CELL CULTURE ADVANCES FUEL DISEASE RESEARCH

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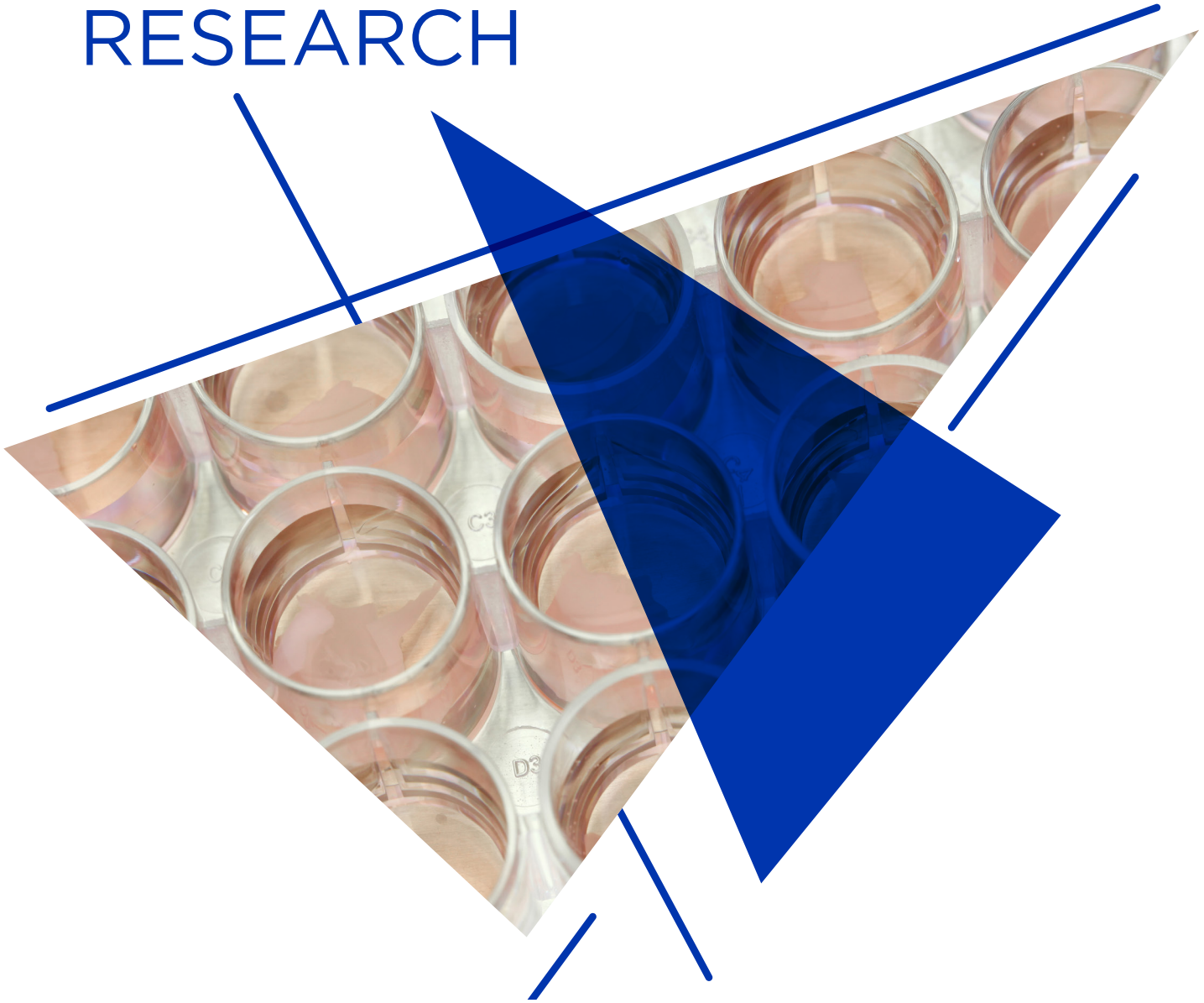

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DESIGNING CLINICALLY COMPLIANT LIVER ORGANOIDS

Three-dimensional culture models using a non-immunogenic matrix bring researchers a step closer to lab-grown transplantable organoids.

