NDnano
Notre Dame Nanoscience and Technology

NDnano Summer Undergraduate Research
2023 Project Summary

1. Student name & home university: Angela Taglione, University of Notre Dame

2. ND faculty name & department: Professor Donny Hanjaya-Putra (AME)

3. Summer project title: Micro-Organoids by Gel Droplet Platform for Cancer Drug Screening

4. Briefly describe new skills you acquired during your summer research:

Over the summer, I learned how to make spheroids to create a platform for high-throughput drug screening. This involved learning the process of cell encapsulation, where controlled interactions are cultivated to mimic natural settings. As these spheroid cultures developed, I also learned how to maintain their vitality through passaging cells and changing cell media. Additionally, I was introduced to staining methods to see the effectiveness of the encapsulation and viability of the spheroids. I was also able to learn about the synthesis of NorHA, utilizing NMR and freeze drying.

5. Briefly share a practical application/end use of your research:

Spheroids are used to model the tumor's microenvironment. Utilizing cancer cells obtained directly from patients, these spheroids can accurately replicate the distinct traits of the individual's tumor through cell encapsulation. The encapsulation serves as a conducive environment for the cells to grow while maintaining their phenotype fidelity. This personalized approach involves conducting drug screening on the patient-specific spheroids which allows for the identification of optimal drugs to effectively treat the patient's tumor.

6. 50- to 75-word abstract of your project:

3D cultures like spheroids are used to represent the tumor's microenvironment; however, production is limited due to time consumption and reproducibility. To solve this issue, we evaluated the production of microgels using an elliptical pipette for cell encapsulation by droplet emulsion. Good cell viability, increased cell proliferation, and successful infiltration of large-sized molecules into the porous gaps of the microgel suggest successful creation of an reproducible and inexpensive gel-droplet platform for high-throughput cancer drug screening.

7. References for papers, posters, or presentations of your research:

Caldwell AS, Aguado BA, Anseth KS. Designing Microgels for Cell Culture and Controlled Assembly of Tissue Microenvironments. Adv Funct Mater. 2020 Sep 10;30(37):1907670. doi: 10.1002/adfm.201907670. Epub 2019 Dec 17. PMID: 33841061; PMCID: PMC8026140.

Chen, L., Zhang, C., Yadav, V. *et al.* A home-made pipette droplet microfluidics rapid prototyping and training kit for digital PCR, microorganism/cell encapsulation and controlled microgel synthesis. *Sci Rep* 13, 184 (2023). https://doi.org/10.1038/s41598-023-27470-1

Ding S, Hsu C, Wang Z, et. al. Patient-derived micro-organospheres enable clinical precision oncology. Cell Stem Cell. 2022 Jun 2;29(6):905-917.e6. doi: 10.1016/j.stem.2022.04.006. Epub 2022 May 3. PMID: 35508177; PMCID: PMC9177814.

Qazi, T. H., Wu, J., Muir, V. G., Weintraub, S., Gullbrand, S. E., Lee, D., Issadore, D., Burdick, J. A., Anisotropic Rod-Shaped Particles Influence Injectable Granular Hydrogel Properties and Cell Invasion. *Adv. Mater.* 2022, 32, 2109194. https://doi.org/10.1002/adma.202109194

Rima XY, Zhang J, et. al. Microfluidic harvesting of breast cancer tumor spheroid-derived extracellular vesicles from immobilized microgels for single-vesicle analysis. Lab Chip. 2022 Jun 28;22(13):2502-2518. doi: 10.1039/d1lc01053k. PMID: 35579189; PMCID: PMC9383696.

Micro-Organoids by Gel Droplet Platform for Cancer Drug Screening

Several alternatives have been applied for modeling disease progression. In the context of cancer progression modeling, spheroids provide a more physiologically relevant tumor microenvironment by incorporating cell-cell interactions, extracellular matrix (ECM), oxygen/nutrient gradients, and hypoxic regions which better reflect the complexity of solid tumors. They also can exhibit increased drug resistance compared to 2D cultures which allow a better prediction of the efficacy of anticancer agents. However, the spheroids' production is limited due to their reproducibility and time consumption.

