Integrative High-Throughput 3D Bioprinting for Size-Controlled Cancer Spheroids: Correlating Biomarker Expression and Drug Sensitivity

Peilin Tian[a, b], *Bram G. Soliman*[a, b], *Eric Y. Du*[a, b], *Maria Kavallaris*[b, c], *Richard D. Tilley*[a, b], *J. Justin Gooding*[a, b]*

a. School of Chemistry, UNSW Sydney, NSW 2052, Australia
b. Australian Centre for NanoMedicine, UNSW Sydney, NSW 2031, Australia
c. Children's Cancer Institute, Lowy Cancer Research Centre, UNSW Sydney, NSW, 2052, Australia

peilin.tian@unsw.edu.au *justin.gooding@unsw.edu.au*

Introduction: Liquid biopsy, from which blood- or plasma-based biomarkers can be measured, has recently gained attention as a less invasive alternative to the traditional solid biopsy for cancer diagnosis. Cancer size is linked to poor prognosis, influencing oxygen and nutrient gradients and biochemical signal distribution[1]. Analysing cancer size *in vitro* to reflect tumour heterogeneity enhances understanding of biomarkers, cancer progression, and drug sensitivity, thereby improving liquid biopsy applications. *In vitro* models mimicking tumour heterogeneity are critical for interpreting biomarker changes, cancer progression, and drug resistance. A high-throughput 3D bioprinting platform has enabled the generation of uniform cancer spheroids with tailored sizes, facilitating biomarker study and drug evaluation[2].

Materials and methods: Using a drop-on-demand 3D bioprinter, solutions of alginate and calcium chloride were sequentially layered form hydrogel models. MCF-7 cells were printed into the cavity of models, leading to spheroid development within 3 days (Fig.1A). We manipulated the bioprinter settings to control the spheroid size through the cup cavity diameter. These spheroids underwent drug exposure to doxorubicin and tamoxifen (0-120 µM) for 5 days. We then assessed cell viability via luminescence with CellTiter-Glo®. Immunostaining was performed by using a Zeiss LSM 800 confocal microscope for CD44 and CD133 visualization.

Results and discussion: Fine-tuning bioprinter pressure between 30-45kPa enabled the generation of MCF-7 spheroids with precise cavity sizes of 300 ± 30µm, 600 ± 40µm, and 900 ± 50µm, leading to a size variation of 5%-10% and enabling the construction of 96 models within 2 hours. This study delineated a direct relationship between spheroid size and chemotherapeutic response, as reflected by variable IC_{50} values; larger spheroids had elevated IC_{50}s, suggesting a size-dependent resistance to drugs (doxorubicin: 11.86, 20.65, 99.35µM Fig.1B; tamoxifen: 14.69, 16.07, 22.66µM Fig.1C, $P<0.001$). Immunostaining outcomes indicated enhanced signal intensity for CD44 and CD133 in larger spheroids, positing a potential mechanism for the observed drug sensitivity.

Conclusions: The deployment of the 3D bioprinting technology showcased here facilitates the high-throughput creation of singular cancer spheroids with stringent size regulation. The developed model accurately reflects *in vivo* size-dependent drug sensitivity, offering a foundation to explore this crucial factor in biomarker discovery. This investigation emphasizes 3D bioprinting's significant promise as a robust tool for comprehensive therapeutic evaluation.

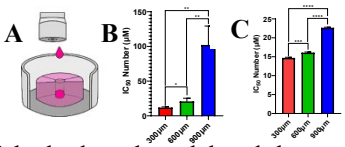

Figure 1: A) the construction of the hydrogel model and the process of adding a cell solution to form spheroids **B)** the doxorubicin study, demonstrating that larger spheroids exhibit decreased sensitivity to the drug **C)** the tamoxifen study, further reinforcing the observation that spheroid size inversely correlates with drug sensitivity.

References:

[1] Däster, Silvio et al. Oncotarget vol. 8,1 (2017): 1725-1736.
[2] Utama, Robert H et al. iScience vol. 23,10 101621. (2020).