Liver transplantation is the only treatment option for patients with severe liver failure.[1] Due to a lack of livers available for donation, there is an urgent need for cell-based therapies. In an effort to develop an alternative for liver transplantation, Kristina Schoonjans and Matthias Lutolf from the Ecole Polytechnique Fédérale de Lausanne recently developed a new approach for growing transplantable liver organoids—tiny, self-organized three-dimensional (3D) tissue cultures derived from stem cells.

Liver organoids preserve key physiological features of the liver, self-renew, and self-organize, making them a good source of functionally mature and easily expandable cells for transplantation.[2-4] To grow organoids, scientists must create physiologically relevant culture environments that promote stem cell differentiation and self-organization. There is no universal method for growing organoids, and 3D tissue culture requirements are complex.

To generate an optimal physiological environment to support organoid cultures, researchers combine multiple components, such as extracellular matrix, signaling factors for maintenance and growth, and differentiation factors for stem cell-based models. Conventional matrices such as Matrigel suffer from structural instability, batch-to-batch variation, and clinical incompatibility as they are animal-derived and potentially immunogenic.[3]

In their recent publication in *Nature Communications*, Schoonjans, Lutolf, and their colleagues described a method for generating liver organoids by establishing a chemically defined 3D culture system for mouse and human hepatic progenitors.[1] They developed a new type of inert polyethylene glycol (PEG) hydrogel matrix scaffold to support cell growth by adding the minimal integrin recognition peptide RGDSPG (Arg-Gly-Asp-Ser-Pro-Gly) to the medium. Liver organoids cultured in this PEG-RGD matrix displayed hepatocyte-specific functions, such as producing and secreting urea and glycogen accumulation.

The PEG-RGD matrix-supported organoid growth without structural deterioration and provided efficient mechanical stability for long-term organoid culture. Compared to commercially available substrates, the PEG-RGD chemically defined matrix was more stable and lacked the batch-to-batch variation observed in conventional matrices. These data provide a proof-of-concept for maintaining human hepatic progenitor cells in vitro with synthetic matrices.

Obesity and diabetes studies indicate an intricate cross-talk between adipose tissue and the liver, demonstrating a relationship between excess fat accumulation, insulin resistance, and liver fibrosis.[1] In the current study, the researchers mimicked the stiffness of healthy and fibrotic livers associated with obesity and diabetes by altering the PEG-RDG matrix by adding various growth factors. The team embedded cells from freshly isolated liver biopsies directly in the PEG-RGD hydrogels to generate clinically relevant human liver organoids. The organoids grown in a rigid matrix behaved more like diseased liver cells with a compromised potential to regenerate and signs of inflammation and injury. By recapitulating the fibrotic liver microenvironment, researchers can use this cell culture method to test potential treatments that may stop or reverse the course of liver disease.[2,4]

Schoonjans and Lutolf's team established a standardized framework for culturing and studying hepatic

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progenitor cells in a defined environment that mimics healthy and diseased hepatic conditions. This protocol using the PEG-RGD matrix advances regenerative medicine by providing a non-immunogenic way to grow transplantable organoids. In the future, clinical researchers must establish the efficacy and safety of transplantable organoid-based therapies grown with this clinically compliant culture matrix. Nevertheless, this matrix opens an exciting new horizon for clinical applications of liver organoids.

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A LIVING ANEURYSM

Researchers create the first ever bioprinted, living aneurysm and watch it heal in real-time.

The thought of a brain aneurysm is scary. It is unpredictable and unpreventable. Brain aneurysms are caused by the silent bulging of a weakened blood vessel. If untreated, the bulge quietly expands, and if ruptured, it could result in stroke, brain damage, or even death.

