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Microencapsulated Tumor Organoids Maintain Drug Sensitivity Stratification

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Abstract:

Drug sensitivity profiling on patient-derived organoids mirrors patient response for gastrointestinal cancers and is currently being evaluated in clinical trials to guide treatment decisions. However, long expansion times are typically required to achieve adequate cell numbers. Accordingly, miniaturization of organoid cultures can be leveraged to reduce assay time and increase the number of compounds tested. Microencapsulation by droplet microfluidics has been applied to produce miniaturized organoid cultures, but the predictive accuracy has yet to be fully established. Here, we show that drug response can be preserved while reducing pancreatic cancer organoid culture volume by 100-fold. Testing five standard-of-care chemotherapeutics in miniaturized 0.5 μ L cultures versus standard 50 μ L cultures, we find that rank-ordering by drug sensitivity produced near identical stratification. Overall, these data demonstrate the potential for platforms based on this strategy to accurately pharmacotype tumor biopsies while reducing the time needed to inform treatment.

Introduction:

Quality tumor models for the evaluation of therapeutic responses are paramount to the success of precision medicine. Examples include 2D patient-derived cell lines, patient-derived xenografts (PDXs), and patient-derived organoids (PDOs). 2D cell lines are cost-effective and simple to culture but fail to represent the tumor microenvironment accurately, while PDXs, created by transplanting patient tumor tissue into immunodeficient mice, better mimic tumors but have very long expansion times (9-18 months) and complicated workflows.(1) PDOs are 3D models created by seeding cells from primary or metastatic tumors in a hydrogel matrix. This fairly simple workflow replicates structural features of tumors well and has shown high accuracy in predicting drug responses for various cancers,(2–4) but still requires significant expansion time (eg. 2 months to screen 5 drugs) that limits their use with rapidly progressing cancers.(4)

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive cancer displaying extremely rapid progression.(5) Despite accounting for only 3.3% of new cancer cases in the U.S.,(6) PDAC is the 3rd leading cause of cancer-related death,(7) with a 5-year survival rate of just 12%.(8) Late diagnosis is a major challenge, with 88% of cases detected after metastasis, making surgical resections unfeasible.(9) Targeted agents only offer limited clinical benefits,(10) and conventional chemotherapy remains the standard of care treatment. However, the efficacy of chemotherapy is limited due to high tumor heterogeneity and rapid acquisition of chemoresistance.(11–13) Furthermore, in the absence of predictive biomarkers, oncologists choose from the existing standard-of-care chemotherapy options based largely on patient performance status and comorbidities. Even after surgery, recurrence rates are as high as 40% within a year of margin-free resection.(14) Accordingly, optimizing predictive modeling by preserving the accuracy of PDOs while expediting drug screening can enhance chemotherapy outcomes and reduce side effects for PDAC.(15,16)

Organoids can be cultured and assayed in smaller volumes of matrix with fewer cells to increase throughput and reduce expansion time. While manual pipetting is plagued by high error when handling low volumes, 3 μ L PDOs with an 18% coefficient of variation have been produced with automated pipetting.(17) Further reduction can be achieved with microfluidics, which can generate monodisperse extracellular matrix (ECM) droplets in the range of tens to hundreds of nanoliters. Initial studies screening microencapsulated tumor organoids expanded in microfluidic droplets show promise for various solid tumors,(1,18) but the predicted accuracy remains unclear due to a lack of data comparing model response with the clinical drug response of the patients from whom the organoids were derived. Standard format organoids have been demonstrated to correlate strongly with patient drug response,(2–4) offering a gold standard for the evaluation of alternative models.

Here, we show that viable mouse and human PDAC PDOs can be formed in 0.5 μ L droplets of Cultrex basement membrane extract (BME) generated with 3D-printed T-junction microfluidic devices. Organoid formation was validated by comparing morphology against conventional 50 μ L cultures. After establishing protocols with murine PDAC organoids, the sensitivity of five standard-of-care drugs was compared on human PDAC organoids using the area under the curve (AUC) over a wide range of 19 dosages. Critically, the dose responses from miniaturized

cultures correlated closely with standard culture models for both cell types. While variation was possible between the two formats, drug sensitivity rankings were largely consistent between conventional and miniaturized cultures. These findings suggest that reducing cell and matrix volume 100-fold from conventional PDO culture maintains pharmacotyping accuracy. In addition, our approach may better preserve tumor heterogeneity and enhance patient-specific accuracy.

Results:

Microfluidic systems can be used to reliably miniaturize PDOs: Previous efforts to generate miniaturized BME volumes with microfluidics employed small droplets (~30 nL) containing fewer cells (20-30) and thus required multiple droplets (10-100) per well in order to reach signal thresholds.(1,2) In contrast, we targeted larger 500 nL droplets seeded with ~200 cells each and exactly one droplet per well, which we achieved by manual dispensing. From Poisson statistics, this latter approach fundamentally produces substantially lower coefficient of variation (CV), due to fewer Poisson processes and lower CV per process because of the higher expected value. For example, to seed 200 cells per experimental condition, the former approach might employ ~20 cells per droplet (22% CV) and ~10 droplets per well (32% CV) for a total CV of 39% (product of two random variables). In contrast, in our approach the minimum CV due to Poisson contributions is only 7% (~200 cells per droplet). In practice, consideration of Poisson statistics likely requires employing a higher number of cells per condition in the former approach.