To tackle this issue we proposed the production of microgels using an elliptical pipette for cell encapsulation by droplet emulsion for high-throughput drug screening. The controlled cell microenvironment created by the uniform microdroplets enhances the reproducibility of the micro-organoids. The microdroplets are uniformly created in < 5 minutes for a single channel. This can be applied to a larger scale to use multi-channel systems at an efficient rate. Furthermore, the uniformly distributed size of the microdroplets can control the size of the tissue constructs to increase their reproducibility.

Uniform microdroplets ranging from 280 to 360 µm were generated using an elliptical pipette with norbornene-modified hyaluronic acid (NorHA) polymer. Prostate (LNCaP) and ovarian (RFP-OVCAR5) cancer cells were seeded on an Aggrewell for generating spheroids of sizes lower than 200 µm to avoid hypoxia. The spheroids were encapsulated using the polymer solution and combinations of DTT and MMP-sensitive crosslinker to allow for ECM degradability and increase the rate of the cell proliferation (**Figure 1**). Viability assays were performed after day 1. The amount of viable cells was ~83% (**Figure 2**). WST-1 proliferation assays were carried out by estimating the mitochondrial activity of the cells exhibiting an increase in the cell count from 24 hours to 48 hours after the spheroid encapsulation (**Figure 3**). The cell permeability evaluation performed using TRITC-Dextran (4400 KDa) confocal imaging showed the large-sized molecules were successfully able to infiltrate the porous gaps within the microgel (**Figure 4**).

A preliminary drug screening with doxorubicin (DOX) and enzalutamide (ENZ) tested the effectiveness of the treatments on the enclosed LNCaP micro-organoids (**Figure 5**). DMSO was used as a positive control due to its implementation as drug solvent and cytotoxicity. It was observed that by increasing the concentration of DOX and ENZ up to 1 µM, the viability of the micro-organoids was severely decreased, being 14% and 32% for the DOX and ENZ, respectively. In contrast, the viability with the positive control at 25 µM was >64%. These

accomplished results demonstrate the successful creation of a platform for high-throughput drug screening and can be applied to various cell-lines and tumor microenvironments.

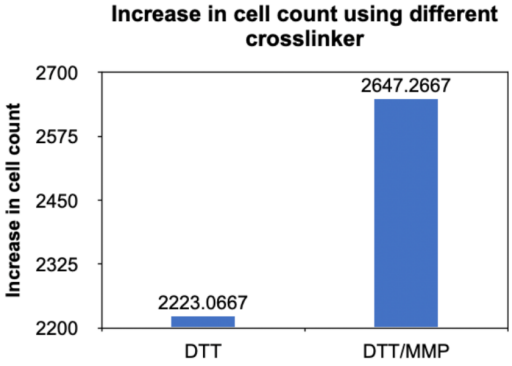

Figure 1. Optimizing cell proliferation using a combination of crosslinkers. The results suggest combinations of DTT and MMP-sensitive crosslinker allow for ECM degradability and increase the rate of the encapsulated cell proliferation.

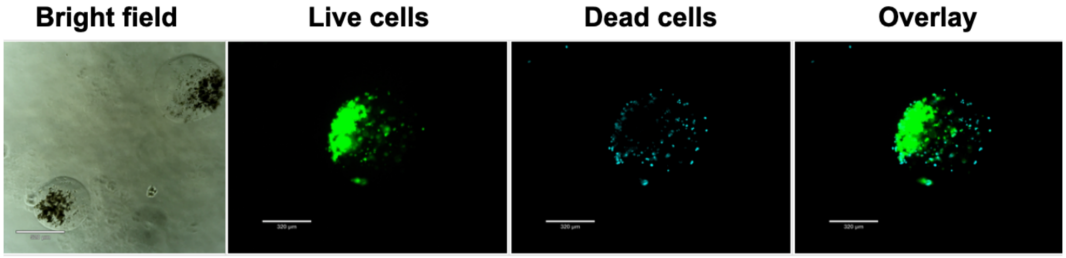

Figure 2. LNCaP spheroid viability assays. Viability assays were performed after day 1 for the LNCaP spheroids. The amount of viable cells was ~83% using a dead stain at 62.5 nM. The spheroid size was estimated to be ~172 µm.

