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Article

"Organ-in-a-Column" Coupled On-line with Liquid Chromatography-Mass Spectrometry

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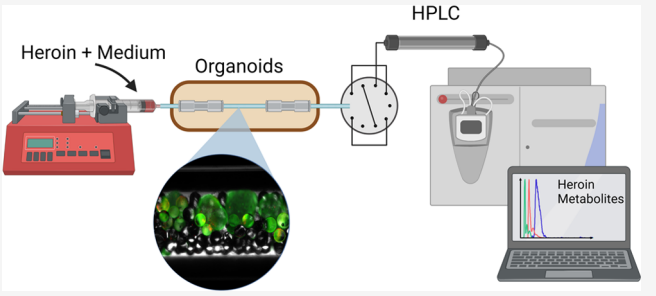
ABSTRACT: Organoids, i.e., laboratory-grown organ models developed from stem cells, are emerging tools for studying organ physiology, disease modeling, and drug development. On-line analysis of organoids with mass spectrometry would provide analytical versatility and automation. To achieve these features with robust hardware, we have loaded liquid chromatography column housings with induced pluripotent stem cell (iPSC) derived liver organoids and coupled the "organ-in-a-column" units on-line with liquid chromatography-mass spectrometry (LC-MS). Liver organoids were coloaded with glass beads to achieve an even distribution of organoids throughout the column while preventing clogging. The liver organoids were interrogated "on column" with heroin, followed by on-line monitoring of the drug's phase 1 metabolism. Enzymatic metabolism of heroin produced in the "organ-in-a-column" units was detected and monitored using a triple quadrupole MS instrument, serving as a proof-of-concept for on-line coupling of liver organoids and mass spectrometry. Taken together, the technology allows direct integration of liver organoids with LC-MS, allowing selective and automated tracking of drug metabolism over time.



Drug discovery and development is an extremely costly process, and the number of new drugs reaching the market per billion dollars spent on research and development is consistently low. Moreover, the efficacy/toxicity of drugs can vary significantly in target patients, calling for personalized drug testing.[1] Key bottlenecks of efficient drug development include the limited predictive value of traditional cell cultures and animal models for human drug metabolism and personalization of the model systems.[2] Hence, novel technologies for predicting personalized drug metabolism are being explored.

Organoids, here broadly defined as *in vitro* three-dimensional (3D) models that exhibit features of the mature organ in question, are rapidly emerging as powerful tools for drug discovery and personalized testing. Organoids can be readily derived from stem cells and carry the potential for serving as relevant and personalized testing materials.[3] Typically, organoids are 200−500 μm in size, consisting of organ-specific cell types.[4] For example, liver organoids can consist of hepatocytes, hepatic stellate cells, endothelial cells, and cholangiocytes and can be used as a tool for assessing aspects of drug metabolism and toxicity.[5,6]

For measuring small molecules such as drugs and their metabolites, liquid chromatography-mass spectrometry (LC-MS) is a method of choice in analytical chemistry. Mass spectrometric analysis of organoids has been performed indirectly ("off-line"), i.e., samples are collected and handled semi-manually prior to MS.[7−11] Off-line handling can be time consuming and prone to variations, depending on the method, sample size, and analyte stability. Direct on-line MS analysis of organoids would potentially offer the advantage of increased automation and possibly improved throughput. Verpoorte and co-workers have previously combined organs/organ models, chip microfluidics, and separation science for studying metabolism of liver slices[12] and pharmacology in a gut-on-chip.[13] We have recently coupled the liver organoids with sample preparation techniques, such as electromembrane extraction (EME),[14] which we also found to be compatible with on-line coupling of organoid-containing chips to LC-MS for studying organoid drug metabolism.[15] However, EME requires an electrical current driven transfer of metabolites through an oil membrane into an MS-compatible solution, which can potentially limit the spectrum of analytes that can be analyzed.[15] Additionally, a key challenge for coupling chips with

