Integrative High-Throughput 3D Bioprinting for Size-Controlled Cancer Spheroids: Investigating How Spheroid Size Influences Drug Sensitivity

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Introduction: Liquid biopsy, measuring blood- or plasma-based biomarkers, is significantly gaining attention as a minimally invasive alternative to solid biopsy for cancer diagnosis. Tumor size closely correlates with poor outcomes, shaping oxygen and nutrient gradients, and biochemical signaling[1]. Modeling *in vitro* tumor size to mirror heterogeneity enhances our grasp of biomarker behavior, progression, and drug response, benefiting liquid biopsy. Developing *in vitro* models that accurately mirror tumor complexity is crucial for interpreting biomarker shifts, disease evolution, and therapy resistance. Moreover, high-throughput 3D bioprinting enables standardized cancer spheroids of controlled dimensions, thereby advancing investigations into biomarkers and drug efficacy[2]. These models refine experimental accuracy.
Materials and methods: Rastrum 3D bioprinter (Inventia) was used to sequentially deposit alginate and calcium chloride solutions, creating layered hydrogel constructs. MCF-7 cells were then printed into the hydrogel cavities, promoting spheroid formation within 3 days (Fig. 1A). By adjusting the bioprinter parameters, specifically the cavity diameter, spheroid sizes could be precisely controlled. The formed spheroids were treated with doxorubicin and tamoxifen at concentrations ranging from 0 to 120 μM for 5 days. Cell viability was subsequently quantified using a luminescent CellTiter-Glo® assay.
Results and discussion: By fine-tuning the bioprinter pressure between 30 and 45 kPa, MCF-7 spheroids of approximately 300 ± 30 μm, 600 ± 40 μm, and 900 ± 50 μm in diameter were consistently produced, achieving a 5%–10% size variability and enabling the fabrication of 96 models within 2 hours. These measurements demonstrated a clear association between spheroid size and chemotherapeutic response, as indicated by progressively higher IC50 values in larger spheroids, signifying increased drug resistance (doxorubicin: 11.86, 20.65, and 99.35 μM; tamoxifen: 14.69, 16.07, and 22.66 μM, all $P < 0.001$; Fig. 1B, 1C).
Conclusions: In summary, this study demonstrates a high-throughput 3D bioprinting technique that enables the rapid and precise production of single cancer spheroids with strict size control. The resulting model accurately mirrors *in vivo* size-dependent drug sensitivity, laying the groundwork for further investigations into how tumor dimensions influence therapeutic outcomes. These findings highlight the considerable potential of 3D bioprinting as a comprehensive tool for drug testing and advancing cancer research.

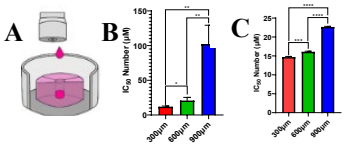

Figure 1: A) the construction of the hydrogel model and the process of adding a cell solution to form spheroids **B)** the doxorubicin study, demonstrating that larger spheroids exhibit decreased sensitivity to the drug **C)** the tamoxifen study, further reinforcing the observation that spheroid size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