Brain aneurysms are difficult for researchers to study. Two-dimensional cell culture methods are often ineffective models of three-dimensional vessel bulging, and animal models do not fully recapitulate human blood vessel shape and physiology. To overcome these challenges and inform future treatments, a team of researchers from Livermore National Laboratory, Duke University, and Texas A&M developed the first-ever living model of a human aneurysm.[1]

Led by William Hynes, a research engineer at Lawrence Livermore National Laboratory, the research team created the model using bioprinting, a novel technique that combines 3D printing with living cellular systems. Similar to a 3D printer, bioprinting prints a three-dimensional structure. Only instead of printing with hard plastic, bioprinters use a protein-based hydrogel that provides a soft environment similar to the *in vivo* conditions of the body.

In the recent study, the researchers bioprinted the shape of blood vessels, and in particular, an aneurysm, using a combination of protein-based hydrogel and a special type of ink called sacrificial ink. The ink lined the inside of the bioprinted vessels. When cooled, the ink dissolved, leaving behind the vascular shape and cavities needed to model a working blood vessel. The researchers then seeded the bioprinted structure with human endothelial cells, which coated the channels to form blood vessels and replicate an aneurysm.

An estimated one in 50 Americans suffers a brain aneurysm. Treatment options are limited, with variable success. In one common type of treatment, physicians attach a metal clip to the base of the aneurysm to redirect blood flow and prevent the vessel from bursting. However, this treatment is highly invasive and involves opening the skull and exposing the brain. It also may not be possible for aneurysms in inaccessible regions.

In a less invasive but no less precarious alternative treatment, surgeons feed a thin metal catheter through an artery in the groin up through the body to the aneurysm in the brain. They then pack the bulging vessel with coils or stents. The cells of the blood vessel grow over the clotted bulge, effectively sealing it off and preventing its expansion. The success of this procedure depends on the unique and variable geometry of each patient's blood vessels.

Hynes's team tested their ability to repair the bioprinted aneurysm in the first-ever surgical intervention performed on an artificial living tissue. They used the coiling procedure with a microcatheter and platinum coils to pack the bulge. They then watched as the endothelial cells grew for eight days over the packed region, circumventing the bulge, and effectively healing the aneurysm.

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Using this new model, the researchers also simulated blood flow. Amanda Randles, an assistant professor at Duke University and one of the authors of the study, developed a high-performance computer code, called HARVEY, to simulate blood flow within the bioprinted aneurysm.

Typically, scientists validate these types of computer models by inducing aneurysms in animals and performing surgery. However, it is difficult to measure blood flow dynamics within a living animal. The geometry of animal vessels is variable and not reproducible. To circumvent this, researchers often use 3D printed silicone tubes, but while ves-

sel shape can be more easily controlled, 3D silicone tubes do not fully replicate human biology.

With this new bioprinted aneurysm, the researchers directly validated blood flow throughout the aneurysm in a dynamic living system, verifying that the outcome of the procedure would likely be the same in a living patient. At low blood flow rates, they observed very little blood flow movement into the aneurysm. At increased blood flow rates, they observed an increased circular flow of blood through the aneurysm as would be expected in a living patient with a brain aneurysm.

Using a combination of computational modeling and 3D-bioprinting, the researchers predicted that physicians could scan the brains of patients and create real-world models of each patient's brain and vasculature. These personalized models could then be used to simulate patient-specific blood flow rates, identify ideal coil type and packing volume, and ensure the best possible treatment outcomes. This would also allow physicians to perform multiple practice runs of a procedure before performing it on the patient.

The clinical applications of this 3D-bioprinted aneurysm model are still far from reality. Right now, the model servers as proof of principle and a platform to accelerate research and understanding of human brain aneurysms. The researchers plan to next investigate two-dimensional blood clotting in response to coil-based treatments and to compare a variety of coil types to achieve better clotting and improved patient outcomes.

