HIGH THROUGHPUT SCREENING MODELS RAMP UP LEAD DISCOVERY

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AUTOMATING IN VIVO SCREENS AND CHALLENGING DOGMA

Scientists built a microfluidic lab-on-a-chip device that accelerates compound screens and phenotype analyses in *C. elegans* models of reproductive aging.

By Deanna MacNeil, PhD

Caenorhabditis elegans is a tiny nematode species that makes big contributions to molecular research. These worms have simple genomes, well-delineated developmental processes, brief lifespans, and many conserved biological pathways across the animal kingdom, including human processes such as aging, reproduction, and neurodevelopment. Because they are easy and affordable to grow in a dish, they are exceptional model organisms for in vivo compound screens, but traditional *C. elegans* culture methods can hinder high throughput assays.[1]

"There are limitations to what you can do when you're looking on a plate, and with millions of worms," said Coleen Murphy, a molecular biologist at Princeton University who studies aging processes using *C. elegans* models. Conventionally, scientists culture *C. elegans* on solid agar plates and visually screen the effects of genetic or environmental perturbations, seeking out and scoring phenotypic changes by watching the worms under a microscope. While this approach is robust and informative, it is tedious and groups many worms on the same plate, making it difficult for researchers to investigate related phenotypes in individual animals.[1]

As a solution to conventional culture limitations, Murphy's team built a new high throughput tool. In work published in *Lab on a Chip*, the researchers created and validated a lab-on-a-chip device called CeLab, which enabled them to automate worm assays for both individual- and population-level studies.[2] Designed by bioengineer Salman Sohrabi, who was a postdoctoral researcher in Murphy's laboratory at the time of this work, CeLab contains 200 separate incubation areas connected to microfluidic ports for manipulations, compound screens, and phenotype analyses.

The researchers performed proof of principle experiments to validate their system, comparing plate-based lifespan, mating, reproductive span, and drug testing assays with CeLab techniques. They demonstrated comparable measurements between plates and CeLab, found that CeLab accelerated screening, and scored individual worm phenotypes that could not be captured in population plate assays.

"There are two sides to the screen: you need to have a good phenotype and a good way to do it. Because sometimes you have really nice phenotypes, but they're really hard to set up for automation," said Alex Parker, a neuroscientist from the University of Montreal, who uses *C. elegans* models to screen neurodegenerative disease therapeutics and who was not involved in the study. *C. elegans* are excellent models for lifespan research, but their large brood size typically necessitates that researchers manually separate individual worms from their progeny while conducting lifespan studies on fertile organisms. Parker pointed to Murphy's solid foundation in *C. elegans* research as key to CeLab's automation success. "It's a very nice system because they had the benefit and the experience of knowing the field for a long time, and what are the problems with trying to automate."

CeLab's strengths also allowed Murphy's team to investigate reproductive aging paradigms, such as the disposable soma hypothesis, which is the evolutionary concept of a trade-off between how long an animal lives versus how large it grows and how often it reproduces.[3] Although some lifespan studies in *C. elegans* have supported the disposable soma hypothesis, the relationship between aging and reproduction remains contentious across the animal kingdom.[3] Murphy and her team found that lifespan and reproductive span were uncoupled in worms cultured on CeLab chips, and individual lifespans were actually correlated with higher progeny numbers. "They've got a new way to study this, and it's already paying off in interesting observations," said Parker.

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>
> —Coleen Murphy
> Princeton University

Their observations support the idea that lifespan does not come at the cost of reproductive fitness, but rather that if an animal is healthy, it is more likely to live longer, reproduce longer, and produce more progeny. "This idea that at an individual level, the disposable soma hypothesis is incorrect, it was mind blowing because it's so well accepted in the field that there must be a trade-off," Murphy said. "It's just so cool to observe … and it fits much better with all the observations in humans."