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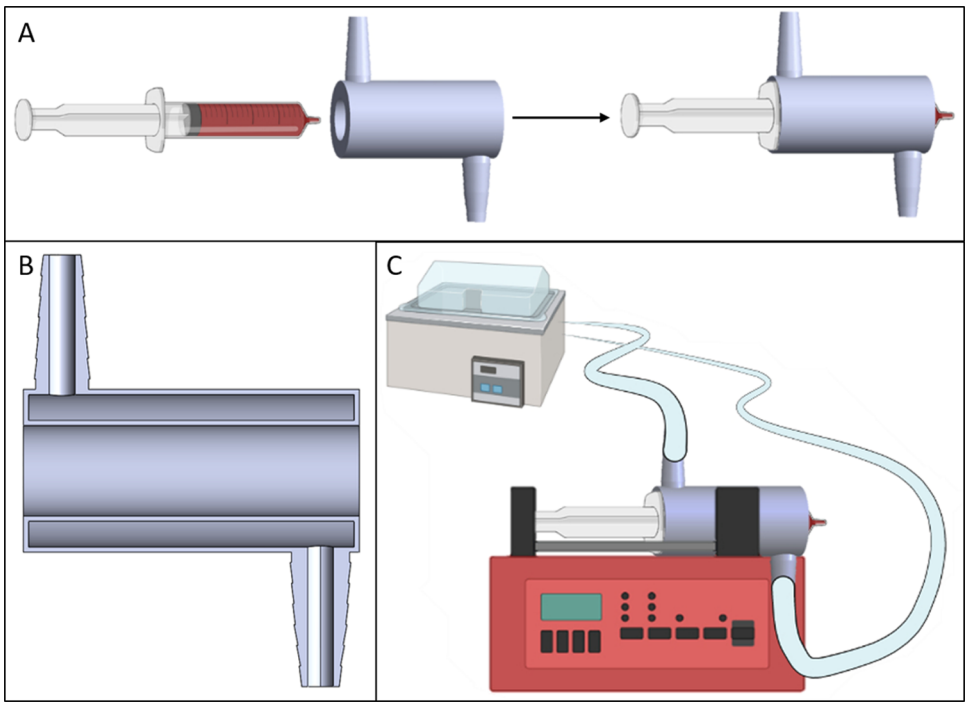

Figure 1. (A) 3D-printed syringe cooler was tailored to fit the syringe cylinder, ensuring a snug fit. 3−10 mL Luer lock syringes from B. Braun Omnifix were used. (B) Cross section of the syringe cooler's double-walled design. The wall thickness was 1 mm. The water chamber was 5 mm thick. (C) The syringe cooler was connected to a refrigerated circulation bath from Haake.

MS ensures practical and robust connections and standardization. Therefore, we have explored placing the liver organoids directly into standardized/commercial tubing and connectors of liquid chromatography (LC), perhaps the most applied fluidics platform in analytical chemistry. Specifically, LC column housings were loaded with organoids generated from iPSC-derived hepatocyte-like cells (iHLC organoids) and sandwiched between an upstream drug delivery system and a downstream connector to a traditional LC-MS setup. The system, termed "organ-in-a-column,"[16] allowed in-column cultivation of liver organoids for an extended period, "on-line" exposure to drugs, and monitoring of drug metabolism using mass spectrometry.

EXPERIMENTAL SECTION

Consumables and Basic Hardware. Stainless steel (SS) unions, reducing unions (1/16″ to 1/32″), SS ferrules and nuts (all for 1/16″ tubing and for 1/32″ tubing), SS tubing (1/32″ outer diameter (OD), 0.020″ inner diameter (ID), and 0.005″ ID), perfluoroalkoxy alkane (PFA) tubing (1/16″ OD, 0.75 mm ID), and 1/16″ SS screens (2 μm pores) were purchased from VICI Valco (Schenkon, Switzerland). SST Vipers (130 μm × 650 mm) were purchased from Thermo Fisher Scientific (Waltham, MA). A chromatographic column (1 mm × 5 cm) packed with Kromasil C4 (3.5 μm silica particles, 100 Å pore size) was purchased from Teknolab (Ski, Norway). Luer lock syringes (3−10 mL) were purchased from B. Braun Melsungen AG (Hessen, Germany). Acid-washed glass beads (150−212 μm) were purchased from Sigma-Aldrich (St. Louis, MO).

Reagents and Solutions. Formic acid (FA, ≥98%) was purchased from Merck (Darmstadt, Germany). Water (LC-MS grade) and acetonitrile (ACN, LC-MS grade) were purchased from VWR International (Oslo, Norway). Tough 1500 3D-printer resin was purchased from Formlabs Inc. (Somerville, MA). For liquid chromatography, mobile phase (MP) reservoir A contained 0.1% FA in HPLC water (v/v), and MP reservoir B contained ACN/HPLC water/FA (90/10/0.1%, v/v/v).

