NDnano
Notre Dame Nanoscience and Technology

NDnano Summer Undergraduate Research
2024 Project Summary

1. Student name & home university:

Keyang(Swindar) Zhou, University of Notre Dame

2. ND faculty name & department:

Prof. Kaiyu Fu, Department of Chemistry and Biochemistry, College of Science
Prof. Yichun Wang, Department of Chemical and Biomolecular Engineering, College of Engineering

3. Summer project title:

Developing Electrochemical Aptamer Biosensors in 3D Cell Culture for High-Throughput Drug Testing

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

During my summer research, I significantly developed both experimental and communication skills within an interdisciplinary context that spanned biochemistry, chemical engineering, and computer science. I acquired hands-on experience in the development of Electrochemical Aptamer Biosensors (EABs), contributing to the design of photomask layouts in Klayout, which were essential for fabricating nanoporous and planar electrodes. I gained proficiency in conducting square wave voltammetry (SWV) measurements using a potentiostat, which is critical for detecting current signals. To streamline data processing, I developed a Graphical User Interface (GUI) that allows users to efficiently filter results and identify patterns through 3D visualization. Additionally, I completed cell culture training to manage the MCF7 cell line, ensuring successful integration of EABs into the 3D cell culture. To further enhance my communication and public speaking skills, I presented my research at the Undergraduate Research Symposium and competed in the 3MT final competition.

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

The practical application of my research lies in transforming traditional 2D cell cultures into advanced 3D models that closely mimic human tissues and incorporate real-time monitoring capabilities. This innovation is poised to enhance drug screening processes by providing more reliable data on drug-cell interactions and mechanisms, which is critical for the development of effective treatments for complex diseases. By improving the accuracy of preclinical testing, my approach aims to reduce the high failure rates and substantial costs associated with traditional drug testing, thereby laying the groundwork for next-generation high-throughput drug screening platforms.

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

High-throughput in vitro drug testing is vital for pharmaceutical development, yet traditional 2D cultures often fail to replicate the complexity of human tissues, leading to unreliable results. My project leverages 3D Cell Cultures to create a more accurate testing environment, complemented by Electrochemical Aptamer Biosensors (EABs) for real-time detection and monitoring. The integration of an automatic data

analysis tool with a user-friendly GUI enhances precision in preclinical testing, potentially improving therapeutic outcomes.

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

[1] Sun, D., Gao, W., Hu, H., & Zhou, S. (2022). Why 90% of clinical drug development fails and how to improve it? Acta Pharmaceutica Sinica B, 12(7), 3049–3062. https://doi.org/10.1016/j.apsb.2022.02.002

[2] Harrison, R. Phase II and phase III failures: 2013–2015. Nat Rev Drug Discov 15, 817–818 (2016). https://doi.org/10.1038/nrd.2016.184

[3] DiMasi, J. A., Grabowski, H. G., & Hansen, R. W. (2016c). Innovation in the pharmaceutical industry: New estimates of R&D costs. Journal of Health Economics, 47, 20–33. https://doi.org/10.1016/j.jhealeco.2016.01.012

[4] Fedi, A., Vitale, C., Giannoni, P., Caluori, G., & Marrella, A. (2022). Biosensors to Monitor Cell Activity in 3D Hydrogel-Based Tissue Models. Sensors (Basel, Switzerland), 22(4), 1517. https://doi.org/10.3390/s22041517

[5] Schoukroun-Barnes, L. R., Macazo, F. C., Gutierrez, B., Lottermoser, J., Liu, J., & White, R. J. (2016). Reagentless, Structure-Switching, electrochemical Aptamer-Based sensors. Annual Review of Analytical Chemistry, 9(1), 163–181. https://doi.org/10.1146/annurev-anchem-071015-041446

One-page project summary that describes problem, project goal and your activities / results:

Traditional 2D cell cultures have been the cornerstone of in vitro drug testing due to their simplicity and consistency, but they fall short in replicating the complex cell-cell and cell-extracellular matrix (ECM) interactions found in vivo. This limitation often leads to unreliable drug testing outcomes, contributing to high failure rates in clinical trials due to a lack of efficacy or unexpected toxicity [2]. In contrast, 3D cell cultures, such as organoids, provide a more physiologically relevant environment by using biocompatible hydrogels to create Inverted Colloidal Crystal (ICC) scaffolds with uniform, size-controlled organoids. These scaffolds better mimic tissue-like microenvironments and offer high yield, versatility, and reproducibility for drug testing. However, the geometric miniature and structural complexity of 3D cell cultures pose challenges in obtaining accurate, real-time data on cellular activities. To overcome this, it is essential to integrate a sensing device for continuous biomarker monitoring [4].

The project aims to address these challenges by focusing on four key areas. First, cell culture training has equipped me with the necessary skills to work with the MCF7 breast cancer cell line, learning essential techniques for seeding cells in ICC scaffolds. Hydrogels, formed through crosslinking, provide a safe environment for cell growth and mechanical support, while two-photon imaging and ImageJ analysis allow for accurate measurement of cell proliferation within the scaffolds. These ICC scaffolds are highly efficient, yielding over 3,000 organoids per mL and 7,616 spheroids per well in a 96-well plate, making them ideal for high-throughput drug testing.

Second, the design, fabrication, and optimization of Electrochemical Aptamer Biosensors (EABs) were explored. EABs were chosen for their stable sensitivity and chemical stability in dynamic environments [5]. Nanoporous gold electrodes, with their significantly increased surface area, enhance signal detection by 41 times compared to traditional planar electrodes, making them particularly effective for monitoring

subtle changes in cellular activities. A unique electrode layout was also developed for photomask creation using photolithography.