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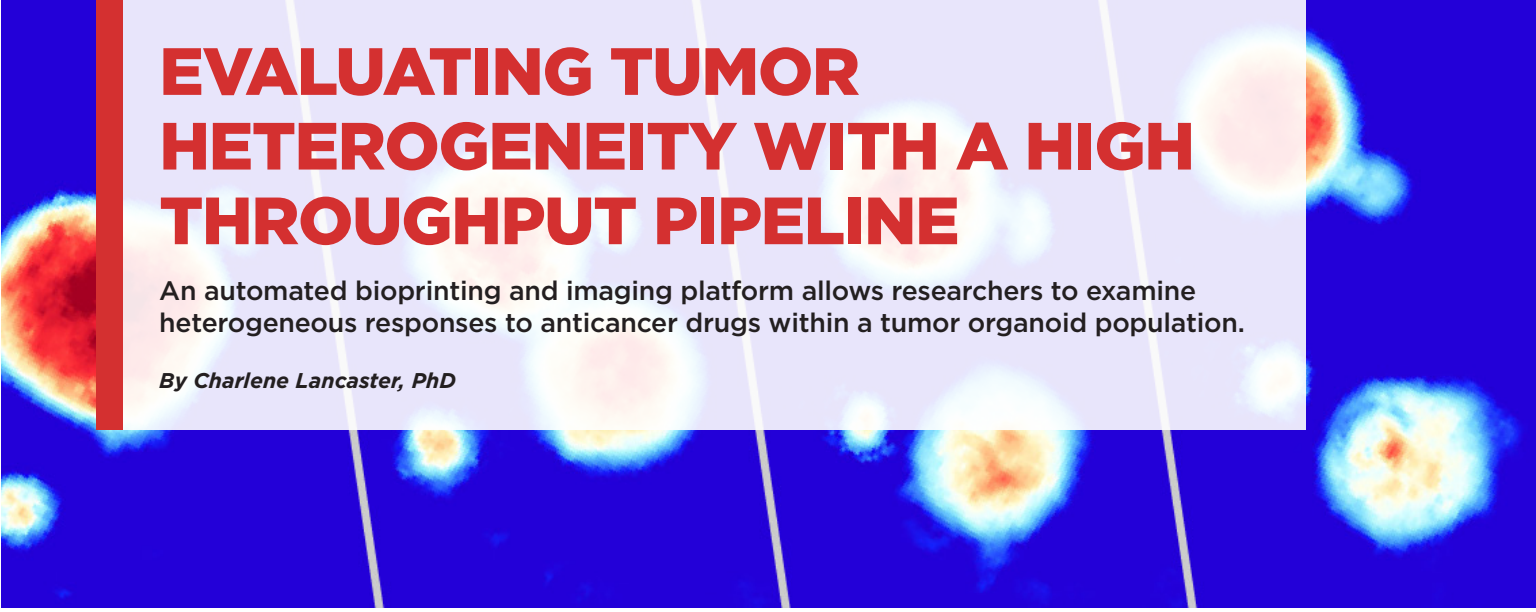

EVALUATING TUMOR HETEROGENEITY WITH A HIGH THROUGHPUT PIPELINE

An automated bioprinting and imaging platform allows researchers to examine heterogeneous responses to anticancer drugs within a tumor organoid population.

By Charlene Lancaster, PhD

The success of nonclinical drug testing relies heavily on using models that accurately recapitulate complex biological processes. With the passing of the Food and Drug Administration (FDA) Modernization Act 2.0 in 2022, researchers are no longer required to employ animal models to screen drug candidates.[1] Instead, the FDA is encouraging scientists to use other models, such as three-dimensional organoids and artificial intelligence-based methods, which are more predictive of the human response.[2] As a result, researchers will need to improve existing methods and develop new protocols to optimize the efficiency of nonclinical testing. In a proof-of-concept *Nature Communications* paper, scientists developed an automated high throughput assay to monitor tumor organoid growth label-free.[3]

"3D screening in a high throughput manner is still very challenging," said Alice Soragni, a cancer biologist at the University of California, Los Angeles (UCLA) and co-corresponding author of this study along with Michael Teitell at UCLA. Normally, researchers manually seed organoids within a 3D extracellular matrix, such as Matrigel, which is either spread across the surface of a microplate's well or placed as a drop in the center of the well. However, this methodology prevents scientists from implementing automated liquid handlers, which would disturb the organoids grown in the center of each well. To overcome this hurdle, Soragni, Teitell, and their interdisciplinary team developed a strategy to bioprint cancer cell line clusters suspended in culture medium and Matrigel into a microplate's wells. The bioprinter extruded the mixture in the shape of a hollow square, leaving a clearing in the center of each well. Because they could now employ automated liquid handlers, the researchers could increase the pipeline's throughput.

