Evaluating Nanoparticle Penetration in Tumor Spheroids

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Introduction: The lack of an appropriate in vitro model of the tumor microenvironment is one of the largest obstacles in evaluating preclinical cancer drug screenings.[1] Cancer cell monolayers do not effectively mimic the limited drug penetration properties of the complex tumor structures found in cancer patients. 3-D multicellular tumor spheroids (MCTS) serve as a more effective model as they better resemble cancer in structure as well as limited drug penetration. In our experiments, we created heterospheroids composed of 4T1 breast tumor cells and 3T3 fibroblasts, as well as homospheroids of each cell type. Tumors feature stromal and extracellular matrix components in addition to cancer cells in ratios that vary between different types of cancer. Fibroblasts are the major component of cancer stroma as well as producers of extracellular matrix. Since heterospheroids feature 3T3 fibroblasts, they may better model the diverse tumor microenvironment.[2] We also synthesized fluorescent PLGA nanoparticles that were added to our spheroid cultures. Using confocal microscopy and ImageJ's fluorescence measuring tools, we qualitatively and quantitatively evaluated the drug penetration properties of our spheroids.

Materials and Methods: Spheroids were grown using the hanging-drop method, in which a 20 uL droplet of cell mixture is suspended upside-down on the lid of a 48-well plate. Homospheroids composed of a single cell type were grown at varying concentrations of 500, 1000, 2000, and 4000 cells per 20uL. Heterospheroids, which featured both 4T1 and 3T3 cells, were composed of 2000 total cells, with varying proportions of the two cell types (10%, 20%, 30% 3T3). MTS assays, which measure cell proliferation by reacting with the byproducts of oxidative phosphorylation, were run on all spheroid types. Area and circularity were calculated using the tracing tools on ImageJ. Fluorescent PLGA nanoparticles were synthesized using acetone as a solvent, polyvinyl alcohol (PVA) as a surfactant, and DiI as the fluorescent dye. For confocal imaging, the spheroids were stained using calcein. Nanoparticles were incubated with spheroids for 24 hours before imaging with the confocal microscope.

Results and Discussion: 4T1 and 3T3 homospheroids grew at all 4 concentrations. Data from MTS assay and spheroid tracing on ImageJ showed that 4T1 spheroids proliferated successfully over time but were not circular in structure (Fig 1A-B). Heterospheroids grew at all 3 conditions, and area was greatest in the 20% 3T3 condition (Fig. 1C). Confocal imaging revealed that nanoparticles successfully penetrated the spheroids they were incubated with (Fig. 1E). Through utilization of ImageJ's fluorescence measuring tools, we found that nanoparticle fluorescence was more concentrated in the core of the spheroid than the periphery of the spheroid (Fig. 1C-E).

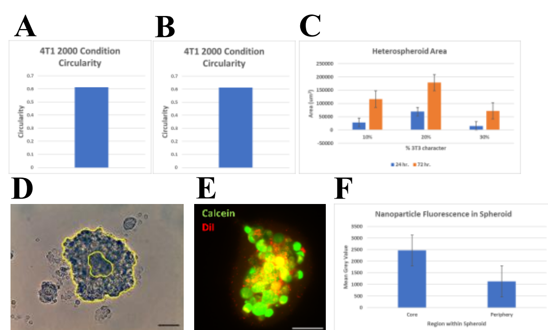

Figure 1. A. B. 4000 condition of 4T1 homospheroids had highest area and 2000 condition had lowest circularity C. Heterospheroid area was greatest in 20% 3T3 condition. B. Heterospheroid circularity followed same trends as area. C. Area of the spheroid within inner boundary represents core of the spheroid, and area between inner and outer boundaries represents periphery (Scale bar = 100 um). D. Confocal image of a spheroid that has been incubated with nanoparticles (Scale bar = 50 um). E. Using ImageJ's mean grey value measurement tool, we found that nanoparticles were more concentrated in the core of the spheroid than the periphery

Conclusions: 4T1 spheroids grown using the hanging-drop method grow successfully in terms of area and proliferation over time but are not very circular in their structure. Heterospheroids composed of 2000 cells/20 uL display the highest area in the 20% 3T3 condition. When spheroids are incubated with PLGA nanoparticles for 24 hours, nanoparticles are more concentrated at the core of the spheroid than the periphery. Moving forward, we will be studying nanoparticle penetration in heterospheroids that consist of 4T1 cells and 3T3 fibroblasts in order to further elucidate mechanisms in the highly diverse tumor microenvironment that inhibit effective drug delivery.

Acknowledgements: Support for this research was provided by the NSF Research Experience for Undergraduates Award #1461192

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