Third, a Graphical User Interface (GUI) was developed to automate data processing in both real-time and post-experiment settings. The GUI simplifies the workflow, allowing users to automate file extraction and processing, apply pre-trained or custom algorithms, and filter graph displays based on key parameters like peak height, frequency, and concentration. Additionally, the GUI offers advanced visualization options, enabling the generation of 3D graphs with customizable parameters, which provides deeper insights into cellular behavior.

Finally, this project explores the integration of EABs with 3D cell cultures to obtain real-time, spatially-resolved data on cellular signaling within ICC scaffolds. The work conducted here not only advances the field of drug testing but also holds the potential to revolutionize clinical research by providing more reliable and efficient methods for drug development.

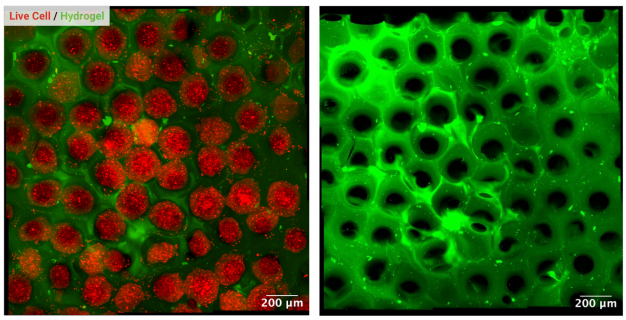


Figure 1. ICC scaffold under the Two-photo confocal microscopy (Cell line: HepG2)

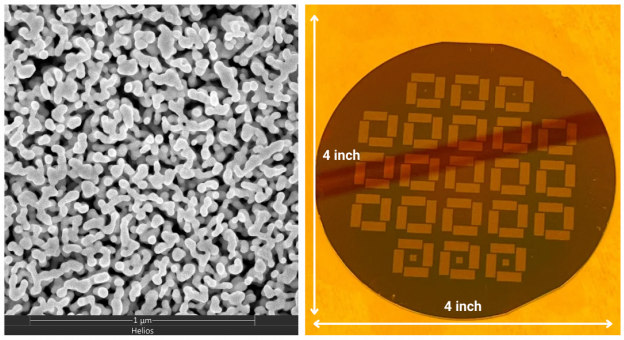


Figure 2. Nanoporous SEM image and the photomask created with photolithography

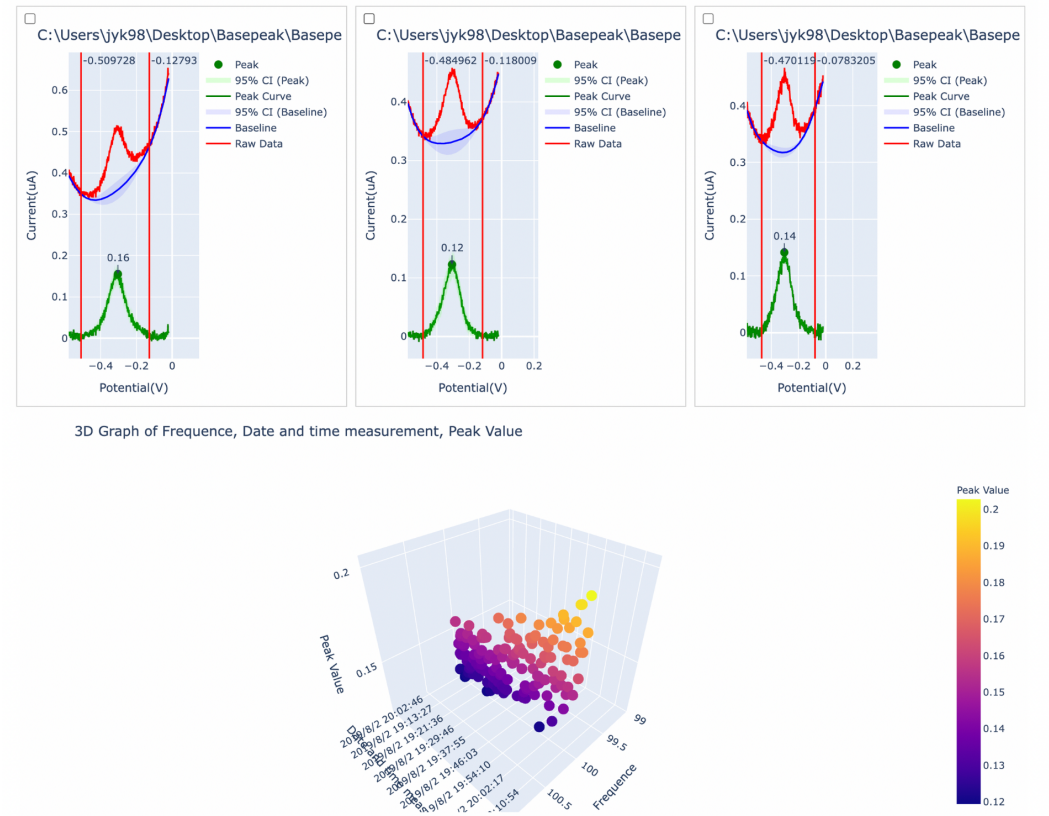


Figure 3. GUI visualization for EABs signal processing