Heroin HCl, 6-acetylmorphine HCl, and morphine were obtained from Lipomed AG (Arlesheim, Switzerland). Heroin-d9, 6-acetylmorphine -d6, and morphine-d3 (used for heroin stability experiments only) were purchased from Cerilliant (Austin, TX). Fetal bovine serum-free medium (William's E medium, supplemented with 0.1 μM dexamethasone and 1% insulin−transferrin−selenium mix) and L15 base medium (prepared according to ref 17) are hereafter referred to as organoid medium.

Organoids and Spheroids. iHLC organoids originating from three cell lines (iHLC-1 = WTC-11, iHLC-2 = WTSIi013-A, and iHLC-3 = WTSIi028-A, Wellcome Trust Sanger Institute) were differentiated toward liver organoids using a modification of the protocol by Ang et al.[18] iPSC line AG27 was differentiated to form liver organoids containing induced hepatocyte-like cells (iHLCs) as described by Harrison et al.[11] and was used in initial experiments (Figures 4A,B and SI1).

Cryopreserved primary human hepatocytes (PHH, Gibco, catalogue no. HMCPMS, lot HU8287) were thawed in hepatocyte thaw media (Gibco, catalogue no. CM7500), according to the manufacturer's protocol. Uniform PHH spheroids were created by aggregation in house-made agarose microwells, as described before,[19] and cultured in Williams E medium (Thermo Fisher Scientific, catalogue no. A1217601) supplemented with 0.5% FBS (Thermo Fisher Scientific, catalogue no. 41400045), 2 mM L-glutamine (Thermo Fisher Scientific, catalogue no. 35050038), 10 μg/mL insulin, 5.5 μg/mL transferrin, 6.7 ng/mL sodium selenite (Thermo Fisher Scientific, catalogue no. 41400045), and 0.1 μM dexamethasone (Sigma-Aldrich, catalogue no. D4902).

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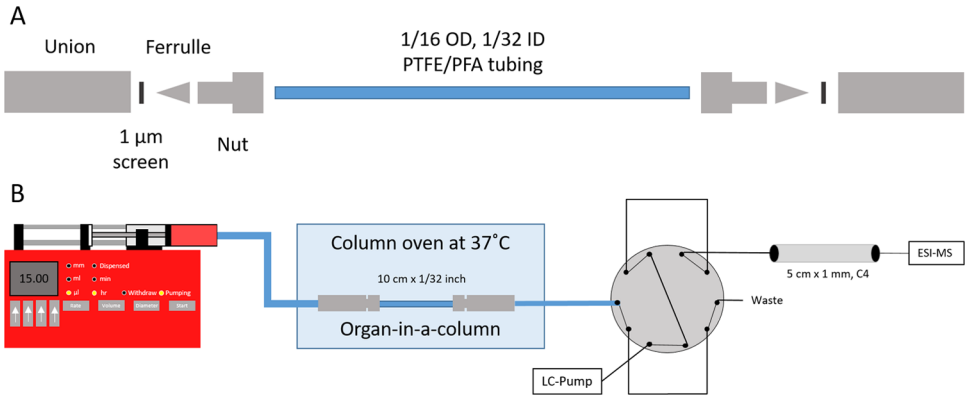

Figure 2. (A) Column housing for "organ-in-a-column". (B) Illustration of "organ-in-a-column" coupled on-line with liquid chromatography-mass spectrometry (see the Experimental Section for more details).

Instruments and Advanced Hardware. The Dionex UltiMate 3000 UHPLC system and the TSQ Vantage MS with the HESI-II ion source were purchased from Thermo Fisher Scientific. A syringe pump (AL-1000) was bought from World Precision Instruments (Sarasota, FL). A 2-position 10-port valve (for 1/32″, C82X-6670ED) was purchased from VICI Valco. A SUB Aqua 5 Plus water bath was purchased from Grant Instruments (Cambridge, U.K.). A Form 3B 3D printer and wash and cure station were purchased from Formlabs Inc (Somerville, MA). A PST-BPH-15 column heater was purchased from MS Wil (Aarle-Rixtel, the Netherlands). A refrigerated circulating water bath was purchased from Haake (Berlin, Germany).

