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Abstract:

Prior work within our lab has demonstrated the ability to print both murine and human mammary organoids and tumoroids *in vitro* that can also be reliably transplanted into a murine host for translational studies. Peripherally, this bioprinting system has also been used for 3D printing neurons, stem cells, cancer cells, and a primary cell line rich with fibroblasts, but each of these efforts were with cells of ectodermal lineage. Thus, the system's capacity for use on cells of other origins had been untested. To address this, we have now developed protocols for cells of endodermal and non-epithelial mesodermal/mesenchymal lineage. In this work, we find that we can produce reliable organoids, tumoroids, and other *in vitro* structures from them, thus expanding the functional range of our open 3D bioprinting platform. Therefore, we demonstrate that our system is versatile for adaptation to multiple cellular systems and can be applied to the work of labs that wish to study development and pathologies in other organ systems.

Results

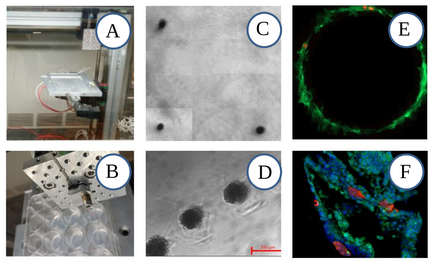
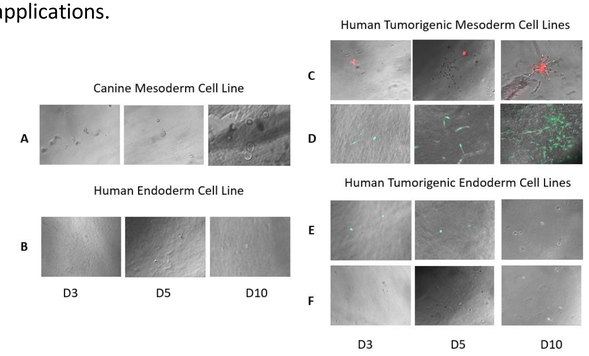


Figure 1 (left): Custom Bioprinter and printing applications. A) Bioprinter, into which can be loaded well plate and micro-extruder. B) Custom Bioprinter extruding bio-ink into gel, mid-print. C) Grid layout of MDCK cell spheroids. D) Arrayed Pan-02 spheroids, immediately post-print. E) Matured chimeric ring organoid composed of MCF-12A and ERBB2 cells. F) Histological slide of chimeric, 3D bioprinted structure composed of EPH4 and ERBB2 cells.

This 3D bioprinter is versatile in print modalities (lines, rings, grids, and more) and diagnostic applications.

Figure 2 (right): Manually mixed (not bioprinted) 3D cultures of cell lines derived from the canine and human mesoderm and human endoderm in rat tail collagen I at 1.5 mg/ml at days 3, 5, and 10. A) MDCK cells. B) FHC (ATCC® CRL-1831™) cells. C) JJ012-RFP cells D) SW1353-GFP cells. E) B. Caco-2-GFP cells. F) Pan-02 cells.

It was found that manual 3D culture was insufficient for forming reliable, repeatable 3D structures from cells of mesoderm and endodermal lineages.



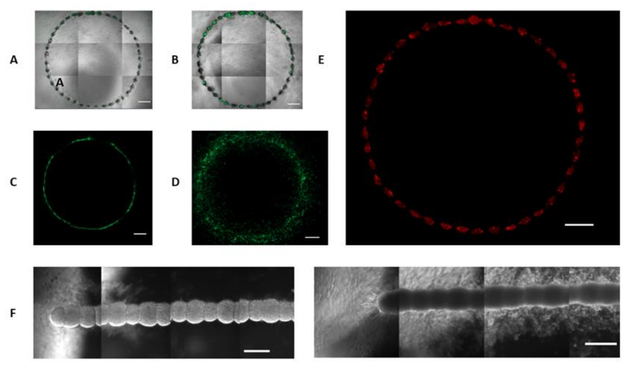


Figure 3 (left): Spheroids printed in rings and lines, from cells of mesoderm and endoderm lineages. (Magnification bar reads 500 µm). A) Caco-2-GFP cells printed in a ring at Day 1. B) Caco-2-GFP cells at Day 7. C) SW1353-GFP cells printed in a ring at Day 1. D) SW1353-GFP cells at Day 7. E) JJ012-RFP cells, printed in a ring at day 7. F) JJ012 spheroids printed closer than normal at Day 1. The spheroids do not merge. G) JJ012 spheroids printed closer than normal at Day 7. The spheroids have merged and can be seen with tendrils reaching outward when examined up close. Reliable tumoroids and other structures were shown to be stably generated from cells of mesoderm and endodermal lineages.