The researchers also wanted to develop a method for observing how individual tumor organoids respond to therapy in real time. Scientists commonly use endpoint assays to assess a drug's effect on an organoid population, but these tests fail to evaluate intra-sample heterogeneity. Soragni and her colleagues instead employed high-speed live cell interferometry, which is a label-free optical technique that uses phase shifts generated when light passes through an object to calculate its mass. Using this imaging method and machine learning-based quantitation, the researchers evaluated how individual tumor organoids responded to treatment with anticancer drugs by monitoring their biomass. Cell populations arising from the same line are often assumed to be homogenous, but after treatment the researchers observed organoids increasing, decreasing, or maintaining their size. These findings highlighted the pipeline's potential for detecting heterogeneous drug responses.

"It is very nice to have an interdisciplinary group working together to figure out what we can do better," said Lanlan Zhou, a cancer biologist and the director of the Organoid Core Facility at Brown University, who was not involved in the study. Zhou was excited to see what the group would do next and hoped they would test their platform's ability to assess more complex organoid models that better represent the tumor microenvironment in vivo. "You do have to think about the tumor microenvironment because the tumor is not only made of tumor cells. It is made of many cells including some normal cells. For example, they have immune cells, they have fibroblasts, they have blood vessels."

Soragni, Teitell, and their team are currently investigating how patient-derived organoids will react to bioprinting in hopes that they can use this platform to predict patient drug responses. This will be of particular importance for rare cancers, such as sarcomas. "For the majority of rare cancers, we have very little knowledge. We do not know what are the drivers of disease, we do not know how heterogeneous they are, we do not know how they respond to therapy," Soragni said. "Looking with this type of platform at rare cancer is going to give us a lot of information. It is going to teach us a lot of biology and it is going to also help us potentially come up with therapies we could be looking at clinically."

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TARGETED GENE INTEGRATION FOR HIGH-THROUGHPUT APPLICATIONS

A new approach using two types of recombinases lets scientists insert larger DNA payloads into human pluripotent stem cells faster than ever before.

By Nathan Ni, PhD

Targeted genomic editing made great strides in recent decades, especially thanks to the advent of endonuclease-based gene-editing systems such as CRISPR-Cas9. However, targeted insertion of larger payloads remains problematic, hampering what researchers can do in terms of capability and throughput. Richard Davis, a stem cell researcher at Leiden University Medical Center (LUMC) and Associate Investigator at The Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), believes that a new strategy, termed serine and tyrosine recombinase-assisted integration of genes for high-throughput investigation (STRAIGHT-IN), can give scientists a powerful new tool for research and clinical applications.[1]

Precision genome editing relies on homology-directed repair (HDR) to incorporate donor DNA into targeted regions.[2] However, HDR targeting efficiency decreases significantly as insert size increases,[3] so inserting multi-kilobase payloads remains difficult. Site-specific recombination can address this issue, and Davis and his team at LUMC used the strengths of two major site-specific recombinase (SSR) classes to build STRAIGHT-IN. "Serine recombinases can rapidly introduce constructs, but the whole vector—backbone, plasmid, everything—will integrate," Davis said. "We knew from experience that if these [unnecessary] sequences were retained, there will be silencing down the road preventing expression. So, we used tyrosine recombinases to excise these auxiliary sequences which were not required in the final cell line."

Davis and his team's findings, published in *Cell Reports Methods*, showed that STRAIGHT-IN facilitated the targeted integration or substitution of multi-kilobase genomic fragments while leaving only traces (under 300 base-pairs) of plasmid backbone DNA.[1] This capability is important for creating more physiologically relevant systems. "We wanted to keep all of the [endogenous] introns and regulatory elements; to retain the entire genomic context of the gene," Davis explained. Davis also noted that in the past, large payloads could be inserted but often required potentially phenotype-altering manipulations of cellular genomes. This was particularly problematic for stem cell researchers. "These additional modifications could involve knocking out or overexpressing certain genes, which would then affect their phenotype and their ability to differentiate," said Davis.

