an 5x objective. (**B**) 3D structure of lung organoids cultivated in ClinoReactor.

Protocol

Preparation and transfer of lung organoids

1. If lung organoids were in culture with matrices like e.g., Matrigel, this matrix has to be removed to get a dynamic culture.
2. To remove the residues of the matrix, the lung organoids were incubated with 0.5 mL TrypLE Express Enzyme for 5 min at 37°C in a falcon tube.

3. After this incubation step, 0.5 mL medium was added, and the organoids were pipetted up and down multiple times.
4. Centrifuge the falcon tube for 5 min at 1250 rpm.
5. Remove supernatant and resuspend the organoids in medium.
6. Transfer organoids to a conditioned ClinoReactor through top port.
7. Ensure no air is left in cell chamber, close top port and sterilize using 70% ethanol.
8. Place ClinoReactor in ClinoStar and adjust rotation speed corresponding to size of the organoids.

Medium change

1. Change 5 mL media through the top port using needle and syringe twice a week.
2. In case the ClinoReactor has to be changed and there are still residues from the matrix, the procedure with TrypLE can be repeated.
3. Increase rotation speed as organoids grow to ensure optimal media flow.

Reagents and Materials

- 15 mL falcon tube
- 20 mL syringes
- G18 2" needles
- P200 and P1000 tips
- Gibco™ TrypLE™ Express Enzyme
- Conditioned ClinoReactors
- ClinoStar
- Upper and lower respiratory lung organoids from HUB Organoids® *

Basic Medium:

Reagent	Amount
Sigma Aldrich™ Advanced DMEM/F12 (SCM162)	500 mL
Glutamax 100x	5 mL
HEPES 1M	5 mL
Pen/Strep 100x	5 mL

Complete organoid growth medium (for 50 mL):

Reagent	Amount
Basic medium	38.421 mL
Rspondin cond. medium	5 mL
Noggin cond. medium	5 mL
ThermoFisher Scientific™ B27 (50x)	1 mL
Nicotinamide (1M)	250 µL
NAC (500mM)	125 µL
Tocris™ A83-01 (500µM)	50 µL
Sigma Aldrich™ SB 202190 (30mM)	1.7 µL
Miltenyi Biotec™ Human FGF-7 (25µg/mL)	50 µL
Miltenyi Biotec™ Human FGF-10 (100µg/mL)	50 µL
InvivoGen™ Primocin (50mg/mL)	50 µL
MCE™ Y27632 (100mM)	5 µL

*HUB Organoids (https://www.huborganoids.nl/). "HUB Organoids® are developed directly from patient material thanks to our exclusive IP-protected technology that allows adult stem cells (ASCs) present in most epithelial tissues to replicate and differentiate in vitro giving rise to functional "mini-organs in a dish".

Conclusion

In conclusion, the ClinoStar system enables us to grow 3D lung organoids without the use of any scaffolds, hydrogels, or other matrices. This new lung model is only dependent on cellular self-aggregation and is a useful supplement to other established lung model systems like air-liquid interface (ALI). We will use this new lung model in our future SARS-CoV-2 studies.

References

1. Zhou J, Li C, Sachs N, Chiu MC, Wong BH, Chu H, et al. Differentiated human airway organoids to assess infectivity of emerging influenza virus. Proc Natl Acad Sci U S A 2018; 115:6822-7.
2. Sachs N, Papaspyropoulos A, Zomer-van Ommen DD, Heo I, Bottinger L, Klay D, et al. Long-term expanding human airway organoids for disease modeling. EMBO J 2019; 38.
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4. Fulcher ML, Gabriel S, Burns KA, Yankaskas JR, Randell SH. Well-differentiated human airway epithelial cell cultures. Methods Mol Med 2005; 107:183-206.

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