Rapid and Controlled Generation of Single Spheroid Cultures in a High-throughput Manner

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Introduction: Liquid biopsies based on blood or plasma biomarkers offer a low-risk alternative in cancer diagnosis and treatment comparing to tissue biopsies.[1] However, *in vitro* cancer models that accurately replicate cancer heterogeneity are necessary for understanding the relationship between the change of biomarkers, cancer progression, and drug resistance.[2]

Materials and Methods: A high-throughput 3D bioprinting system was utilised to create single cancer spheroids with controlled size for drug screening. Spheroid-in-cup models were printed on a drop-on-demand 3D bioprinter (Inventia Life Sciences). Cups were prepared by sequential dispensing of alginate and calcium chloride (Figure 1). Cell suspensions (MCF-7/MCF-10A/MDA-MB-231) were automatically dispensed into the lumen. Spheroids were cultivated for 7 days.

Results and Discussion: Valve opening time was kept minimal for optimal droplet fidelity, as prolongation caused unwanted droplet satellites. The pressure was adjusted to fabricate cups with reproducibly varied lumen sizes (300 µm ± 30 µm, 600 µm ± 40 µm, and 800 µm ± 50 µm). 96 cups were fabricated within 1.5 hours, and spheroids formed within 48-72 hours. The control over lumen opening size afforded a method to generate spheroids with controllable size.

Conclusion: Our method utilizing a 3D bioprinting system enables high-throughput and rapid formation of single cancer spheroids with controllable size. The generated spheroids accurately reflect *in vivo* heterogeneity and facilitate the detection of biomarkers release.

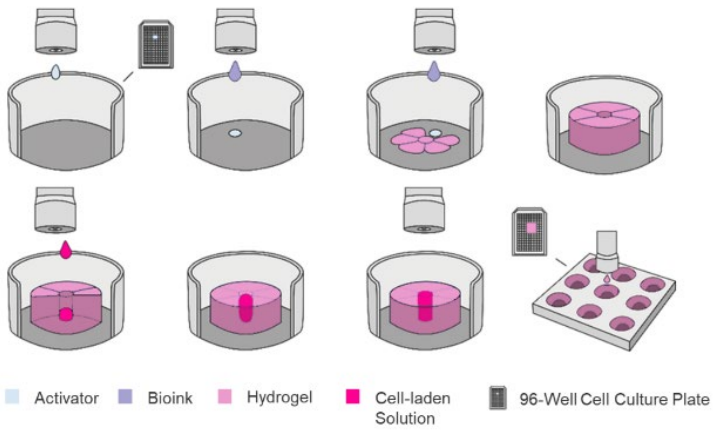

Figure 1: Schematic of the bioprinting process involves bioprinting hydrogel matrix to form a cup, depositing cells onto it, and embedding the top half of cup. The structure is incubated at 37°C and 5% CO_2 until spheroid is formed.

References:

[1] Alix-Panabieres, C., Perspective: The future of liquid biopsy. *Nature*, **2020**. 579(7800): p. S9-S9.

[2] Franklin, M.R., et al., Immuno-oncology trends: preclinical models, biomarkers, and clinical development. *J Immunother Cancer*, **2022**. 10(1).