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# 3D Bioprinting and Implantation of Mouse Mammary Epithelial Structures Using a Custom Accessible 3D Bioprinting Platform

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# 3D Bioprinting and Implantation of Mouse Mammary Epithelial Structures Using a Custom Accessible 3D Bioprinting Platform

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## Abstract:

Prior work has shown that our bioprinting system can reliably produce human mammary organoids and tumoroids with high precision. However, this was not previously applied to mouse models, which are also important with respect to translational research in cancer drug development. To address this, we have produced protocols for the development of *in vitro* structures from murine mammary epithelial and tumor cells. Additionally, we assessed the translatability of both human and murine bioprinted organoids into mouse mammary fat pads over a period of 6 weeks. Our lab found that our produced organoids are reliable, they can survive *in vivo*, and meaningfully integrate within host systems. Therefore, we have demonstrated that our system is adaptable to both human and murine models, as it offers a unique methodology for *in vivo* transplantation of human or murine organoids into mice, which can boost research efforts in cancer therapy research.

## Results

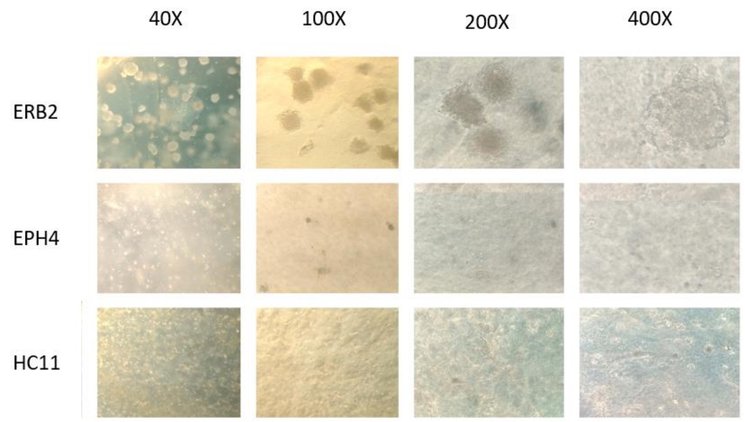
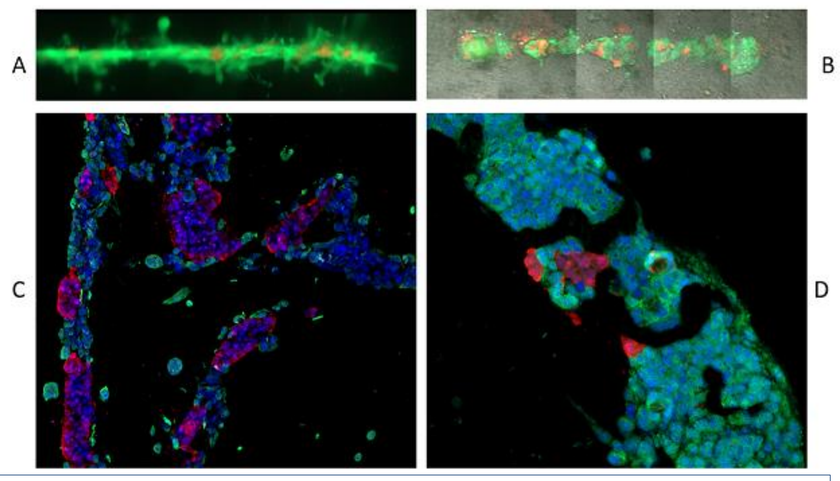



Figure 1 (left): Manually mixed (not bioprinted) 3D culture of mouse mammary non-tumorigenic and tumorigenic lines (EPH4, HC11, and ERBB2) at Day 14, at magnifications of 40X, 100X, 200X, and 400X.

Manual mixes failed to form organized, repeatable structures that were sufficient for implantation. This mirrors the findings of Reid et al (2018), in which human mammary cell lines that were subjected to manually mixed 3D culture also failed to produce desirable structures.

