Automation of 3D bioprinting assays for high-content imaging and assessment of compound effects

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Summary

The automation of the 3D cell models results in a significant reduction in the time and effort involved, as well as an increase in assay precision and throughput. Here we describe methods for an automated generation of organoids and 3D models using automated 3D bioprinting. Cells mixed with hydrogel-based inks or matrices were dispensed or printed into a 96-well plate using the multi-tool robotic platform, BioAssemblyBot®400 (BAB400). The BAB400 platform enabled efficient dispensing/printing of cells into domes, lines, or other patterns, plate handling, and media addition and exchange. This assay was used for compound testing and evaluation of the anti-cancer effects of various drugs. The results showed the workflow for automated bioprinting/dispensing 3D cellular models with ECM matrices for anti-cancer drug screening workflows. An increase in throughput and ease of operation was achieved through automation. Also, imaging and data analysis methods provided valuable information about complex compound effects in 3D printed and cell-tissue-engineered models.

Methods

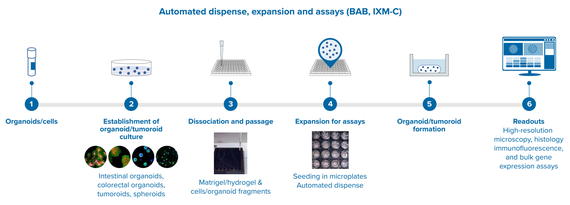


Figure 1. BAB400 pipette tool dispense workflow integrated with Imager for endpoint assays.

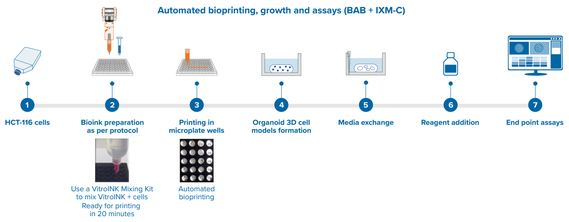
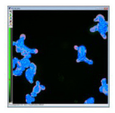


Figure 2. BAB400 ambient tool 3D printing workflow integrated with Imager for endpoint assays.

The integrated system included a high-content confocal imager (ImageXpress® Micro Confocal system) and enabled automated seeding, bioprinting, liquid handling, plate transferring, as well as high-content imaging.

BioAssemblyBot 400

ImageXpress Micro Confocal High-Content Imaging System



MetaXpress High-Content Image Acquisition & Analysis Software

BAB400

The BioAssemblyBot 400 (BAB400) by Advanced Solutions is a cGMP-certified multitool. A multipurpose platform that can be used to build 3D models. Its pneumatic dispense and 3D printing capabilities enable it to build and handle different 3D models or organoids without damage.

The Human Machine Interface (HMI) allowed for effective path design and implementation for the pipette tool and Pick and place tools. The TSIM – Tissue structure information modeling software enabled 3D structure designing for 3D printing with the ambient tool and Bioapps Maker the easy-to-make and easy-to-automate solution could be used to automate a sequence of steps for independent dispensing, maintenance, and imaging of the 3D models.

ImageXpress (imaging)

Transmitted light (TL) of fluorescent images were acquired on the ImageXpress Micro Confocal High-Content Imaging System (IXM-C)(Molecular Devices) using MetaXpress® High-Content Image Acquisition & Analysis Software. For CRCs and intestinal organoids, Z-stack images were acquired with the 4X or 10X objectives using confocal mode. MetaXpress was used for all analyses.

Organoid and cell culture

Cellesce patient-derived colorectal organoids were maintained as per their proprietary protocol; Mouse Intestinal organoids were obtained from Stem Cell Technologies and were maintained according to their recommendations. Patient-derived Triple Negative Breast Cancer (4IC) cells were obtained from Dr. Matthew E Burow's Lab at Tulane University and maintained accordingly. The HCT-116 (human colon cancer cell line) cells and CHO-GFP cells (Chinese hamster ovary cells) were maintained according to ATCC protocol.

Bioink with cells preparation

The cells were lifted off the plate surface, spun, and mixed with media to obtain a 30 million/300µL concentration in the case of Vitroink and HCT-116s; 10 million/300µL concentration for Gelatin ink and CHO-GFPs. These cell mixtures were then mixed with readily available VitroInk RGD and in-house prepared Gelatin-Fibrin inks to form a cellular bioink and printed on Greiner CELLSTAR® microplate – nontissue culture treated (Cat: GN655185) and cell culture treated Corning 3603 plates, respectively.