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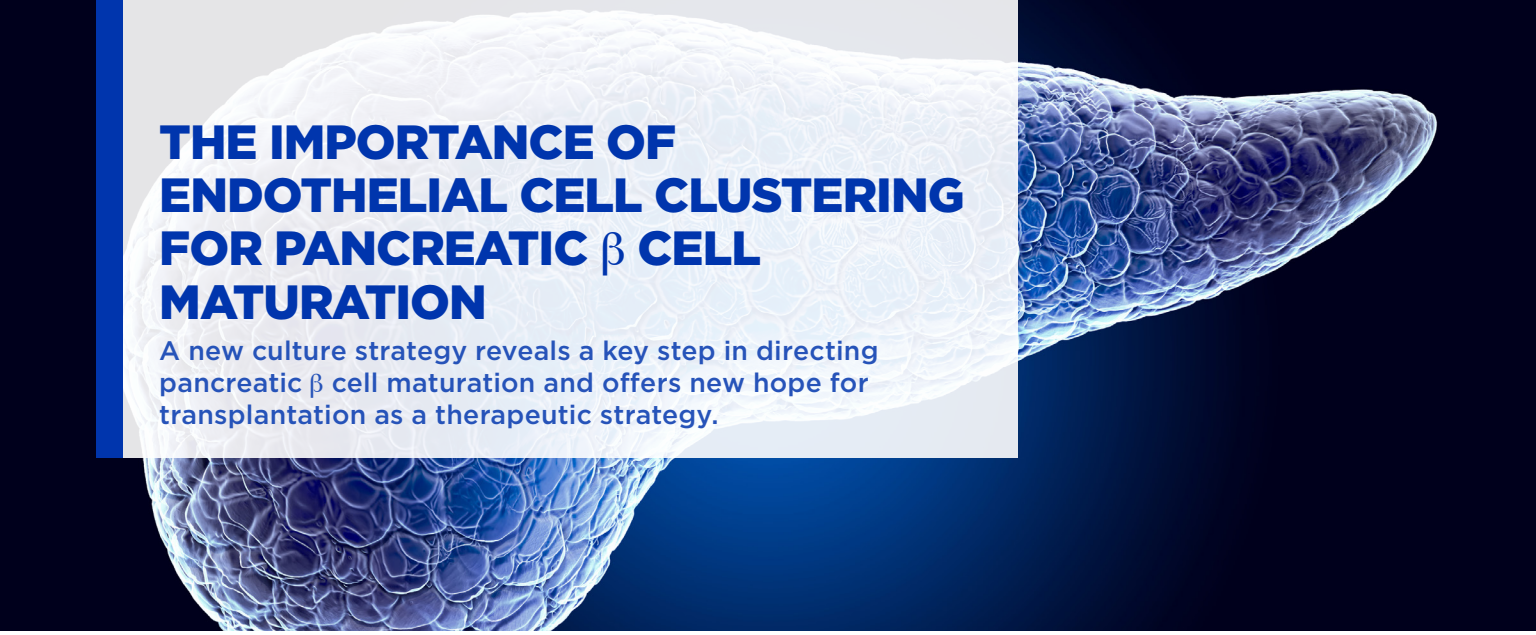

THE IMPORTANCE OF ENDOTHELIAL CELL CLUSTERING FOR PANCREATIC β CELL MATURATION

A new culture strategy reveals a key step in directing pancreatic β cell maturation and offers new hope for transplantation as a therapeutic strategy.

Pancreatic β cell destruction or dysfunction lies at the core of diabetes mellitus, and so their recovery or regeneration lies at the core of a cure for the disease. Ever since scientists learned to isolate and culture stem cells, they have striven to produce mature functional β cells.[1] However, guiding stem cells to differentiate into endogenously-comparable insulin-producing β cells has been difficult. In a key development published in *Nature Cell Biology*,[2] Matthias Hebrok's research team at the University of California, San Francisco's Diabetes Center outlined how mature functional β cells can be produced by using pancreatic islet organogenesis-mimicking cell culture conditions to create islet-sized clusters. The resulting β cells showed paracrine function upon proper stimulus for several months after being transplanted into murine kidneys.