Basement membrane extract (BME) has complex rheological properties, displaying strong temperature dependence, shear thinning, and orders of magnitude higher viscosity than is typical in droplet microfluidics,(19) thus complicating the physics of droplet generation. Using a T-junction droplet generator, we found that conditions that work well for generation of water droplets can fail when trying to form BME droplets (Fig. 1). Droplet formation in a T-junction is a function of the capillary number $Ca = U\mu/\gamma$, where U (m s^{-1}) is the flow velocity, μ (kg ms^{-1}) is the dynamic viscosity, and γ (kg s^{-2}) is the interfacial tension between the dispersed and continuous phases.(20) At lower Ca , monodisperse droplets are generated in the squeezing and dripping regimes, whereas higher Ca produces less uniform droplets in the jetting regime or no droplets in the threading regime (Fig. 1A). (21–23). This behavior has previously been described as a question of whether the interfacial velocity γ/μ is sufficiently greater than the flow velocity U to allow droplets to snap off cleanly.(21) Under our experimental conditions (~12 °C, 10-100 s^{-1} shear rate), we estimate BME viscosity to be 1-10 Pa s, or 3-4 orders of magnitude higher than that of water.(19,24,25) The resulting increase in Ca is consistent with the difference in droplet formation between water and BME seen in Fig. 1.

In order to maintain low Ca for quality droplet generation in the face of high BME viscosity, flow velocity can be reduced or interfacial tension can be increased. We studied the effects of both parameters on droplet generation. Specifically, we modulated interfacial tension by adjusting surfactant concentration,(26) as surfactants serve to reduce the interfacial tension between liquids. Concurrently, we studied reductions in total flow velocity while maintaining a 1:10 flow ratio of the dispersed and continuous phases. As shown in Fig. 1, both adjustments produced

substantial changes in BME droplet generation while minimally affecting water droplet generation.

Lower BME droplet frequencies were produced at lower flow rates: 0.44 Hz at 20 $\mu\text{L}/\text{min}$, 0.2 Hz at 10 $\mu\text{L}/\text{min}$, and 0.06 Hz at 1 $\mu\text{L}/\text{min}$. Without surfactant, droplet generation exhibited only a slight decrease in droplet volume as flow rate and capillary number increased, consistent with the squeezing regime (Fig. 1A). Instead, droplet size was determined primarily by the flow ratio of the dispersed and continuous phases, again consistent with the expected behavior in the squeezing regime.(22) Smaller 317 nL droplets (14.3% CV, $n=68$) were produced with a 1:15 flow ratio, increasing to 609 nL (10.6% CV, $n=50$) with a 1:10 flow ratio, and further increasing to 720 nL (16.2% CV, $n=30$) with a 1:5 flow ratio (Fig. 2A).

We chose to produce droplets without employing surfactant in order to establish low capillary number in the face of high BME viscosity. However, surfactants maintain the stability of emulsions and prevent droplet coalescence. To avoid droplet fusion, BME plugs were collected single-file in a spool of 1/32" ID PTFE tubing (Fig. 2B) and incubated for 30 minutes (37 °C, 5% CO₂) to gel the BME.(27) During incubation, the tubing was plugged on both ends by ~1 cm cubes of PDMS punctured to a depth of ~5 mm with a 1-mm biopsy punch. Following gelation, the BME droplets could be aggregated without fusion (Fig. 2C). Droplet volumes were measured after gelation and dispensing by measuring length and width from images and estimating height from the tubing diameter.

Miniaturized murine organoid cultures mirror the morphology and drug sensitivity of conventional organoid cultures: Following the optimization of BME droplet generation, we utilized the murine mT3 organoid system derived from the genetically engineered $\text{Kras}^{+/G12D};\text{Trp53}^{+/R172H};\text{Pdx1-Cre}$ (KPC) mouse model of PDAC.(28) The mT3 cells were suspended in BME to form cell-laden droplets at a density matched across the miniaturized and standard formats so that viability assay results could be appropriately compared between formats. We next confirmed that cell encapsulation and organoid formation in the microfluidic organoids mirrored that seen in traditional 3D cultures via brightfield microscopy (Fig. 3A, B).

To validate our system, we first compared an established model(28) of acquired gemcitabine resistance (mT3 GemR) to mT3 cells from the parental lines (mT3 sensitive). For standard organoid cultures, we used a 50 μL BME and cell solution (Fig. 3C), and for miniaturized organoid cultures, we generated cell-laden BME droplets that averaged 0.5 μL (Fig. 3D). Accordingly, the reduction in volume between standard and miniaturized organoid was roughly 100-fold. Importantly, in both culture models, the GemR mT3 cells maintained their resistance to gemcitabine (Fig. 3C, D).

Next, we sought to determine if we could achieve a comparable level of dose response to chemotherapy in both the standard and miniaturized cultures (Fig. 3D-E). After 4 days of treatment, organoids expanded from the sensitive line showed more marked responses to gemcitabine in both formats. The IC₅₀ of KPC MT3 sensitive organoids in standard and miniaturized cultures was 10.03 nM and 4.15 nM, respectively. Conversely, the resistant cell

line's tolerance to gemcitabine was evident in both formats, as the IC_{50} doses were 2177 nM and 140.4 nM, respectively; the dose response in both formats showed considerable shifts between the resistant and sensitive cell lines, suggesting the sensitive line's response can be attributed to the drug. Interestingly, for both cell lines, the dose response curves for miniaturized cultures are shifted left (indicating higher sensitivity) relative to standard cultures, which may be due to reduced gemcitabine diffusion distance in miniaturized cultures and increased effects of local hypoxia in the larger conventional cultures.(29) Of note, a 4-day treatment period was chosen as it allows for chemotherapeutic efficacy without sacrificing BME droplet stability (Fig. S2).