The researchers designed STRAIGHT-IN with human induced pluripotent stem cells (hiPSCs) in mind, as the cells' potential for disease research had been hampered because they were more difficult to genetically modify compared to immortalized cell lines. "STRAIGHT-IN technology is going to play a role in making [hiPSC] models more useful," said David Largaespada, a geneticist at the University of Minnesota Medical School who was not associated with this study. "For example, we have trouble mimicking what happens in human cancer genomes in mice because murine chromosomes are different. With hiPSC models, we could potentially model things more relevant to human cancer like gene amplification events, enhancer hijacking events, and so on."

In addition to capability, Davis and his team wanted to improve model generation speed and throughput. They developed a procedure where they replaced the gene of interest with a landing pad cassette containing SSR recognition and attachment sites, creating a template for future manipulation. Now, either the original gene of interest or a variant could be inserted without the need to develop a wholly new procedure each time, and the sequence would be placed within its endogenous genomic context. "Typically, Cas9 targeting requires three to six months," Davis explained. "But having a system where someone can rapidly reintroduce the gene with different variants is far more rapid, even with the time necessary to establish it." This approach also allowed multiplexing, which Davis and his colleagues demonstrated by introducing plasmids for twelve different mutations to a cell population, and recovering eleven of these from just a single transfection.

STRAIGHT-IN's potential is perhaps best displayed by its high ceiling. Davis and others in the field are already tackling the immediate hurdles such as looking at potentially better SSRs and working on delivery into differentiated cells. Davis's team has already introduced STRAIGHT-IN v2,[4] which removed the need for a clonal isolation step by achieving 100 percent efficiency. Finally, Largaespada noted that Davis and his team have made both vectors and cell lines with already-integrated SSR sites available to other scientists: "I think it will be quite easy to [bring in] and to scale—it would be quite doable for many labs, and we're considering it ourselves."

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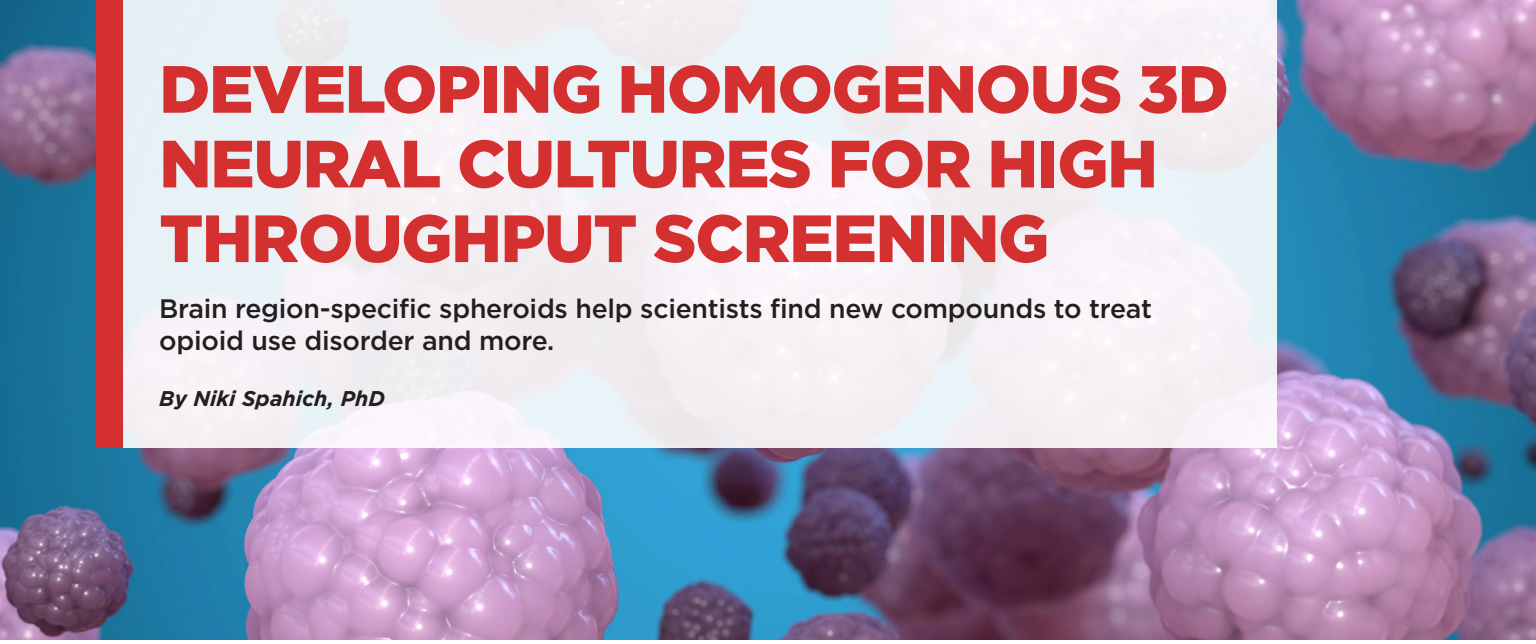