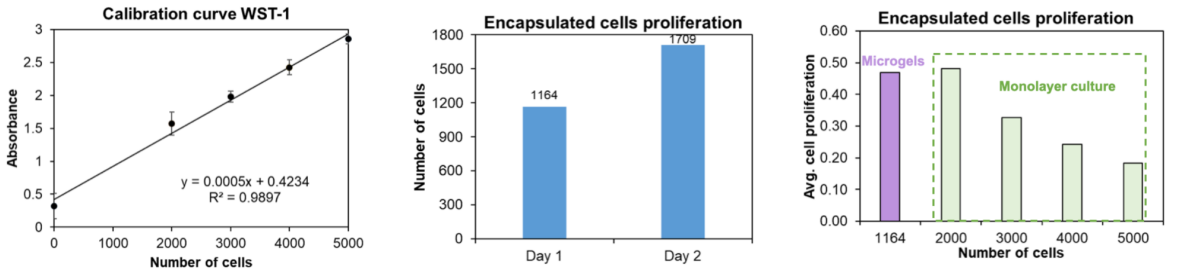

Figure 3. RFP-OVCAR5 WST-1 cell proliferation assays. Proliferation assays were carried out by estimating the mitochondrial activity of the RFP-OVCAR5 cells exhibiting an increase in the cell count from 24 to 48 hours after the spheroid encapsulation. The cell dentistry was $4x10^6$ cell/mL.

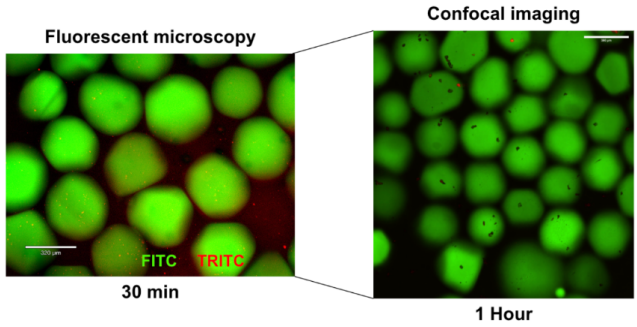

Figure 4. Microgel Permeability Evaluation. The assessment of microgel permeability using TRITC-Dextran (4400 kDa) indicates the uptake of labeled molecules within the microgel structure.

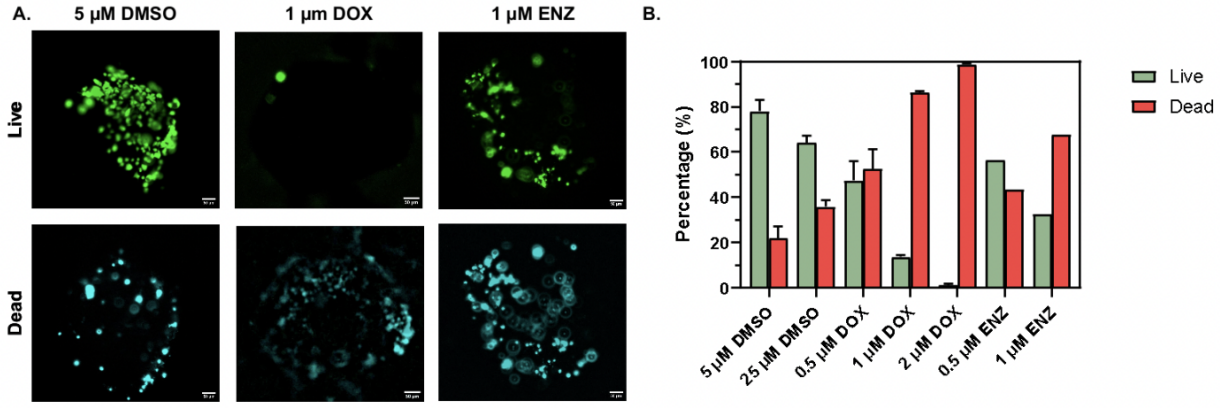

Figure 5. LNCaP micro-organoid drug screening. Doxorubicin (DOX) and enzalutamide (ENZ) were employed to evaluate the responsiveness of the enclosed LNCaP micro-organoids to cancer treatments with DMSO as the positive control. **A**) Micrographs depicting viability for the DMSO, 1 µM DOX, and 1 µM ENZ treatments. **B**) Measurement of the ratio between live and dead cells across varying concentrations of DMSO, DOX, and ENZ.