Diabetes mellitus type I and II feature blood glucose dysregulation caused by pancreatic β cell pathology. Current therapeutic approaches involve using exogenous insulin to manipulate blood glucose levels, but insulin administration lacks the rigorous control provided by endogenous β cells. As such, researchers want to generate β cells *de novo* for transplantation. Although scientists have identified how to direct pluripotent stem cell differentiation into β cells, the resulting β cells lacked sufficient functionality.

Hebrok's group looked at human and rodent β cell maturation patterns and noticed significant cellular reorganization during development. In particular, they observed endocrine cell clustering, followed by compaction, and then the formation of definitive islets. These mechanisms were largely absent from previous attempts to generate β cells in vitro from human pluripotent stem cells (hPSC). Believing this to be important for endogenous-like functionality, the researchers mimicked endogenous endocrine cell clustering by reaggregating hPSC-generated immature β cells into what they termed "enriched β-clusters" (eBCs).

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To create eBCs, the team differentiated hPSCs until monohormonal C-peptide-expressing immature β cells arose. They then extracted the insulin-producing cells from this population using fluorescence-activated cell sorting (FACS) and seeded them in microwells at a density recapitulating the average number of β cells in one human islet. The seeded cells reaggregated and self-organized into islet-sized eBCs after two days. eBCs were exclusively endocrine, with duct or acinar cells entirely absent. Immunofluorescent staining confirmed that the vast majority of eBC cells were monohormonal C-peptide-expressing immature β cells, with only small populations expressing α cell or δ cell markers, and even then, only in conjuction with C-peptide. Transcriptomic analysis of eBCs also revealed a gene expression profile aligning with mature β cells.

Previously generated hPSC-derived β cells responded poorly to stimulatory challenges, often exhibiting limited or no paracrine activity. In contrast, eBCs responded well to challenges with insulin-production stimulators such as glucose and KCl. Delving into the insulin secretion mechanism, Hebrok's team saw that cytosolic calcium flux post-glucose stimulation in eBCs was similar to that reported in human islets and differed dramatically from the calcium flux seen in immature or progenitor β cells. Similarly, closing K^+_{ATP} channels eBCs elicited insulin secretion, much like it does in mature adult β cells. Finally, as would be expected from mature β cells, eBC insulin secretion stopped at low glucose concentrations.

Despite confirming in vitro that eBCs were functionally and genetically similar

to mature β cells, the researchers needed to test how these cells would hold up in vivo. To test this, they transplanted eBCs into the kidney capsules of non-diabetic mice to examine growth, cellular composition, and functionality. Hebrok's team observed elevated responses to glucose challenge as early as three days post-transplant, and this capability was maintained 30 days after transplant. (A separate experiment showed function at eight months post-transplant.) eBC transplantation also ameliorated hyperglycemia caused by streptozotocin administration, further confirming the ability of the transplanted cells to regulate systemic glucose levels.

Interestingly, Hebrok's group found that hPSC-derived cells that reaggregated and clustered without being screened for insulin production did not respond to glucose challenges, highlighting the importance of endocrine cell enrichment to β cell maturation. Exploring this further using RNA sequencing, they found that 937 genes were differentially expressed between eBCs and non-enriched cells, which is almost the same number of genes that differ in expression between eBCs and clusters of immature cells (1,068). RNAseq results led Hebrok's team to isolate mitochondrial function as a potential cause for this. Indeed, a respiration assay showed that eBCs and mature human islets, but not immature clusters, increased oxygen consumption upon glucose stimulation. Further investigation revealed superior mitochondrial morphological organization in eBCs as well as higher counts and mass.

