Creating Standardized 3D Bioprinting of Breast Cancer Spheroids Addressing Model Disparities for Therapeutic Screening

Dominika Lee[1], Adam Mohammed[2], Ufana Riaz[2,3], Manika Shrestha[3], Delores Grant[1,3], Qing Cheng[1,3], Eric Saliim[1], Nicole Salazar[1,3]

[1]Biological and Biomedical Sciences Department, [2]Chemistry and Biochemistry Department, [3]JLC-BBRI, North Carolina Central University, Durham, NC

Abstract

Breast cancer disproportionately impacts women of African ancestry, particularly in the incidence and mortality of aggressive subtypes such as triple-negative breast cancer. Despite this disparity, preclinical cell line repositories remain largely derived from women of European ancestry, limiting treatment and testing equity. This study establishes a standardized extrusion-based 3D bioprinting workflow using the CELLINK BioX platform to generate reproducible breast cancer spheroid models for therapeutic testing. MDA-MB-231 and MCF-7 cells were suspended in a Gelma bioink and printed into 96-well U-bottom plates using controlled parameters including extrusion pressure, temperature, and deposition height. Constructs were cultured for 72 hours and imaged every 6 hours using live-cell imaging to assess spheroid formation, structural integrity, and viability. Bioprinted constructs demonstrated consistent morphology and progressive compaction, confirming successful 3D tumor organization. These models provide a scalable platform for chemotherapeutic evaluation, beginning with doxorubicin exposure to assess treatment response in a three-dimensional microenvironment. Future studies will incorporate gold nanoparticle mediated therapeutic strategies to evaluate enhanced drug delivery and targeted cytotoxicity. By combining standardized 3D modeling with therapeutic screening, this workflow advances reproducible tumor modeling while supporting broader efforts to improve representation and equity in breast cancer research.

Background Information

Hypothesis: Three-dimensional bioprinted breast cancer spheroids will form structurally stable and viable tumor constructs that mimic the in vivo tumor microenvironment. These models will provide a reproducible platform for evaluating chemotherapeutic response and may help address disparities in breast cancer research by enabling the development of representative tumor models.

Specific Aim: To develop and validate a standardized extrusion based 3D bioprinting workflow using variable bioink to generate viable breast cancer spheroids from cell lines of diverse ancestry for downstream therapeutic screening.

Methods and Data

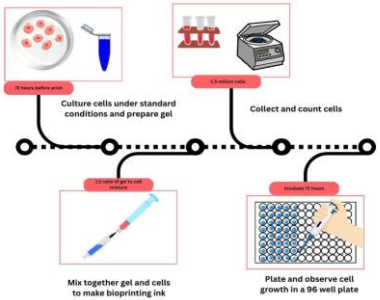

Figure 1. Protocol for Cell preparation and culture prior to 3D bioprinting.

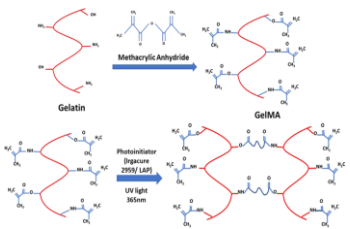

Figure 2. Crosslinking process of GelMA ink.

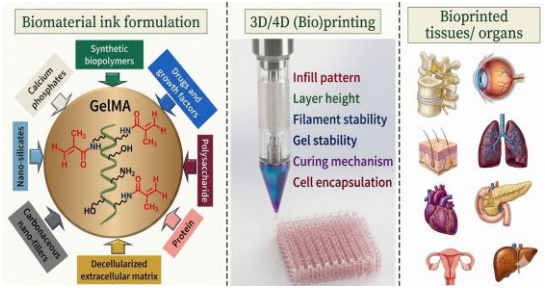

Figure 3. Applications in Bioprinting.

Results and Conclusions

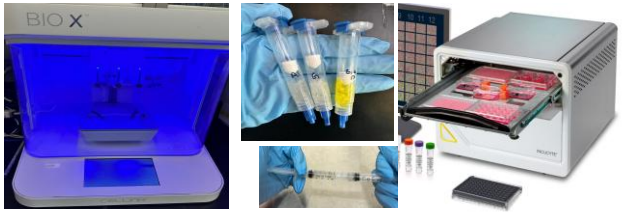

Figure 4. Tools used for protocol development for 3D bioprinting, as illustrated in Figure 1, Breast cancer cell lines were cultured under standard laboratory conditions prior to printing. Cells were harvested and prepared for suspension with different materials including GelMA, GelMA-LAP, Alginate 5%, and PEGDA bioinks to support extracellular matrix mimicry and enable three-dimensional tumor growth following extrusion-based printing using BioX printer and visualized with an Incucyte.

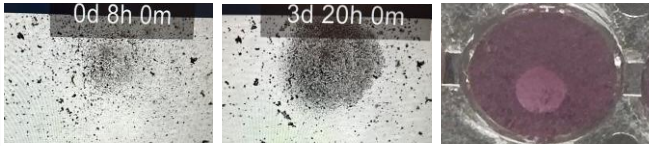

Figure 5 . Time-course development of bioprinted breast cancer spheroids. Representative images demonstrate spheroid formation following extrusion-based printing. Early time points show dispersed cells immediately following printing, while later time points illustrate cellular aggregation and compaction into dense three-dimensional tumor spheroids. This progression indicates successful 3D tumor organization and viability within the printed constructs.

Conclusions: This study establishes and initiates a workflow for extrusion-based 3D bioprinting of breast cancer spheroids using bioink materials. The resulting constructs demonstrate spheroid formation and structural stability, supporting their use as physiologically relevant *in vitro* tumor models. Next steps include expansion to multiple cell types, materials, and high-throughput development.

IMPACT: Two-dimensional cell culture has long served as the standard *in vitro* model for studying disease mechanisms, virology, and drug interactions, but its relevance to in-vivo biology is limited. Three-dimensional bioprinting now enables the creation of tissue-like spheroids that more accurately replicate native architecture, allowing disease research, particularly in cancer, to better approximate *in vivo* conditions.

Acknowledgements

Funding: Leroy "Pop" Miller Endowed Professorship, NIH NIBIB UG3EB037531, RCMI Pilot Award

National Institute of Biomedical Imaging and Bioengineering

Technologies to Shape the Future of Health