Results

Matrigel dome assays and imaging

Matrigel dome 3D Expansion (BAB400) ➔ Assay Development

We have successfully used pipetting tool to plate organoid fragments/cells in Matrigel domes into 96 well format (Figure 3 and Figure 7). Briefly, cells/ organoids were first expanded, then mixed with GF-reduced Matrigel/ Hydrogel ECM and seeded into Matrigel domes in 96-well plate or 384 well formats.

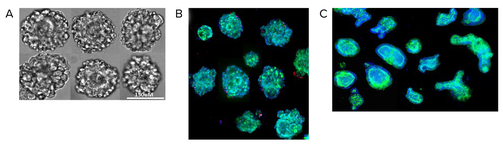


Figure 3. A. Cellesce Colorectal Organoids (CRCs) in Matrigel dome after one week in culture, TL image (10X). B. Organoids 7 days in culture stained with Hoechst dye (blue) and Cyto3D (Acridine orange and Propidium Iodide), 10X. C. Mouse Intestinal organoids dispensed with pipette tool after one week in culture, TL image (10X).

Colorectal cancer organoids matrigel dome assay

Patient-derived Cellesce Colorectal organoids were thawed, and mixed with GF-reduced Matrigel at a concentration of 20K organoids/mL and were dispensed at 7uL/ well volume into 96 well plates (center of wells). After 2 days, a series of drug dilutions (Romidepsin, Cisplatin, 5FU, and Trametinib) were added to the plates with a media exchange. On day 6, the wells were stained with Cyto3D and Hoechst 3322 for about 30mins to perform a live dead assay using IXM-C. The imaging assays evaluated the size and viability of organoids. The viability of the organoids measured was > 90%. Assay validation criteria: Z'-value between control and treated samples were found to be > 0.5 for the count of viable cells or another measurement.

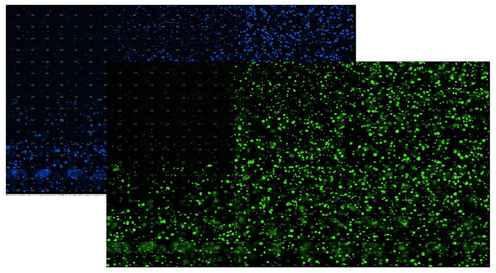
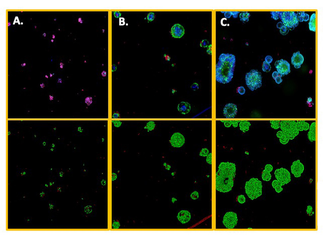


Figure 4. Plate representations of uniformly seeded CRCs treated for 4 days with Romidepsin (10µM), and Cisplatin (100 Mm) in 5-fold dilutions (~150 organoids/well).



Z value: [Z-prime = Z']	
Romidepsin (10µM)	Cisplatin (100µM)
0.6	0.5

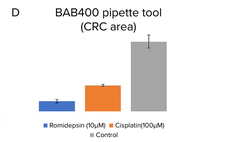
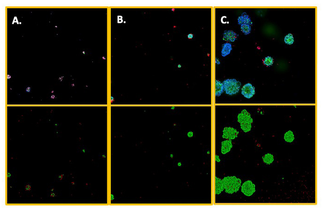


Figure 5. CRCs confocal images, 4X (top panel) and their representative cell scoring analysis masks generated by MetaXpress software (bottom panel) A. Romidepsin (10µM) B. Cisplatin (100µM) C. Untreated Controls. D. Graphical representation of the same.



5FU (5mM)	Trametinib (40µM)
0.6	0.7

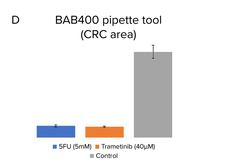


Figure 6. CRCs confocal images, 4X (top panel) and their representative cell scoring analysis masks generated by MetaXpress software (bottom panel) A. 5FU (5mM) B. Trametinib (40µM) C. Untreated Controls. D. Graphical representation of the same.

4IC cells matrigel dome assay

The patient-derived triple-negative breast cancer cells were harvested using trypsin and suspended in a medium with 30% FBS at a concentration of 5 X 10^5 cells/mL. The 3D cell culture was performed in VitroGel Hydrogel Matrix. Multiple 96- well plates with 4IC cells in VitroGel domes were set up by both the BAB400 and manual dispenses. These were observed on day 3 using the Cyto3D stain for live and dead counts and, when analyzed, there wasn't a significant difference in their viability. The BAB400 workflow had average viability of 93%, whereas the manually dispensed well plates had a viability of 92.4% (Figure 7).