Miniaturized human PDAC organoid cultures mirror the morphology and drug sensitivity of conventional cultures: After validating that our miniaturized organoid system could reproduce a known system with a defined murine model, we next sought to demonstrate compatibility of the platform with human specimens. Here, we used UCI-PDA-PDO1 human PDAC organoids in 50 μ L standard cultures and compared these with 0.5 μ L miniaturized cultures in droplets. Again, we observed a similar 3D organization of the organoids, demonstrating that miniaturization has no detrimental impact on human PDOs (Fig. 4A, B).

To assess the ability of miniaturized organoids to serve as a reliable precision medicine platform, we next compared the dose response of UCI-PDA-PDO1 organoids, in both standard and miniaturized formats, to five standard-of-care first- and second-line therapies: gemcitabine, 5-fluorouracil, oxaliplatin, paclitaxel, and SN-38 (the active form of irinotecan) (Fig. 4C, D). Here, we observed that when stratifying the five chemotherapeutics based on efficacy, as determined by area under the curve (AUC), both the standard and miniaturized culture models arrived at nearly identical conclusions (Fig. 4E). Increased variance in the miniaturized format produced minor differences in statistical grouping (Fig. 4F). Nevertheless, the same three drugs are identified as effective in either culture format, suggesting that both models can be used to inform potential therapeutic priorities when used as a prospective model in parallel with patient treatment (Fig. 4F).(4)

Discussion:

The data presented suggest that a 100-fold reduction in the culture and assay volume of pancreatic cancer organoid models can retain accuracy in terms of predicting rank ordering in drug response. In the context of determining personalized cancer treatments, this reduction in cell volume is expected to reduce the cell expansion time that is required prior to drug screening. Additional studies are needed to quantify the exact time-to-result following biopsy collection and to validate predictive accuracy across a larger number of patient-derived organoids. Ultimately, the goal is to determine effective drugs for individual patients within critical windows before succumbing to disease, and ideally before developing resistance to certain lines of chemotherapy.(13) Late diagnoses, rapid progression, and the high mortality rate of PDAC make it an especially important organ system for which to enhance personalized medicine approaches.(10)

The lack of highly predictive clinical biomarkers for PDAC is in part due to high inter- and intratumor heterogeneity. When culturing samples of biopsy tissue as PDOs, some intratumor heterogeneity is lost, which can lead to imperfect pharmacotyping insights.(30) These PDO models also lack immune and stromal cells, which may further impact drug responses.(31,32) As such, pharmacotyping efforts should be viewed as strong, but not perfect, predictors of drug sensitivity. Inclusion of stromal and immune cells in future miniaturized PDO platforms can potentially mitigate part of these limitations.

Single cell sequencing experiments have shown that past studies vastly underestimated the level of complexity of PDAC tumors, with most tumors consisting of a combination of classical and basal hybrid subtypes as opposed to a single subtype.(10,11) This heterogeneity has been shown to drive phenotypic consequences on metabolism, metastasis, and response to therapy.(33–35) Early cultures more closely resemble the phenotypes and clonal heterogeneity of the patient's PDAC tumor, while later cultures tend to express the more dominant clones. Specifically, long-term organoid cultures have been shown to drift towards the classical phenotype.(30) As miniaturized organoids should require shorter culture periods than standard organoids, some heterogeneity may in fact be preserved in the miniaturized organoids, similar to early cultures. Therefore, while existing PDO pharmacotyping systems all lose some primary PDAC intratumor heterogeneity during culture of biopsy samples, miniaturized organoids with a shorter culture duration may be more likely to capture sufficient tumor heterogeneity while also enabling more rapid pharmacotyping.

Microfluidic approaches have been applied to other solid tumor systems, including colon, liver, kidney, and lung cancer.(1,27) Our platform is among the first shown for PDAC, and it was unclear whether miniaturization would perform well with this highly complex tumor organ system. In addition to the previously noted heterogeneity, low cellularity and high fibrosis observed in PDAC biopsies increase the challenges in establishing PDOs.(36) Choi et al. reported encouraging results from assaying PDAC spheroids or organoid fragments in 3 μ L volumes on a microfluidic chip, demonstrating the effectiveness of GSK inhibitors in combination with gemcitabine, and NK cells in combination with TriKE immunomodulators.(37) Our approach differs in that PDAC organoids remain embedded in BME throughout the entire process of growth and assay. Ultimately, our study aims to alleviate the notable absence of quantitative comparisons between miniaturized organoid cultures and gold-standard systems by performing parallel drug sensitivity screens in the two formats. Our data support the accuracy of microencapsulated organoid models, opening the door to future studies leveraging this miniaturized format to reduce the time from biopsy to screening or to screen larger drug libraries.

Limitations:

The miniaturized organoid cultures show increased variance relative to conventional cultures, likely due to factors including heterogeneity in BME cell suspensions, variation in BME droplet volume, BME disintegration over time, and pipetting error, all of which can be more prominent in miniaturized cultures. Additionally, the tumor microenvironment is complex, with cancer cells, immune cells, and stromal cells interacting unpredictably and responding variably to

chemotherapeutic treatments. Under standard epithelial organoid culture conditions, stromal cell populations such as fibroblasts are generally depleted over serial passage, resulting in cultures enriched for epithelial tumor cells.(38) While traditional PDOs do not incorporate immune cells, recent advances in co-culture systems with PDOs, immune cells, and cancer-associated fibroblasts (CAF) help to fill this gap.(39) Our model is currently simplified as a proof of concept, including solely PDAC cells, but future models could incorporate co-cultures or additional ECM components.