Results (Continued)

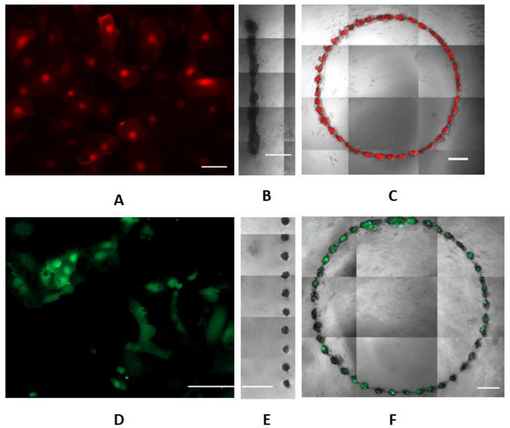


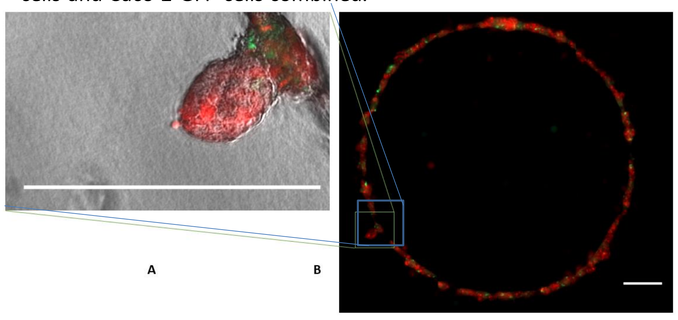
Figure 4 (left): Separate 2D cultures, linear 3D bioprints, and 3D ring bioprints of FHC (ATCC® CRL-1831™)-RFP (Human, Colon, Endoderm, Normal) cells and Caco-2-GFP (Human, Colon, Endoderm, Normal) cells. Magnification bar reads 500 µm.

Each culturing/print modality reveals different ways of viewing and interacting with these cultures. A) 2D cultures of FHC (ATCC® CRL-1831™)-RFP cells. These are large cells, which reveal a polygonal form in this shot. B) 3D bioprinted linear culture of FHC (ATCC® CRL-1831™)-RFP cells. C) 3D bioprinted ring cultures of FHC (ATCC® CRL-1831™)-RFP cells. D) 2D cultures of Caco-2-GFP cells. These are irregularly shaped and long cells. E) 3D bioprinted linear culture of Caco-2-GFP cells. F) 3D bioprinted ring cultures of Caco-2-GFP cells.

When printed, FHC (ATCC® CRL-1831™)-RFP cells create connected lumen, whereas Caco-2 cells create disconnected tumoroids.

Figure 5 (right): Connecting Chimeric, Endoderm Spheroids. A) Close-up of two spheroids seen to be growing towards each other, a few hours after printing. B) The same area, on day 1, 24 hours post-print.

Chimeric spheroids can combine the behavioral properties of two cell lines, to create dynamic 3D bioprinted structures, which in turn create more biomimetic contexts that can be modeled. Herein, spheroids are composed of FHC (ATCC® CRL-1831™)-RFP cells and Caco-2-GFP cells combined.



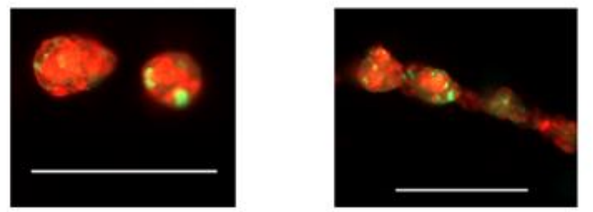


Figure 6 (left): Demonstrated budding and formation of a potential polyp structure on ring of FHC (ATCC® CRL-1831™)-RFP/Caco-2 chimeric tumoroid ring on Day 2 of another ring. Magnification bar reads 500 µm. A) Polyp structure formed. B) Entire FHC-Caco-2 chimeric tumoroid ring.

When FHC (ATCC® CRL-1831™)-RFP cells and Caco-2-GFP cells were printed together, they were able to form a chimeric structure that exhibited budding, which is useful in examining colonic features. This can serve as a model for colon cancer, upon further contextualization.

Summary and Conclusion:

This work contrasted manually mixed 3D culture against automated bioprinting 3D culture, while also building on prior works within the lab that demonstrated the feasibility and benefits of the latter. Specifically, this work expands on that of Mollica et al. (2019); Reid et al. (2018); Reid et al. (2016); Reid et al. (2019), and Palmer et al [2022] which have demonstrated the feasibility of a low-cost bioprinter, the consistency of bioprints, the generation of singular and chimeric mammary organoids and tumoroids, the use of patient-specific ECM, and the successful implantation into and functional, glandular development of mammary organoids in mice. This work expands upon the prior works through the printing of organoids and tumoroids across 3 species and through structures from all 3 germ layers. The combined body of work from the Bruno-Sachs Lab demonstrates the versatility of bioprinter design developed by Reid et al. (2016) for work across all 3 germ layers in projects that promote the exploration and eventual understanding of microenvironmental control of cell fate.

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