Evaluation of Biocompatibility on 3D-printed Inserts using Mouse Intestinal Organoids

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Goal

Testing customized 3D-printed inserts as an alternative to commercialized air-liquid interphase (ALI) culture systems.

Materials & Method

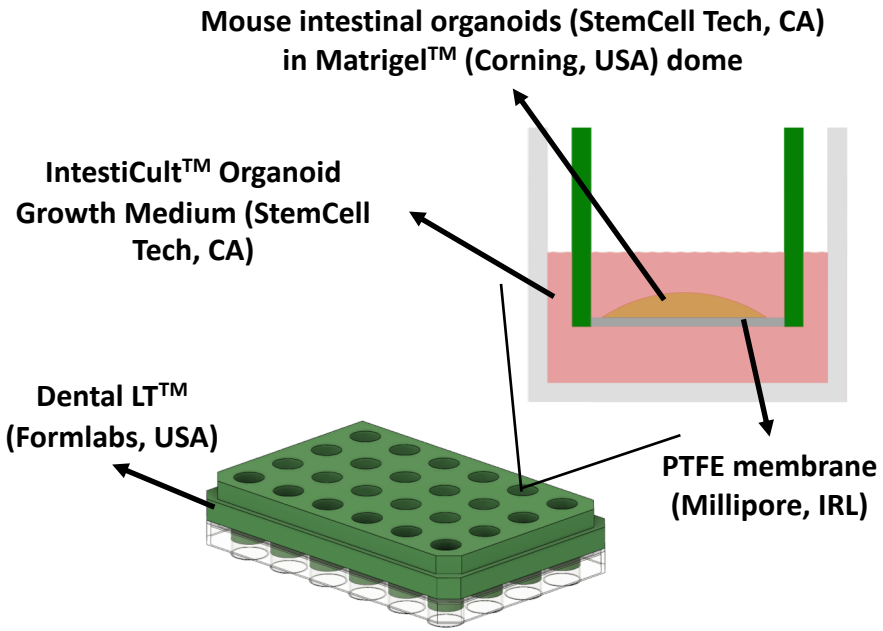

Figure 1. ALI culture of mouse intestinal organoids on 3D-printed 24-well insert plate (*Material:* Dental LT resin; *Printer:* Form 2, Formlabs, USA).

Results

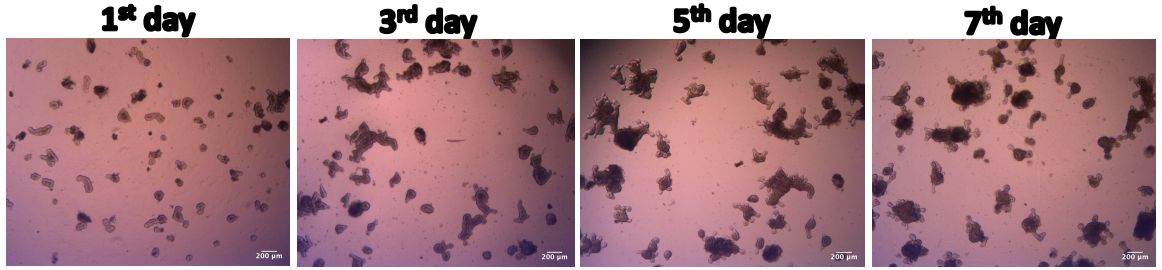

Figure 2. Light microscope images of mouse intestinal organoids on 1st, 3rd, 5th and 7th days (*Scale bar:* 200 μm; 4X magnification; Zeiss Primovert).