Methods:

Cell culture (2D): Murine KPC-mT3 cells were a gift from D. Tuveson (Cold Spring Harbor Laboratory). GemR KPC-mT3 lines were generated in our lab by long-term culture in increased concentrations of gemcitabine. KPC-mT3 cells were cultured in DMEM (Gibco) with 10% fetal bovine serum (Corning).

Human PDAC organoids UCI-PDA-PDO1 were generated by our lab and cultured as previously described.(4) Briefly, de-identified post-pathology tissue from distal pancreatectomies from the University of California Irvine Medical Center was minced into small (~1mm) pieces using a sterile scalpel. These pieces were then digested with Accutase (Gibco) to enzymatically digest these tissue fragments into single cells at 37 °C for 40 minutes. Dissociated cells were seeded in 50 µL domes of BME and cultured in WNT3A/RSPO1-conditioned organoid culture medium with B27 (Thermo Fisher), Nicotinamide (Sigma-Aldrich), N-acetylcysteine (Sigma-Aldrich), Primocin (InvivoGen), mouse Noggin (Peprotech), human EGF (Peprotech), human FGF-10 (Peprotech), human Gastrin I (Tocris), and A83-01 (Tocris) at 37 °C with 5% CO₂.

Cell encapsulation: Prior to cell encapsulation, Cultrex BME, type R1 (bio-technique) was thawed to a liquid at 4°C on ice overnight. Cells at least at 80% confluency were detached using 0.25% Trypsin-EDTA and then resuspended in DMEM with 10% FBS and 1% penicillin-streptomycin (pen-strep) (Gibco). Miniaturized and standard cultures were prepared in parallel from the same suspension of cells in BME. Cells were suspended in Cultrex BME at 400,000 cells/mL, corresponding to 200 cells per 0.5 µL or 20,000 cells per 50 µL. The total cell and BME volume was composed of at least 70% BME for reliable droplet generation and optimal stability of the gelled phase. To create standard organoid cultures, 50 µL of the BME and cell solution was pipetted into a 24-well plate (6.12% CV for n = 24 domes).

Droplet generation: To produce consistent miniaturized organoids, we established a system using a 3D-printed T-junction microfluidic device.(40) First, a T-junction chip was printed with a S240 stereolithography printer in HTL resin (Boston Micro Fabrication) (Fig. S1A). Two sterile 21-gauge 1" needles (B-D) were securely and tightly inserted into the ends of short (approximately 15 cm) pieces of PTFE Masterflex® Transfer Tubing (avantor) with 1/32" inner diameter and 1/16 " outer diameter. The free ends of the respective tubing segments were pinched by the ETFE ferrules (Cole-Parmer) and inserted into the coned and threaded IDEX NanoPort fittings (Cole-Parmer). A much longer (5 m) piece of the PTFE tubing served as the outlet tube for the droplets produced in the main channel, and was similarly connected to an IDEX NanoPort fitting with an ETFE ferrule. The fittings threaded into the IDEX NanoPort Bodies

superglued over the inlets of the T-junction microfluidic chip, connecting the tubing segments to the channels.

Cell suspensions were loaded into a 3 mL syringe (B-D) with a blunt 18-gauge 1" needle (DWK Life Sciences). Similarly, a 20 mL syringe with a blunt 18-gauge needle was filled with 5 mL of 3M Novec HFE 7500 Engineered Fluid (Applied Thermal Fluids), used as the continuous phase. The blunt needles were removed, and the 3 mL and 20 mL syringes attached to 21-gauge needles inserted in the short tubing segments connected to the dispersed and main channels, respectively.

The 2 syringes were secured into syringe pumps (New Era Pump Systems, Wantagh, NY) and flow rates set to 0.3 mL/min for the HFE 7500, and 0.023 mL/min for the BME and cells. This flow ratio generated monodisperse droplets approximately 0.5 μ L in volume (average volume of 494.22 nL with CV 11.95%, n=40). Droplet sizes were reported in volumes instead of diameters since gelation occurred before droplets exit the outlet tubing, with droplets forming ellipsoids as opposed to spheres. To calculate volumes, the lengths and widths of gelled BME droplets were measured on ImageJ, and the height of the droplets was estimated as the inner diameter of the outlet tube (1/32"). An ice pack was kept around the syringe with BME and cells in order to avoid premature gelation as droplets were produced at room temperature. Once the outlet tubing was filled with uniform droplets, both ends of the tubing were inserted into blocks of PDMS with small holes punched by 1.5 mm biopsy punches. These blocks of PDMS ensured that droplets remained evenly spaced inside the tubing, avoiding fusion.

Droplet plating: To initiate BME gelation, the outlet tube, filled with cell-laden droplets and plugged with PDMS blocks, was sprayed generously with 70% ethanol and incubated at 37°C and 5% CO₂ for 30-40 minutes. Following incubation, one PDMS plug was removed from the outlet tube, and the remaining plug held above the well plate inside the biosafety cabinet. The outlet tube was carefully maneuvered manually between wells, allowing just 1 gelated droplet to directly drop into each well assisted by gravity. Once 10 wells in a row were filled, the PDMS plug was reattached to the tubing. With droplets adhering to the circumference of wells, the HFE 7500 in each well was aspirated. Once demulsified, these 10 wells were treated, as described below. The next set of wells was filled in the same manner, until all droplets were dispensed. Wells that were plated improperly (no droplet, multiple droplets) or demulsified improperly (droplet aspirated fully or partially) were marked as outliers and excluded from the data analysis.