In vitro production of insulin-producing β cells from hPSCs has been plagued by issues related to insufficient cell maturing. Hebrok's research team highlighted the importance of clustering endocrine-enriched populations for driving β cell physiological maturation, yielding a cell product that closely mimics human β cells in function and genetics. This finding opens new doors for studying β cell physiology and is an essential step towards β cell transplantation as a therapeutic approach against diabetes.

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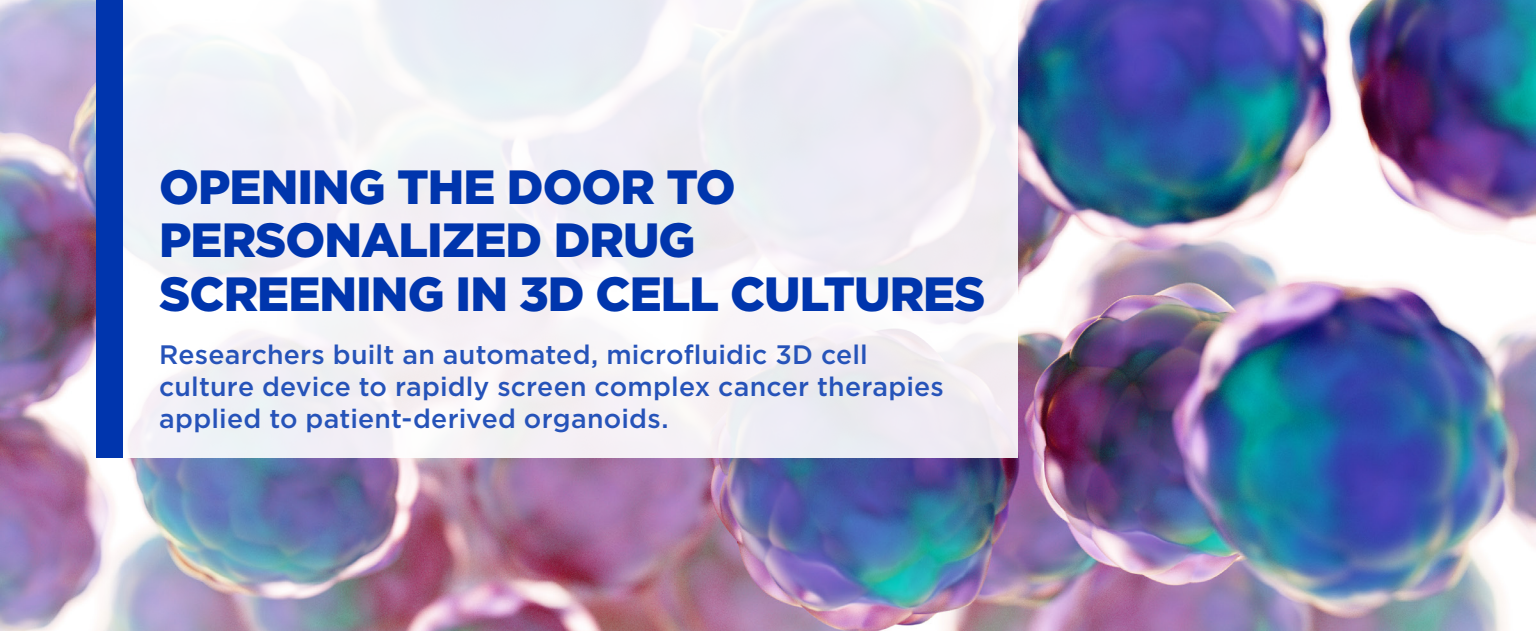

OPENING THE DOOR TO PERSONALIZED DRUG SCREENING IN 3D CELL CULTURES

Researchers built an automated, microfluidic 3D cell culture device to rapidly screen complex cancer therapies applied to patient-derived organoids.