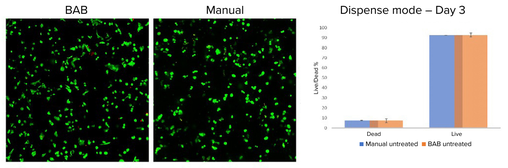


Figure 7. Comparison between BAB400 and Manually dispensed 4IC cells on Day 3 (Images – left) and graphical representation of the same (right) – Acridine Orange (Green – Live) and Propidium Iodide (Red – Dead).

Results

The drugs Trametinib and Idarubicin were added to the multiwell plates on day 3 to make up a final concentration of 4µM and their effects were observed on day 5 after a 48h treatment. We observed a significant difference in viability in both drug treatments when compared to the control (Figure 4). The dead cells percentage in idarubicin 4µM was the highest with 97% of cells in spheroids dead, whereas in the case of trametinib it was 66.4% and the control was 7.5% (Figure 8).

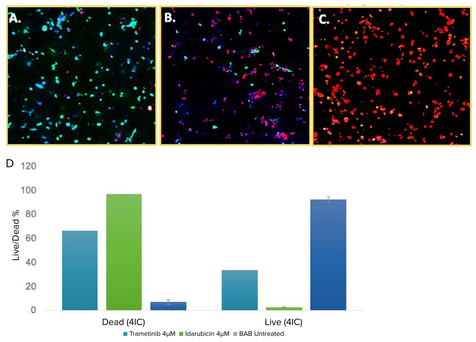


Figure 8. Control (A.) and treated samples were stained with Cyto3D (Green – live, Red – dead) and DAPI (Blue – nuclei), treated – 48 h treatment with Trametinib (B.) and Idarubicin (C.) at 4µM concentration. These were imaged on Day 5 using IXM-C. The graphical representation of data analyzed of the same (D.) using MetaXpress software.

Mouse intestinal organoids matrigel dome assay

MIOs were cultured using STEMCELL Technologies protocols and then seeded into 96-well and 384- well formats using BAB400 pipette tool. These were treated with Drugs on Day 5 for a 48-h duration before performing live dead assay.

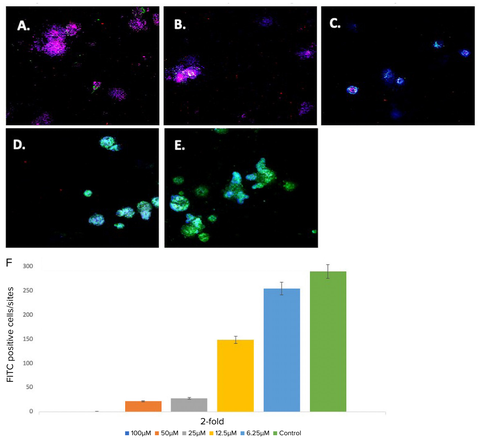


Figure 9. MIOs in Matrigel after one week in culture and 48 h of treatment with Cisplatin. A. 100 µM. B. 50 µM. C. 25 µM. D. 12.5 µM and E. 6.25 µM. Control (Figure 3. C) Stained with Cyto3D (Acridine Orange (Green – Live) and Propidium Iodide (Red – Dead) and DAPI (Blue – Nuclei).

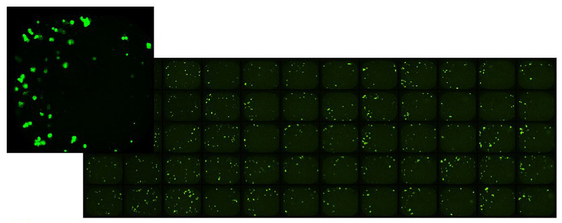


Figure 10. BAB400 dispensed MIOs in Matrigel after 4 days in culture in a 384-well format. Stained with Calcein AM (Green – Live), 4X.

3D printing assays and imaging

3D Printing with BioInks (BAB400) ➔ Assay Development

We have successfully used ambient tool to print cells into patterns using various bioinks into a 96 well format (Figure 11 and 14). Briefly, cells were first expanded, then mixed with inhouse made Gelatin Fibrin Ink/ hydrogel-based Ink and printed into patterns.