Drug treatment: Drug solutions were created via two-fold serial dilutions from 10 mM stock solutions. Oxaliplatin was dissolved in ddH₂O, while gemcitabine, 5-fluorouracil, irinotecan, and paclitaxel were dissolved in DMSO. For standard culture treatments, following the 10-minute gelation incubation period, 500 μ L of the appropriate dosage of drug solution was added to each well. Control wells received 500 μ L of DMEM with 10% FBS and 1% pen-strep. Plates were incubated for 4 days. Standard organoids were cultured and assayed in white opaque 24-well plates to minimize signal crosstalk. Miniaturized organoids were cultured and assayed in white 96-well plates with clear bottoms, as opaque white 96-well plates were not available. A white index card was taped underneath the bottom of each plate to minimize crosstalk. For

miniaturized culture treatments, following demulsification, 100 μ L of the appropriate dosage was added to each well. Control wells received 100 μ L of DMEM with 10% FBS and 1% pen-strep, and plates were incubated for 4 days. Brightfield images at 10x objective captured organoid growth on days 0 and 4.

Dose Response Cell Viability Assay: CellTiter-Glo 3D reagent was diluted in an equivalent volume of phosphate buffered saline (PBS) (Gibco); the total volume per well was composed of $\frac{1}{2}$ drug solution and $\frac{1}{2}$ diluted CellTiter-Glo 3D reagent. In the 96 well plates used for miniaturized organoid cultures, the total well volume was 200 μ L, with 100 μ L drug solution and 100 μ L of diluted CellTiter-Glo 3D reagent. For wells with standard organoid cultures in 24 well plates, the 500 μ L of drug solution was first aspirated and replaced with 250 μ L of DMEM with 10% FBS. Then, 250 μ L of diluted reagent was added to each well. Plates were equilibrated to room temperature (22–25°C) for 30 minutes. Contents of the wells were mixed vigorously at 50 rpm on a MaxQ 4450 Digital Orbital Incubator Shaker (Thermo Fisher) for 5 minutes to induce cell lysis, and plates were incubated for 25 minutes at room temperature to stabilize the luminescent signal. Luminescence was read by a Varioskan Lux plate reader (Thermo Fisher). Relative viability at each dosage was calculated by dividing RLU at respective dosage points by the average RLU of the control wells. The inhibitory dose response curves were fit to three-parameter nonlinear regression functions with GraphPad Prism v9, and used to generate the dose response curves in Figures 2, 3, and 4 of this article as well as Supplementary Figures 2 and 3. The inhibitory dose response curve was fit to a three-parameter nonlinear regression function, and AUC was estimated with the trapezoidal rule using GraphPad Prism v9 (GraphPad Software, La Jolla, CA, US).

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Author Contributions:

The investigation was conducted by A.N. and A.K.M. The original draft was written by A.N., C.J.H., and E.E.H, and was reviewed and edited by A.N., A.K.M, C.J.H., and E.E.H. Methodology was developed by A.N., A.K.M., C.J.H., and E.E.H. Visualization and formal analysis were done by A.N., C.J.H., and E.E.H. A.N. curated data and performed validation. Conceptualization, software, resources, supervision, funding acquisition, and project administration were provided by C.J.H. and E.E.H.

Conflicts of Interest:

There are no conflicts to declare.