Heroin Stability Testing. Stability testing of heroin was performed by incubating solutions of L15 base medium, serum-free organoid medium, and type 1 water. The incubation was performed at 4 and 37 °C. For each solution, 100 μL of freshly made 1 mM heroin (in 0.9% NaCl) or 0.9% NaCl (control) and 900 μL organoid medium were mixed. At 0, 24, 48, and 120 h, 100 μL of samples were collected from all solutions. To precipitate proteins, 10 μL of 1.1 M FA was added to each sample, followed by vortexing and 2 min centrifugation at 14,500*g*. Fifty microliters of the resulting supernatant was transferred to a new vial and diluted to 1 mL with type 1 water. Samples were stored at −80 °C prior to analysis. For these experiments, the determination of heroin, 6-acetylmorphine, and morphine was performed using UHPLC-MS as previously described by Skottvoll et al.[14]

3D-Printed Syringe Cooler. A 3D-printed double-wall syringe cooler was printed and fitted directly to a syringe cylinder (Figure 1A). 3D-printed syringe coolers were designed using SOLIDWORKS CAD software (3DS, Paris, France). Syringe coolers were printed in Tough 1500 resin with the Form 3B 3D printer. A cross section of the double-walled design is shown in Figure 1B. The wall thickness in the main body was 1 mm. The flow-through part of the body was 5 mm thick, and the inner diameter was adapted to fit the individual syringe. Note that the dimensions of e.g. a 3 mL syringe vary greatly between different manufacturers. Cold water (4 °C) was pumped through the cooler with a refrigerated circulating water bath (Figure 1C). To ensure a cold stable temperature from the start, the water bath, organoid medium, and heroin solutions were cooled prior to the start of the experiment. Due to the low flow rate (15 μL/h) and a small ID of the tubing (0.75 mm), the medium was quickly heated to physiological temperature upon introduction to the "organ-in-a-column," which was kept at 37 °C in the column heater (see also the Supporting Information for STL-file.)

"Organ-in-a-Column". For fabrication of the column housing, a 10 cm long piece of PFTE/PFA tubing (1/16″ OD, 0.75 mm ID) was cut and assembled with nuts and SS ferrules. To one end of the tube, a union with a 1 μm SS screen (VICI Valco) was connected. Organoid medium containing approximately 50 iHLC organoids (size range 100−200 μm) was then transferred to a 3 mL Luer lock syringe. Two spatulas of acid-washed glass beads, containing approximately 45 mg of beads, were subsequently added. Through gentle shaking, beads and organoids were mixed in the syringe. The syringe was then connected to the open end of the column. By pressing the contents of the syringe through the empty column housing, the organoid column was finalized. Once the entire contents of the syringe were passed through the column, the inlet was fitted with a SS screen and a union. A schematic of an "organ-in-a-column" is shown in Figure 2A. For idle conditions, a new syringe was filled with fresh organoid medium, connected to the "organ-in-a-column", and placed in a syringe pump. The pump was set to 15.0 μL/h. After filling, the "organ-in-a-column" was kept in the column heater at 37 °C.

"Organ-in-a-Column" Coupled with Liquid Chromatography-Mass Spectrometry. The pump/syringe system described above was connected to LC-MS instrumentation. Eluate from the "organ-in-a-column" was transported to a valve system for fractionation. The valve system contained two sample loops (5 μL), which were filled sequentially. As one loop was being filled, the content of the other loop was pumped to a 5 cm × 1 mm C4 LC column for separation prior to MS detection. The 5 μL loops were overfilled with an additional 2.5 μL to ensure that any LC solvent left in the loop was flushed off and to accommodate possible small fluctuations of syringe pump flow rate (see Figure 2B for a schematic of the setup). For studying heroin metabolism, a fresh stock solution of 1 mM heroin HCl in 0.9% NaCl was prepared prior to each experiment and diluted with organoid medium to 10 μM. The organoid medium/heroin solutions were delivered constantly at 15.0 μL/h by the syringe pump, with fractionation every 30 min. This allowed for 5 μL injections onto the LC-MS system, with an overfilling of a factor 1.5 (=7.5 μL, delivered in 30 min at a 15 μL/h flow rate) to ensure proper loop filling (see Tables 1−3 for LC-MS conditions (C4 1 mm ID separation column, flow rate 50 μL/min)).