Figure 2 (right): *In vitro* chimeric organoids, analyzed by fluorescence microscopy (parts A and B) and by immunohistochemistry (IHC; parts C and D). A) Linear chimeric organoid printed from MCF-12A-GFP epithelium and MMTV-ERBB2-RFP tumoroids. B) Linear chimeric organoid printed from EPH4-GFP epithelium and MCF-7-RFP tumoroids. C) IHC slide of organoid pictured in part A. Cell nuclei are stained blue with DAPI. D) IHC slide of organoid pictured in part B. Cell nuclei are stained blue with DAPI.




These linear organoid structures, one of murine epithelium with human tumoroids and one of murine epithelium with murine tumoroids, were sturdy and were later implanted into murine mammary fat pads. The fat pads were harvested after 6 weeks, and examined via H&E and IHC, for presence of the implanted organoids (see Figure 3).

## Results (Continued)

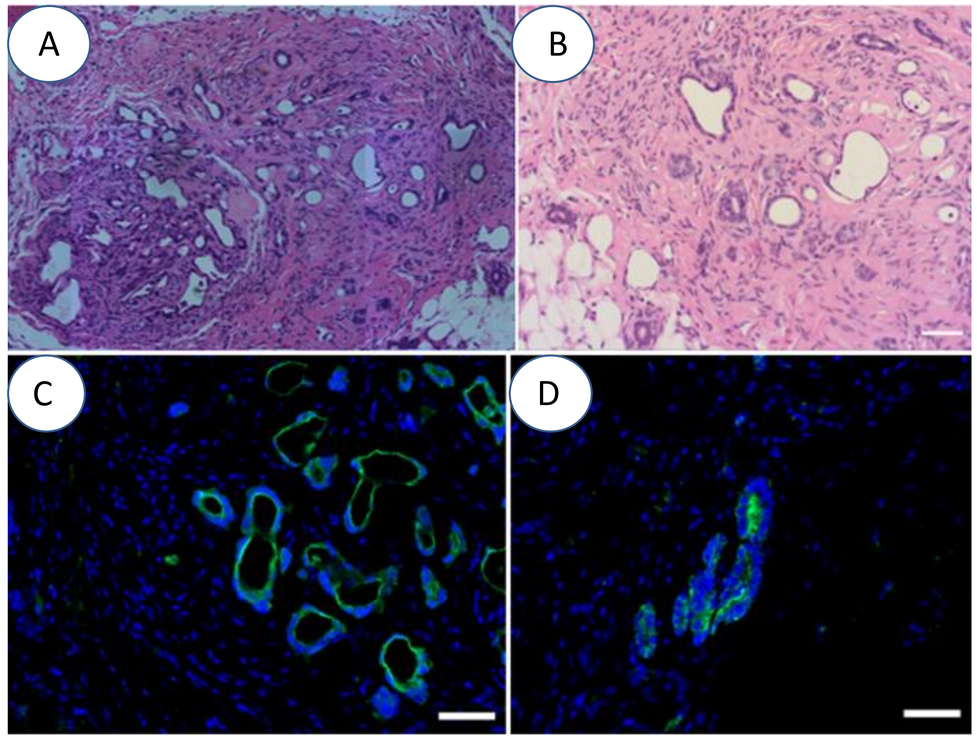



Figure 3 (above): A) Hematoxylin and eosin (H&E) closeup of mammary fat pad section with organoid implants; darker area shows implants in the form of a cyst. B) H&E closeup of gland extract with normal area. C) IHC closeup of B, stained for GFP and DAPI. D) Second IHC Closeup of B, stained for GFP and DAPI. (Bar = 50 µm) IHC guide: DAPI stains blue; GFP stains green.

Organoid implants were found to be detectable 6 weeks after, showing successful integration into murine mammary fat pads.

## Summary and Conclusion:

3D bioprinting demonstrated an ability to organize mouse mammary cells into 3D structures that can then be optimized for further study. Chimeric organoids were implanted within mice and survived for 6 weeks, paving the way for follow-up studies in intact, functional organoid implantation, and follow-up analysis. It is worth reiterating that this is by large a pilot work, but it allows for more optimizable translational medicine and bioprinting. Future work lies in more complete animal studies that would aim to address pure organoid structures, with more mice. Lastly, further molecular diagnostic work is required to understand deeper molecular mechanisms at play in the long-term viability of implantation studies.

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