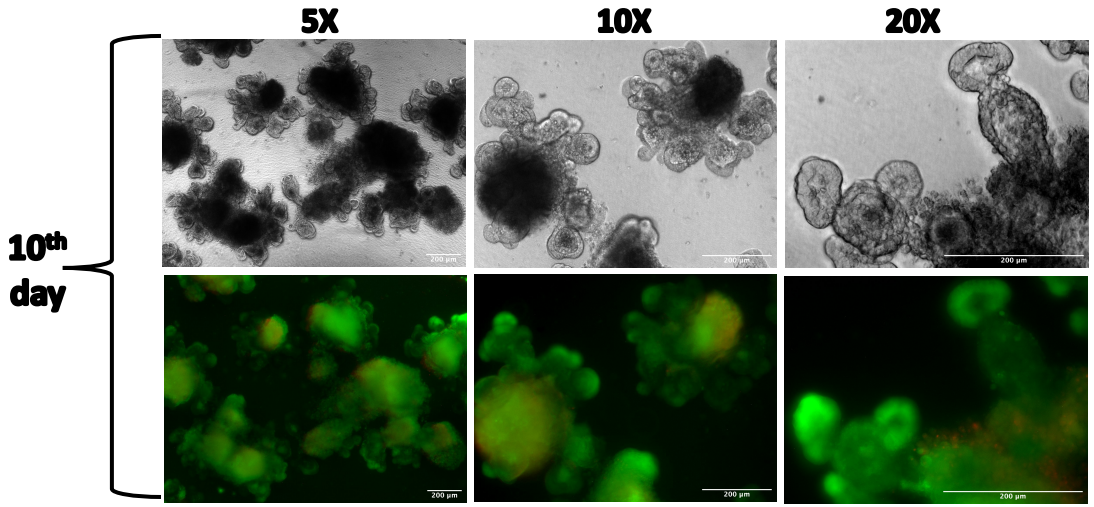

Figure 3. Bright field and Live&Dead staining images of organoids on 10th day (*Green:* Live, *Red:* Dead; *Scale bar: 200 μm* , Zeiss Axio Observer).

Conclusion

- ✓ **No cytotoxic effect on mouse intestinal organoids**
- ✓ **Easy & broad application**
  - **ALI culture**
  - **3D cell culture**
  - **High-throughout screening (HTS)**
- ✓ **Reusable insert**
- ✓ **Low-cost fabricated insert**
- ✓ **Customized design**

Zoom Link: https://dtudk.zoom.us/j/3076664324?pwd=WERkbzB3QkV4WC8xRHhpR05XU0ZTZz09

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Poster Nr.144

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Introduction

Customized 3d-printed inserts are a promising alternative to commercialized air-liquid interphase culture systems [1, 2] to mimic the intestinal tissue for drug absorption/transportation studies [3]. While current models can provide useful information on early biological responses, they have poor predictive capabilities for the physiological features of the intestine [4]. To address these limitations, we aim to provide a customized, cheap, and easy to fabricate/use platform to develop 3D humanized models that are capable of reproducing complex physiological responses *in vitro*.

Experimental Procedure

The design for 3D-culture insert plates (*Figure 1*) was drawn in Fusion 360 software (Autodesk, USA) and printed in FormLabs Dental LT resin using the Form2 printer (FormLabs, USA). After attaching a PTFE membrane (0.4 µL pore size, Millipore, IRL) at the bottom of each insert, the printed insert plates were cleaned with isopropanol followed by UV crosslinking for 2 h at 60°C and sterilized under UV for 20 mins. Intestinal organoids were seeded on the inserts according to manufacturer's instructions (StemCell Tech, CA), and after ten days incubation, cellular viability of organoids was tested *via* Live & Dead staining. Stained organoids were imaged using fluorescence microscopy (AxioVision, Zeiss, DE).

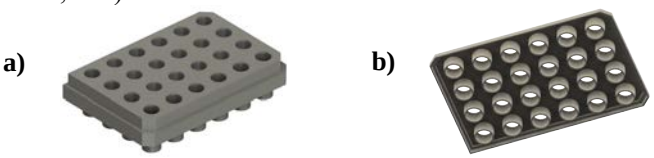

Figure 1. 24-well insert plate. **a)** Top of the insert, **b)** Insert assembled PTFE membrane

Results and Discussion

The design of the 3D-printed platform relies on inserts for commercially available cell culture plates. The biocompatibility of the insert plates was studied by culturing mouse intestinal organoids. During the 10-day incubation period, the size of individual organoids increased, extruding cells into the central lumen structure. Multiple buds and multi-crypt structures were observed. On the 10^{th} day, Live & Dead staining showed only a few dead cells in the buds due to diffusion limitations, for the medium, through the outer layer of oversized organoids. These results show that the platform does not have any significant cytotoxic effect on organoids, and allows for long-term culturing of organoids.

Conclusion

In conclusion, we have shown the fabrication of 3D-printed cell culture insert plates, using a commercial biocompatible resin, and verified their utility in culturing of mouse intestinal organoids. Our design may provide an alternative to the current, commercially available, culture systems to mimic 3D organ systems in the air-liquid interphase for drug testing and tissue engineering applications.

Acknowledgments

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References

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