Laser Guidance and Cell Printing-Based Patterning System for Studying Breast Cancer Cell Redirection

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Introduction

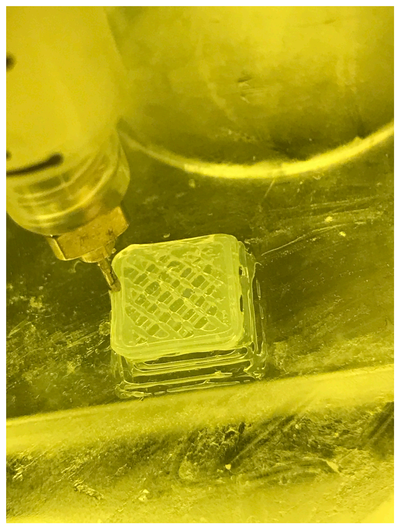
Published literature shows that breast cancer cells, including HER2- positive and triple negative breast cancer (TNBC) cells, can be redirected to halt proliferation and migration of new cancer cells when placed within specific ratios of normal mammary epithelial cells (MECs).[1] Given that breast cancer cells have been shown to have clonal properties, stemming from cancer stem cells (CSCs), there is a heightened importance in understanding the single-cell interactions of breast cancer cells with the microenvironment. This is because many anti-cancer therapies target the bulk tumor cells outwardly growing but have little to no effect on the CSCs.[2]

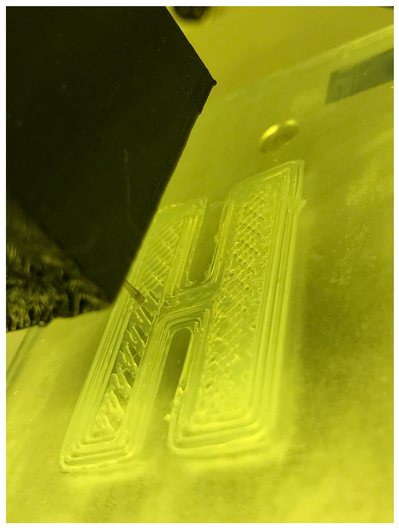
3D bioprinting, alongside laser patterning and microwell arrays, can give rise to a better understanding of the single cell interaction between breast cancer cells and the microenvironment. However, before this biological assay can be accomplished, the engineering of the technological design need to be accomplished. The goal of this research is to develop a G-code based control system to achieve precise arrangement of a single CSC in a MEC culture with a defined cell number in each microarray. Additionally, a synchronized imaging system is required to be able to view the deposition of the different cell types.

Materials and Methods

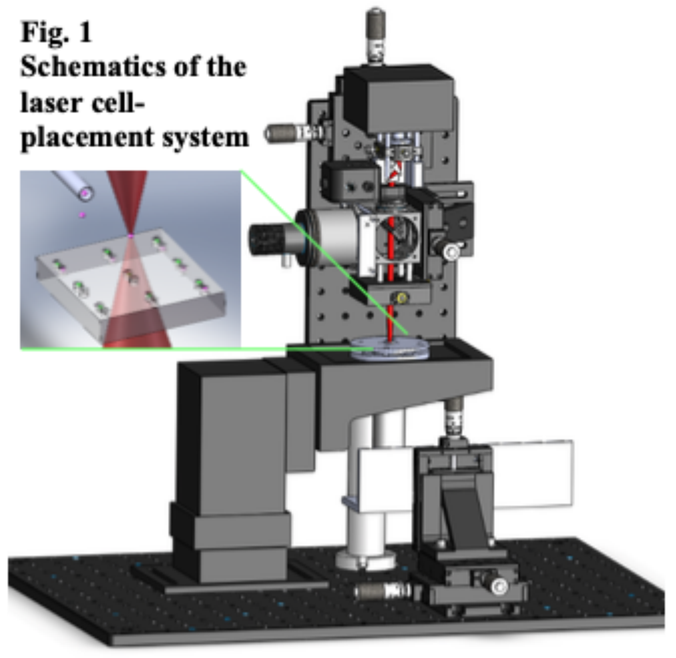
A SunP BioTech 3D bioprinter was modified to house a state-of-the art imaging system, allowing for real-time monitoring of biological cells being printed. Modifications include extrusion head stabilizing units, a custom printing bed that allows for the imaging of biological cells and modifying the extrusion tip to allow for the deposition of low cell density. A custom program was written in C++ to allow for both the manual control with a remote controller and automatic control with the in-house software via G-code. Preliminary testing of the modifications and programming by conducting practice prints using silicone.

Images and Figures





Images 1 and 2: Practice prints of silicone 3D structures using G-code on custom-programed, modified bioprinter



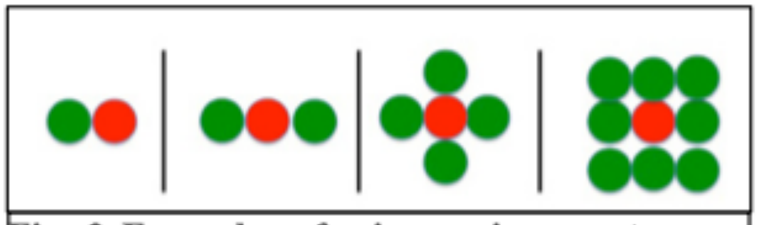


Fig. 2 Examples of microenvironments-on-a-chip cell placements. Red dots=cancer cells, green dots =normal breast epithelial cells.

Results and Discussion

We have developed a custom G-code parser, implemented on an Arduino Mega 2560, to execute G-code produced from Simplify3D on our modified bioprinter with elevated precision for cell-deposition and extruder displacement. Two additional axis for the imaging system were added and synchronized below the bioprinter to allow for the real-time imaging and precise deposition of biological cells. Additionally, the G-code was designed to automatically bioprint duplicate cell micropatterns throughout an entire microwell culture plate using the spacing and depth of each microwell. With this multidisciplinary approach, using laser patterning, 3D bioprinting, and bio fabrication, we were successfully able to develop a cell deposition platform for studying cell-to-cell interaction of breast cancer cells with their surrounding MEC and microenvironment.

Future Work

In the near future, we are going to move to preliminary experimentation of our system by using fixed fibroblast cells suspended in PBS. After successful priting of fixed cells, we will move to use the bioprinter to deposit mammary epithelial cells in a 50 by 50 microarray, with each well measuring 100 µm × 150 µm. Introduction of the breast cancer cell will be done with a laser patterning system, and the proper analysis will be done.

Acknowledgments

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References

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