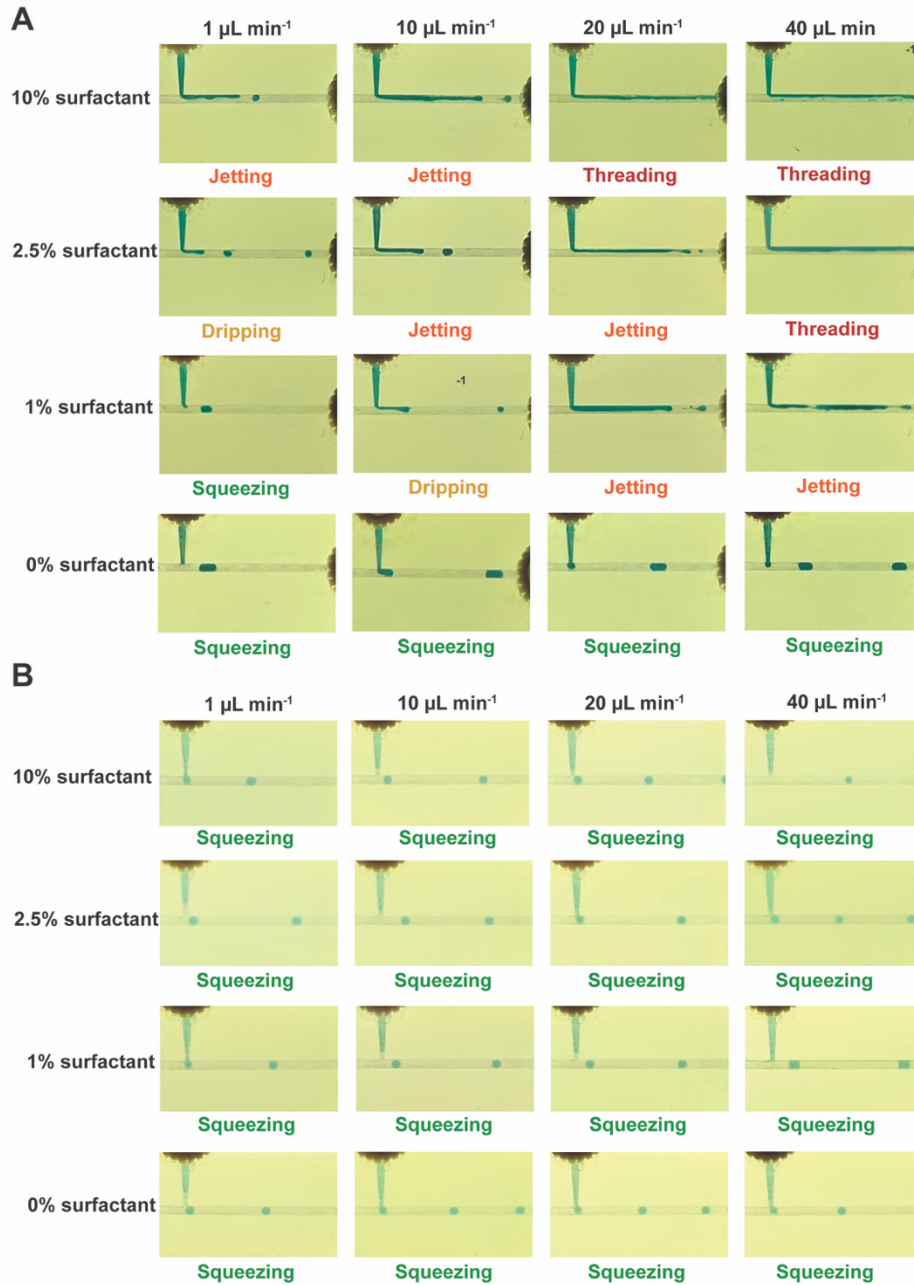


Figure 1. Droplet generation as a function of surfactant concentration and flow velocity. (A) Cultrex BME droplet generation is highly dependent on surfactant concentration and flow velocity. Dispersed phase flow rates are given, and a 1:10 flow ratio of dispersed phase to continuous phase was employed in all cases. (B) Water droplet generation remains constant across a range of surfactant concentrations and flow velocities.