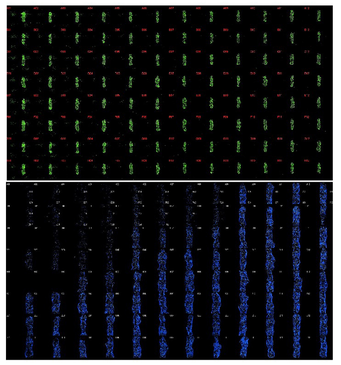
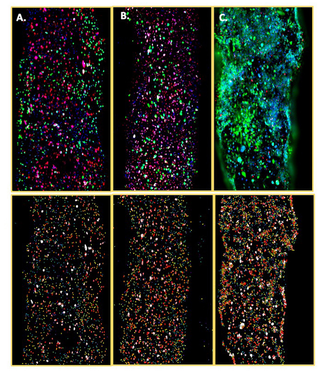


Figure 11. A. 3D-printed patterns imaged using confocal imager, 4X. CHO-GFP cells at a concentration of 5 million cells/mL, mixed with Gelatin Fibrin ink were printed into lines at 6psi, printing speed of 4mm/sec. B. The same plate was treated for 48-h starting on Day 4 with Romidepsin (10µM), and Cisplatin (100 Mm) in 5-fold dilutions and imaged on Day 6 (DAPI – blue/nuclei).

Results

After 4 days, a series of drug dilutions (Romidepsin, Cisplatin, 5FU, and Trametinib) were added to the plates with a media exchange. On day 6, the wells were stained with Cyto3D and Hoechst 3322 for about 30mins to perform a live dead assay using IXM-C. The imaging assays evaluated the size and viability of cells. The viability of the cells in bioink measured was > 90%. Assay validation criteria: Z'-value between control and treated samples were found to be equal or greater than 0.5 for the count of viable cells or another measurement.



Z value: [Z-prime = Z']	
Romidepsin (40µM)	Cisplatin (100µM)
0.7	0.5

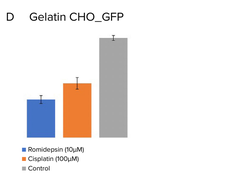
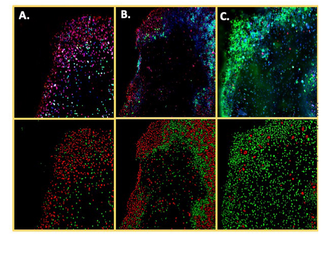


Figure 12. CHOs in Gelatin ink - confocal images, 4X (top panel) and their representative cell scoring analysis masks generated by MetaXpress software (bottom panel) A. Romidepsin (10µM) B. Cisplatin (100µM) C. Untreated Controls. D. Graphical representation of the same.



Z value: [Z-prime = Z']	
5-FU (5mM)	Trametinib (40µM)
0.6	0.6

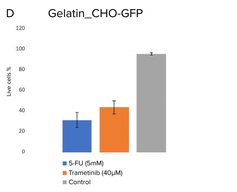
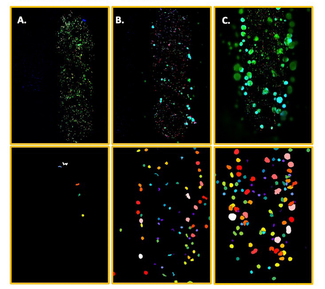


Figure 13. CHOs in Gelatin ink - confocal images, 4X (top panel) and their representative cell scoring analysis masks generated by MetaXpress software (bottom panel) A. 5FU (5mM) B. Trametinib (40µM) C. Untreated Controls. D. Graphical representation of the same.

VitroInk – HCT-116 cells



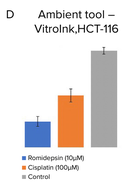


Figure 14. HCT-116s in VitroInk - confocal images, 4X (top panel) and their representative cell scoring analysis masks generated by MetaXpress software (bottom panel) A. Romidepsin (10µM) B. Cisplatin (100µM) C. Untreated Controls. D. Graphical representation of the same.

Summary and conclusions

- The organoids were observed to be uniformly distributed, they were maintained and later treated. Both cytostatic and cytotoxic effects were observed due to the drugs. Trametinib was observed to be the most and cisplatin being the least cytotoxic in the treatments carried out. Cisplatin led to morphological changes in the organoids compared to the controls.
- The gelatin ink with CHO-GFP cells was evenly printed. The gelatin lines were slightly bumpy in comparison to the VitroInk lines because of the different ink properties. Cells in VitroInk formed larger 3D cellular clusters due to the HCT-116 cellular properties whereas the CHOs preferred staying in smaller groups. The drug treatments led to an overall decrease in the number of live cells and 3D arrangements formed in the ink and owed to a lower number of nuclei counts.
- The results showed the overall feasibility of the workflow for automated bioprinting/ dispensing 3D cellular models with ECM matrices for anti-cancer drug screening workflows.
- An increase in throughput and ease of operation was achieved through automation. Also, imaging and data analysis methods provided valuable information about complex compound effects in 3D printed and cell-tissue-engineered models.