Proteomic Analysis of iHLCs and PHHs Using Liquid Chromatography-Tandem Mass Spectrometry. Heroin-

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Table 1. LC Gradient for On-line Studies of "Organ-in-a-Column" Metabolism of Heroin

time (min)	flow (μL/min)	mobile phase (%B)	purpose
0−2	50	3	separation
2−10	50	3−20	
10−20	50	80	wash
20−30	50	3	re-equilibration

Table 2. Multiple Reaction Monitoring (MRM) Parameters Used for the Detection of Heroin and Its Phase 1 Metabolites

analyte	parent ion (*m*/*z*)	product ion (*m*/*z*)	collision energy (eV)	S-lens value
heroin	370.15	286.04	42	104
		210.96	59	104
6-acetylmorphine	328.13	210.96	6	72
		180.97	37	72
morphine	286.14	200.99	5	88
		184.91	48	88

Table 3. General MS Parameters for Detection of Heroin, 6-Acetylmorphine, and Morphine

parameter	value
capillary temperature	300.0 °C
vaporizer temperature	200.0 °C
sheath gas pressure	20.0
ion sweep gas pressure	0.0
auxillary air flow	5.0
spray voltage	Pos: 3000.0 V Neg: 0.0 V
collision gas pressure	1.0 mTorr

treated (harvested after 24 h) iHLC organoids generated from three cell lines (iHLC-1 = WTC-11 (WiCell), iHLC-2 = WTSIi013-A, and iHLC-3 = WTSIi028-A, Wellcome Trust Sanger Institute) and 3D spheroids generated from cryopreserved primary human hepatocytes (PHHs, Gibco, lot HU8287) were prepared in two replicates in addition to controls (untreated iHLCs and PHHs, $n = 1$). Pelleted iHLC organoids and PHH spheroids were prepared by Easy Extraction and Digestion (SPEED),[20] using dithiothreitol (DTT) and iodoacetamide (IAM) for reduction and alkylation, respectively, and 6 μg of trypsin for digestion (overnight at 37 °C). Sample clean-up (after concentration and reconstitution of samples in 100 μL of LC-MS grade water containing 0.25% (v/v) heptafluorobutyric acid) was performed using 100 μL Bond Elut C18 solid-phase extraction pipet tips (Agilent, Santa Clara), following the protocol of the manufacturer. The eluate was concentrated to dryness and dissolved in 4 μL of LC-MS grade water containing 0.1% (v/v) FA.

LC-MS analysis was performed using a timsTOF Pro (Bruker Daltonics, Bremen, Germany) coupled to a nanoElute nanoflow LC system (Bruker Daltonics). Separation was performed with 25 cm × 75 μm, 1.6 μm silica particles, C18, Ion Opticks (Fitzroy, Australia) column operated at 50 °C. Mobile phase A and B reservoirs contained LC-MS grade water and acetonitrile, respectively, both containing 0.1% (v/v) formic acid. A linear gradient from 0−35% B (54 min) was employed (300 nL/min flow rate). MS acquisition was performed in data-dependent acquisition parallel accumulation-serial fragmentation mode, and an injection volume of 2 μL was employed. Data were searched against the human UniProt database (20,431 entries), with PEAKS X+ software version 10.5 (Bioinformatics Solutions), allowing one missed cleavage and a false discovery rate of 1%.