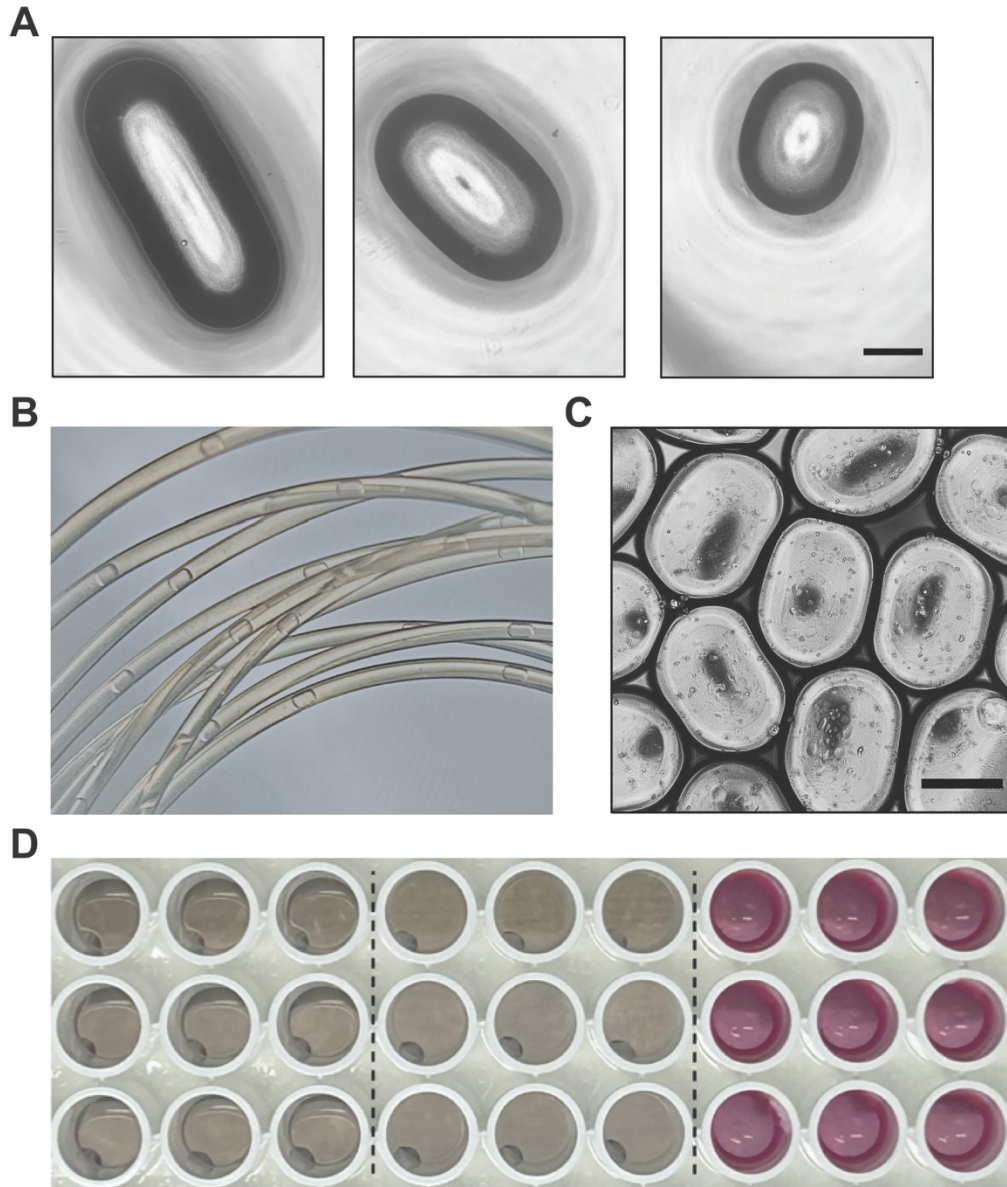


Figure 2. *Preparation of microencapsulated organoids and assays.*

A. Droplet size varies as a function of the dispersed:continuous flow ratio: 1:5, 1:10, or 1:15 (left to right). Scale bar: 200 μm . B. 0.5 μL BME droplets collected in tubing and separated by oil. C. Aggregated BME droplets after gelation. Scale bar: 500 μm . D. BME droplets individually dispensed into 96-well plate (left), after oil removal (middle), and after addition of culture medium containing drug dosages.

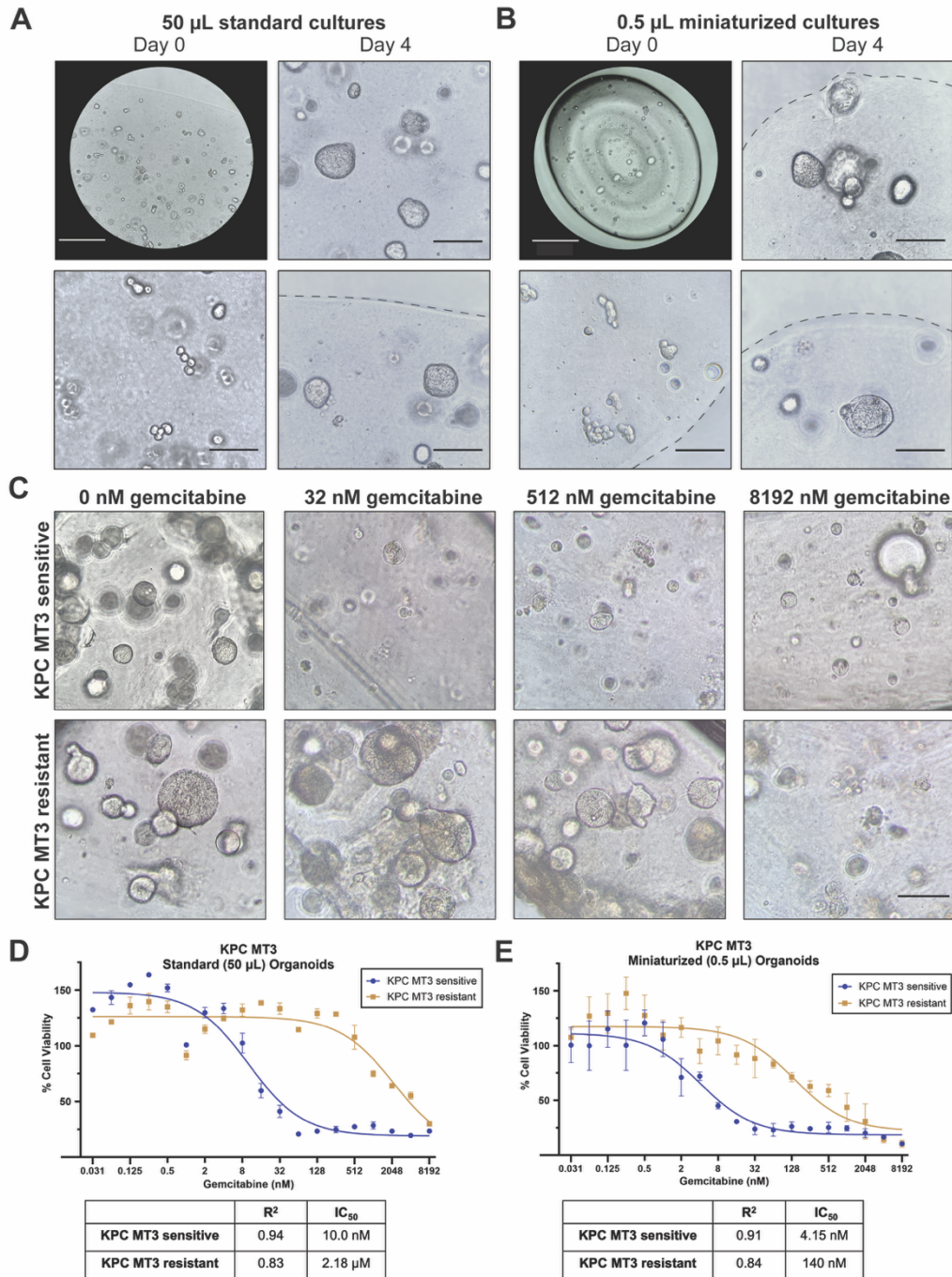


Figure 3. Microencapsulated mouse organoids maintain predictive value of standard cultures.
A. Brightfield images (20x and 10x (top left only) objectives) of untreated 50 μ L standard cultures with KPC MT3 sensitive organoids. White scale bar: 360 μ m. Black scale bar: 100 μ m. B. Brightfield images (20x and 10x (top left only) objectives) of untreated 0.5 μ L miniaturized cultures with KPC MT3 sensitive organoids. White scale bar: 360 μ m. Black scale bar: 100 μ m. C. Brightfield images (10x objective) of 0.5 μ L miniaturized cultures with KPC MT3 sensitive and resistant organoids on day 4 of culture at various gemcitabine concentrations. Scale bar: 100 μ m. D. Dose response of KPC MT3 organoids after 4 days of treatment in 50 μ L standard cultures (n=2 at each dosage). E. Dose response of KPC MT3 organoids after 4 days of treatment in 0.5 μ L miniaturized cultures (n=3 at each dosage).

