Adaptation of a Microfluidic Device for Use in Scalable Cell Encapsulation

Ravi Nataraj[1,], Yuan Tian[2], Mohammadjafar Hashemi[3], Elizabeth A. Lipke[4]*

[1] Undergraduate Student, Department of Chemical Engineering, Auburn University
[2] Postdoctoral Fellow, Department of Chemical Engineering, Auburn University
[3] PhD Candidate, Department of Chemical Engineering, Auburn University
[4] Mary and John H. Sanders Professor, Department of Chemical Engineering, Auburn University

Three-dimensional (3-D) cell culture models have gained recent interest in tissue engineering (TE) and drug discovery applications over traditional two-dimensional monolayer culture [1]. This is because 3-D culture models are more physiologically relevant and better able to mimic the human in-vivo microenvironment. Different cell applications, such as high-throughput screening, injectable cell delivery, and bioprinting, require the scalable production of 3-D systems, but current manufacturing approaches are inadequate. For example, using single-use 3-D bioreactors for scale-up cell production form aggregates that lack size and shape uniformity, resulting in cell structures with dissimilar functionalities. To overcome such challenges, TE methods – e.g. emulsification, photolithography, microfluidics, and micromolding – can create highly consistent microengineered hydrogels with tight spatial control, but microfluidics is especially promising because it uses a continuous-flow approach suitable for scale-up production needs [2].

Previously, our lab has established a novel microfluidic platform capable of producing 3-D engineered tissues by encapsulating cells within monodisperse poly(ethylene glycol)-fibrinogen hydrogel microspheres (MS) [3]. During MS encapsulation, an expensive, high-intensity visible halide lamp has been used for rapid photocrosslinking of the hydrogel; however, issues with light output consistency, heat regulation, lack of adjustable light parameters restricting photoinitiator choice, and bulkiness weaken platform scalability. Such drawbacks cause added maintenance costs, batch-to-batch variability, and may interfere with cell viability. Here, by leveraging an iterative and modular design strategy, we optimized the photocrosslinking module in our platform to support the high-throughput and scalable production of MS.

* Corresponding author: rzn0039@auburn.edu

As an alternative to the halide lamp, high-watt LED modules (LEDSupply) were mounted on an aluminum heatsink assembly for heat dissipation, and light output was precisely controlled using a PWM (pulse-width modulation) dimmer. Optic lens focusers were used with LEDs to help collimate light and reduce unwanted light from clogging the microfluidic device due to premature crosslinking. Similar assemblies were created by altering LED type – visible or ultraviolet-A (UVA) – to accommodate various photoinitiator types (Eosin Y, LAP) for supporting robust crosslinking of different polymers specific to user needs. 3-D printed fixtures were designed to secure the power supply, dimmer, and lab jack to a 15x15 cm2 aluminum breadboard (THOR Labs). As proof of concept, HT29 colorectal cancer cells were encapsulated using both our original and optimized microfluidic platform, and cell viability was compared using live/dead staining. Images of live/dead stained HT-29 cancer cell MS from both platforms were captured using confocal microscopy.

The compact design of the optimized platform (Figure 1) helps minimize its footprint inside the biological hood.

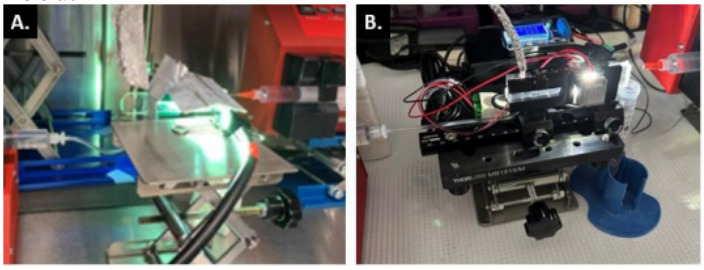

Fig. 1. A. Original and **B.** optimized microfluidic platform setup for acellular microsphere production.

The use of modular LED assemblies allows for quick compatibility with different photoinitiators. Fast ad-

justability and reliable modularity support easy troubleshooting and efficient batch-to-batch transitions. Preliminary results of live/dead confocal microscopy to assess cell viability suggest fewer dead cells in MS produced by the optimized platform (Figure 2).

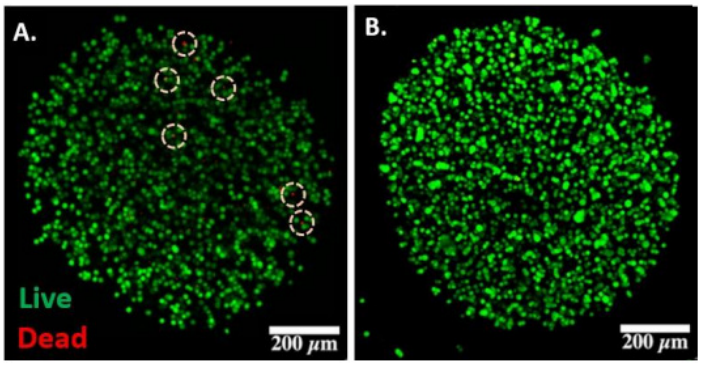

Fig. 2. Confocal microscopy image of a live/dead stained HT29 cancer cell microsphere produced from **A.** original and **B.** optimized microfluidic platform.

Overall, our adjustable and modular microfluidic platform provides quick compatibility with multiple photoinitiators, efficient batch-to-batch transitions, and easy troubleshooting during cell encapsulation. To validate if the above changes impact cell viability, additional assays and experimental tests will be performed to help provide insight into cell growth, metabolic activity, and microenvironment. Future optimization plans include implementing computer vision software to count real-time MS production and designing an automated MS distribution system into any size well plate for high-throughput drug screening applications. By improving both function and utility while maintaining its reliability, our platform has high potential to address large-scale cell production needs.

Statement of Research Advisor

Ravi has been instrumental in driving forward the establishment of a new, adjustable LED light source with consistent light intensity for photocrosslinking of engineered microtissues. Ravi has demonstrated a high capacity for innovation, problem solving, critical thinking, and leadership in the lab environment. His contributions were critical to advancing his light source project from initial design through multiple iterations to prototype.

- Elizabeth A. Lipke, Chemical Engineering, Samuel Ginn College of Engineering

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Authors Biography

Ravi Nataraj is a senior-year student pursuing a B.S. degree in Chemical Engineering at Auburn University. After joining the Lipke Lab, he has played key research roles in optimizing a novel microfluidic platform and has gained experience in computational analysis for characterizing the maturity of engineered cardiac tissue.

Yuan Tian, Ph.D. is a Postdoctoral Fellow in the Department of Chemical Engineering, Samuel Ginn College of Engineering at Auburn University. Yuan received his doctorate training from Dr. Elizabeth Lipke's research group at Auburn University. Yuan's research interest focuses on intravascular targeted cell delivery and scalable production of engineered tissues for drug discovery and regenerative medicine purposes.

Mohammadjafar Hashemi is currently a fifth-year PhD candidate in Chemical Engineering at Auburn University. He obtained his M.Sc. in Polymer Engineering from Sharif University in Iran. With his knowledge in these fields, he specializes in encapsulation of hiPSC microspheroids and cardiac differentiation.

Dr. Elizabeth A. Lipke is the Mary and John H. Sanders Professor in the Department of Chemical Engineering at Auburn University. Dr. Lipke completed her graduate studies at Rice University followed by a postdoctoral fellowship at Johns Hopkins University. Dr. Lipke's research focuses on the use of cell-material interactions to create cellular microenvironments that guide tissue formation and direct cellular function.