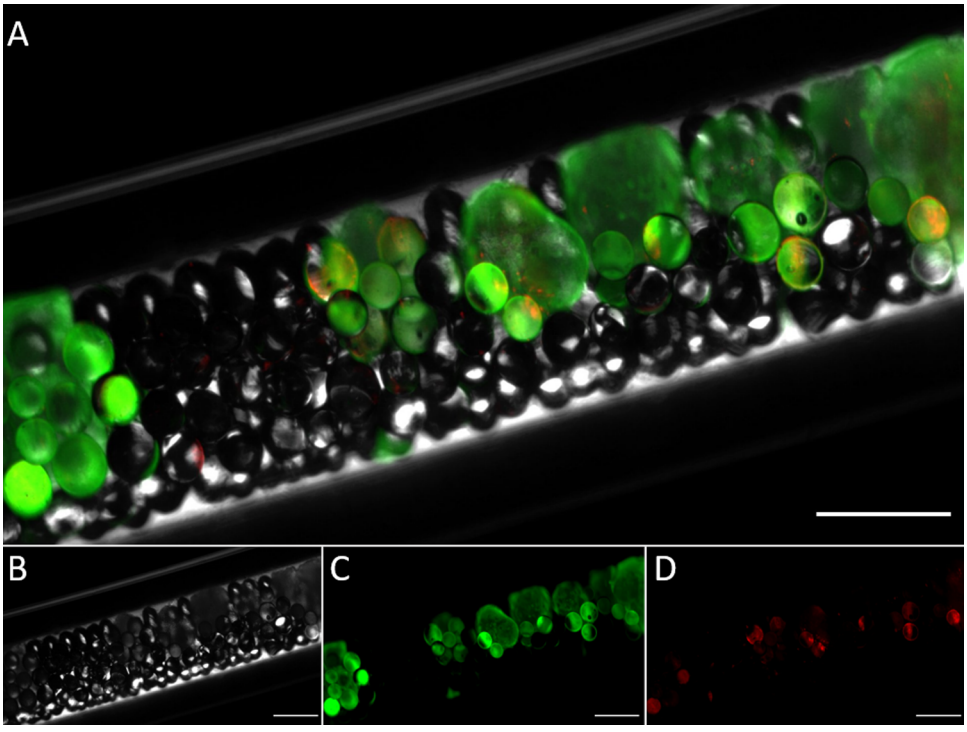

Figure 3. Fluorescence microscopy image of iHLC organoids loaded with glass beads in an "organ-in-a-column". Viable cells were stained with green fluorescent calcein-AM. Dead cells were stained with propidium iodide (red). Brightfield (B), green (C), and red (D) channels are shown below the merge (A). Scale bar is 600 μm.

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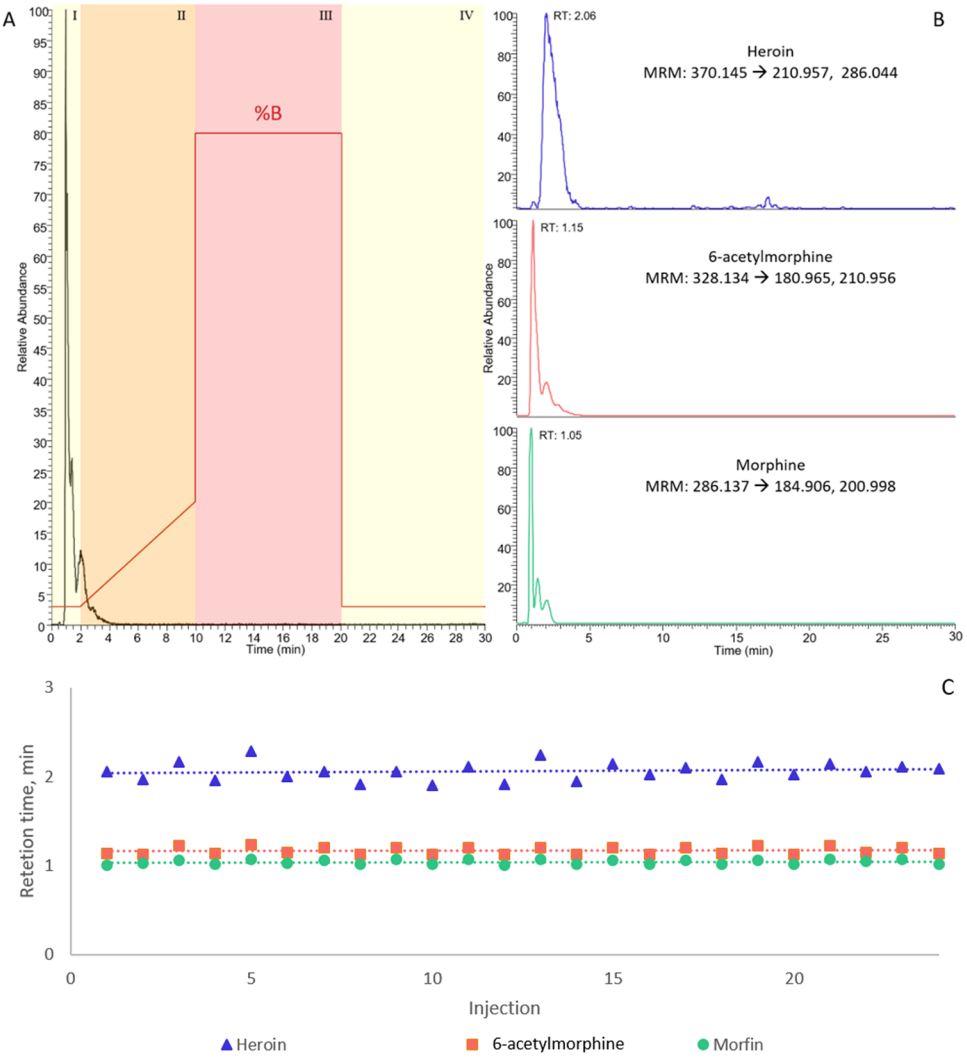