DEVELOPING HOMOGENOUS 3D NEURAL CULTURES FOR HIGH THROUGHPUT SCREENING

Brain region-specific spheroids help scientists find new compounds to treat opioid use disorder and more.

By Niki Spahich, PhD

Drug use disorders are increasing worldwide, with few individuals receiving treatment for these conditions.[1] In particular, abuse of prescription opioids and the rise in popularity of the synthetic opioid fentanyl has led to an opioid crisis in North America.[2]

To battle this epidemic, the United States National Institutes of Health (NIH) launched the Helping to End Addiction Long-term (HEAL) Initiative in 2018. As part of this effort, scientists at the NIH National Center for Advancing Translational Sciences (NCATS) led by Marc Ferrer and Emily Lee developed a new way to screen for opioid use disorder (OUD) treatments, which they described in a recent *Communications Biology* paper.[3] "We were looking for [3D] models that we could repurpose for screening compounds to target this initiative," said Lee. "We realized quickly that the existing models were not adaptable for high throughput screening."

To quickly test tens of thousands of compounds, the researchers needed to measure hundreds of wells in minutes with a plate reader. They found that brain organoids, which grow from stem or progenitor cell populations and differentiate into complex clusters that recapitulate in vivo morphology, were too heterogenous and took too long to mature for use in their high throughput screens.

As an alternative, Lee and Ferrer's team came up with an innovative way to make brain region-specific spheroids suitable for their needs. Spheroids are simple 3D cell clusters that mature in a matter of weeks. Previously, researchers developed neural spheroids similarly to how organoids are made—by allowing pluripotent cells to differentiate within wells, which creates heterogenous populations.

The NCATS team instead formed prefrontal cortex (PFC)-like and ventral tegmental area (VTA)-like spheroids by mixing together already differentiated neural cells at ratios that mimic each brain region's in vivo composition. "One of the interesting and different things that they did was aggregating a discrete number of cells and defining that [population] from the get go," said Madeline Andrews, a neuroscientist at Arizona State University who was not involved in this study. "It demonstrates a new and interesting approach to using stem cell-derived populations to get a slightly more mature kind of cell type faster."

The researchers confirmed that their starting ratios stayed consistent as the spheroids matured. They developed a plate reader assay that used a fluorescent dye to track intracellular calcium oscillations, which indicate neuronal activity.[4] To model OUD, the team grew their spheroids in 384-well plates and exposed them to an opioid receptor agonist over 10 days, which mimicked neuronal stimulation from drug use. The treatment lowered calcium activity in the PFC-like spheroids, which was reversed upon application of a drug used clinically to treat opioid overdoses. In the future, they could expose their spheroids to a collection of chemicals called a compound library to identify additional therapies for OUD.

The brain region-specific spheroid models also proved promising for studying neurodegenerative diseases. Lee and Ferrer's team assembled PFC-like spheroids with GABAergic neurons that contained an Alzheimer's disease (AD) risk allele. These spheroids exhibited defective calcium activity, which was reversed when treated with compounds used to improve cognition in patients with AD, including the clinically-approved drugs Memantine and Donepezil. "We're currently working on expanding to additional diseases, looking at things like epilepsy and amyotrophic lateral sclerosis," Lee said.

The researchers also plan to automate the manual steps in the spheroid maturation process and develop a high throughput screening method for testing assembloids made from their spheroids, as the VTA and PFC regions interact.

"I think [this model] is going to expedite discovery and also the understanding of what these therapeutics can actually do," said Andrews. "Anytime we can push the timeline a little bit faster, it helps science proceed more quickly."

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