Researchers often explore personalized therapies by growing organoids from patient samples. 3D cell culture better replicates the tumor microenvironment and maintains specific patient phenotypes than 2D culture methods, so responses to therapies tested on these organoids mimic those of patient tumors.[1] This technique is currently experimental and only possible on a small scale. To use personalized 3D cultures clinically, researchers need better strategies to quickly grow reproducible organoids and test different drug combinations in a high-throughput manner. A multidisciplinary team led by Savaş Tay from the University of Chicago combined engineering and biology expertise to build an automated, microfluidic 3D cell culture system for preclinical research into personalized therapies.[2] With this device, researchers can dose hundreds of individual organoid cultures at the same time with a variety of complex and clinically-relevant drug treatments.

Current microfluidic systems for 3D culture are insufficient for precise drug screening. They are low-throughput and often incompatible with extracellular matrix materials such as Matrigel that organoids require for mechanical support and biochemical cues. Another limitation of existing systems is their size; few consist of individual wells that are large enough to accommodate mature organoids.[3]

To effectively screen for drug sensitivity, Tay's team built a microfluidic platform that facilitates cell growth in 3D with standardized and reproducible miniature assays. Their new platform consists of two integrated devices. A 200-well 3D culture chamber has a removable top layer containing fluid channels that deposit chemical solutions to the lower chamber. By removing the top layer, researchers can pipette culture reagents and seed and harvest cells from each well. The wells are large enough to accommodate mature organoids and are divided into subsets that accommodate up to 10 patient samples and 20 experimental conditions, allowing for thousands of treatment simulations, including replicate and control wells.

This new platform consists of two integrated devices: a 200-well 3D culture chamber and a multiplexer fluid control device that manages the temporal delivery of multiple solutions to sections of the growth chamber.

The second portion is a multiplexer fluid control device that manages the temporal delivery of multiple solutions to sections of the growth chamber. Using custom software that reads a simple tab-delimited text file, researchers can program the automated delivery of up to 30 chemical solutions, including medium, drugs, and chemical signals, from individual vials hooked up to the multiplexer. A system of channels and valves deliver the solutions in various combinations and temporal sequences according to the programmed schedule. The automated fluid delivery removes the need for manual pipetting, which reduces error and standardizes the timing of delivery.

The researchers housed the device on a microscope within a temperature-controlled system to perform time-lapse phase contrast and fluorescence deconvolution microscopy on the 3D cultures during experimental treatments. Using the microscopy images, they measured many parameters, including proliferation, cell reactions, cell death, and apoptosis.

To test the efficacy of the platform, Tay's team grew pancreatic ductal adenocarcinoma (PDAC) organoids from individual patient cells to maturity.[1] As expected, the researchers observed contrasting morphologies in samples grown from different patients. They next employed a high-throughput assay to track organoid sensitivity to various cancer drug treatments, testing clinically relevant doses of seven drugs for

pancreatic cancer on organoids derived from three patients. The platform delivered drugs individually or in combination consistently over 72 hours or in four-hour pulses. By tracking cell death and apoptosis with fluorescent dyes and live-cell imaging, the researchers observed that combination chemotherapy resulted in increased apoptosis compared to monotherapy.

Some of the most effective chemotherapies are administered in a specific temporal order.[4] Therefore, the team compared patient-derived organoid responses to individual drugs and clinically-used drug cocktails delivered in various sequences. Organoids from different patients responded differently to the treatments, with temporally administered drug cocktails inducing more cell death and apoptosis in some of the patient samples. The results confirmed that temporally-delivered drug treatments may be more effective than constant-dose monotherapy or combination therapy, depending on the patient.

This automated, microfluidic device is the first 3D cell culture platform that facilitates high-throughput screening of complex drug treatments on organoid cultures. In the future, scientists may use it to culture organoids from routine biopsies, assess patient samples at different stages of tumorigenesis, and screen numerous drug treatments to find ideal regimens for individual cancer cases.

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