Figure 4. (A) LC-MS chromatogram of heroin and metabolites derived from an "organ-in-a-column" system exposed to heroin coupled on-line with liquid chromatography-mass spectrometry. The gradient program as a function of organic mobile phase modifier (%B) is shown in red. The four stages of the gradient program are (I) isocratic elution, (II) gradient elution, (III) wash, and (IV) re-equilibration. (B) Extracted ion chromatograms for heroin and metabolites are shown with MRM transitions that were used. The chromatograms were extracted from the same run that is shown in (A). (C) Retention time variability of heroin and metabolites over an entire exposure experiment (24 injections/12 h).

Measurement of Enzyme Expression in iHLC Organoids and PHH Spheroids. RNA was isolated using TRIzol reagent (Thermo Fisher Scientific), according to the manufacturer's protocol. RNA concentration and purity were analyzed using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific). cDNA was synthesized using a high-capacity cDNA reverse transcription kit (Thermo Fisher Scientific, catalogue no. 4368814). Gene expression analysis was performed using a TaqMan Universal mix on a TaqMan ViiA7 real-time PCR system. Petidylpropyl isomerase A was used as endogenous control. The level of expression of genes of interest was quantified by ddCt with normalization to control (vehicle-treated organoids).

RESULTS AND DISCUSSION

Liver organoids were generated from four induced pluripotent stem cell (iPSC) lines and benchmarked to primary human hepatocytes, grown as spheroids. In support of expected liver functionality, enzymes related to the metabolism of heroin (CES1 and CES2) were analyzed and identified by rtPCR and proteomic analysis in iHLC-1−3 liver organoids (see Supporting Information, SI4-SI5).

Liver Organoid Charging of an LC-Column Structure. LC column fittings are specifically designed for leakage-free and simple packing and come in a variety of diameters and lengths, with readily available fittings. Hence, we aimed to investigate whether LC columns could be directly loaded with liver organoids and whether the liver organoids can be grown for an extended period in the columns without losing viability. LC columns have previously been demonstrated as useful housings for studying biological interactions, e.g., Wiedmer and co-workers' studies of drug interactions with in-column liposomes.[21] Liver organoids were used for packing LC column housings: iHLC organoids from four different iPSC cell lines.

For liquid chromatography column housings, a micro LC format was chosen (0.75 mm ID and 10 cm length). Both PFA tubing and PTFE tubing have been used instead of regular steel column housings as their optical properties allow for visual inspection of organoids in situ (see Figure 3 of stained organoids in the column). To prevent clogging, organoid medium containing liver organoids was supplemented with glass beads