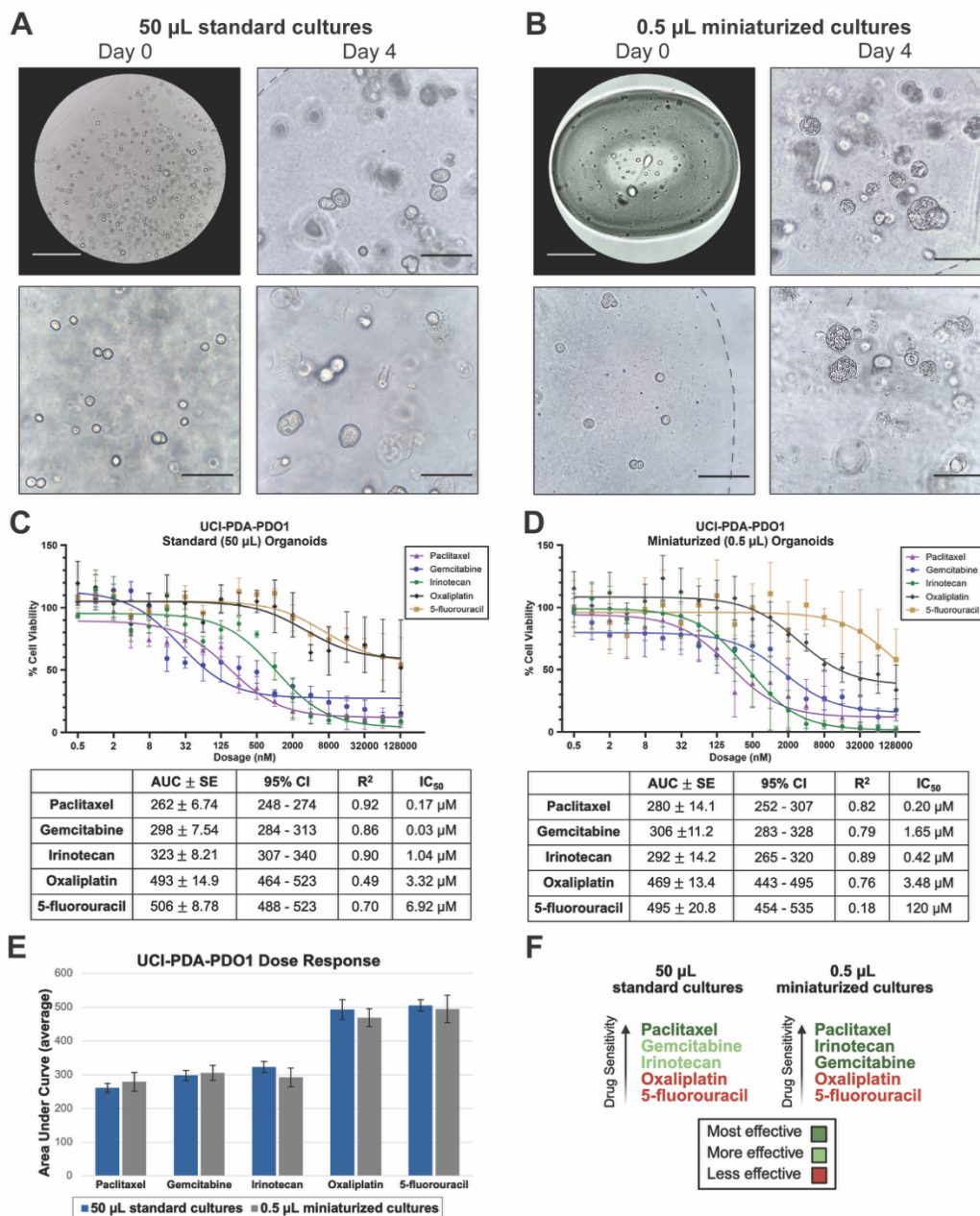


Figure 4. Stratification by chemotherapeutic efficacy is preserved across organoid culture volumes. A. Brightfield images (20x objective) of untreated standard 50 μ L cultures with UCI-PDA-PDO1 organoids. White scale bar: 360 μ m. Black scale bar: 100 μ m. B. Brightfield images (20x objective) of untreated 0.5 μ L miniaturized cultures with UCI-PDA-PDO1 organoids. White scale bar: 360 μ m. Black scale bar: 100 μ m. C. Dose response of UCI-PDA-PDO1 organoids after 4 days of treatment in standard cultures. Average viability plotted for each dosage from 2 experiments with n=2 replicates in each experiment. D. Dose response of UCI-PDA-PDO1 organoids after 4 days of treatment in miniaturized cultures. Average viability plotted for each dosage from 2 experiments with n=3 replicates in each experiment. E. Comparison of UCI-PDA-PDO1 organoid drug sensitivity via area under average dose-response curves, with 95% confidence intervals shown with error bars. F. Rank ordering of response to tested chemotherapeutics for UCI-PDA-PDO1 organoids in both culture formats.

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