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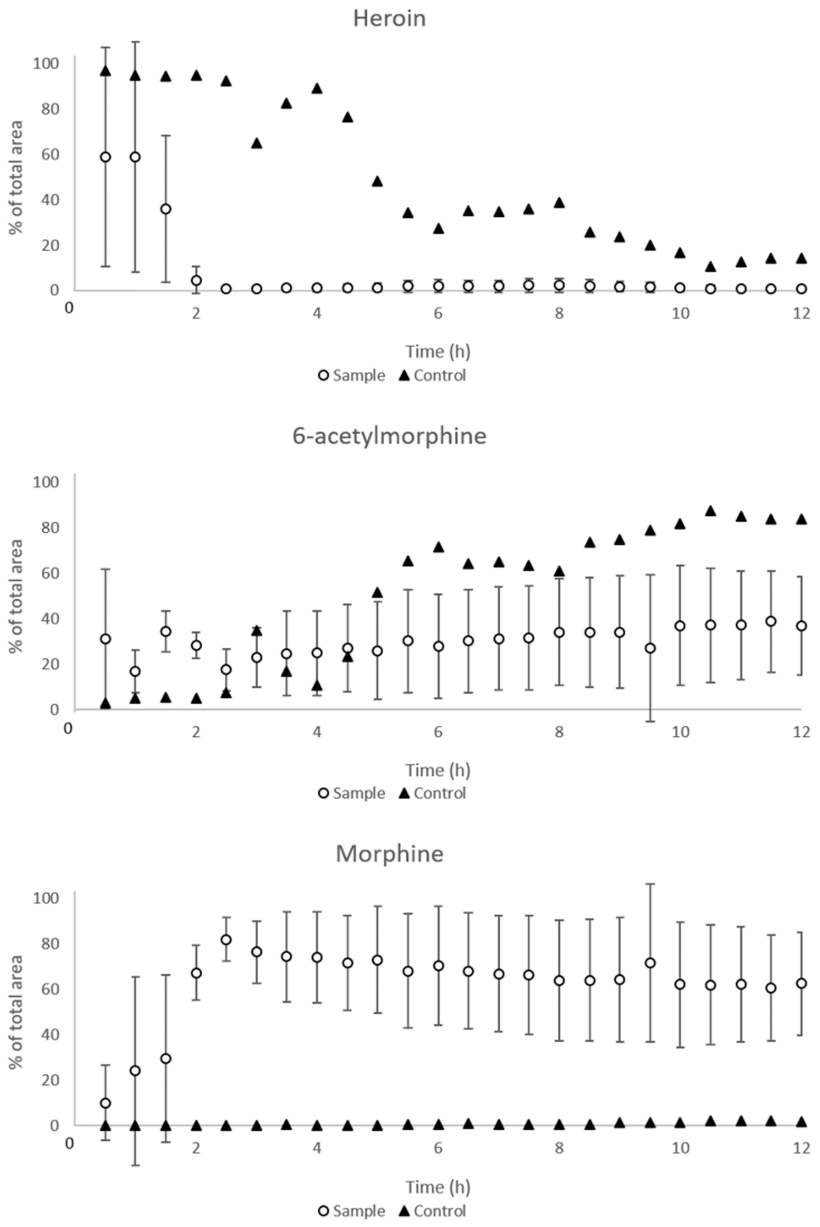

Figure 5. On-line enzymatic and nonenzymatic conversion of heroin (10 μM) to 6-acetylmorphine and morphine in columns containing approximately 50 iHLC organoids (sample) and columns containing no organoids (control). Graphs show average areas of heroin and metabolites normalized to the average total area of heroin, 6-acetylmorphine, and morphine (area-% of avg. total area of heroin, 6-acetylmorphine, and morphine). The average total areas of heroin, 6-acetylmorphine, and morphine are based on three runs performed on different days (12 h experiments) and columns with organoids from the WTC-11 cell line (error bars: standard deviation, n = 3).

(45 mg beads per 10 cm column/50 organoids) prior to packing. After gently mixing the organoid/glass bead containing the organoid medium solution, columns were filled manually with a syringe into an open LC column housing. Postfilling, the column was coupled to the upstream and downstream hardware. The addition of the beads kept the liver organoids well-spaced throughout the column, significantly reducing clogging and increasing the robustness of the system.

After on-line culture and measurements (see below), liver organoids could be readily flushed from the column by removing one of the columns' unions and applying mild pressure with a handheld syringe. Preloading and postflushing inspection of the organoids by microscopy revealed that >80% of the organoids showed substantial amounts of live staining after 7 days within the perfused column in both the absence and presence of heroin exposure (see Supporting Information, SI1 for examples).

Coupling of the Liver Organoid-Loaded "Organ-in-a-Column" to an LC-MS System. The "organ-in-a-column" containing iHLCs was coupled to high-pressure LC through a fractionation valve setup. LC-MS is generally operated at high pressures e.g., 50−400 bars, which is incompatible with organoid culture. A 2-position 10-port stainless steel valve was used to collect and pump liquid fractions to the LC-MS system, not unlike that used for two-dimensional LC separations.[22] The valve system setup efficiently isolated the organoids from nonbiocompatible solvents and high pressure of the analysis system (see Figure 2B).

Organoid medium is complex and can contain considerable amounts of proteins such as albumin. Sample complexity and the

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presence of proteins can cause unpredictable chromatographic performance. A short-chained butyl (C4) stationary phase was thought to be necessary regarding robustness for 100s of injections of protein-rich organoid medium. The column (considered relatively compatible with proteins) allowed for repeatable chromatography of organoid medium spiked with model substance heroin/metabolites at this stage of the project (see Figure 4A). Mass spectrometric detection was performed in multiple reaction monitoring (MRM) mode, which allowed highly selective and sensitive detection of small molecules, such as heroin and its metabolites 6-acetylmorphine and morphine (Figure 4B, from initial experiments with AG27-derived organoids). The mobile phase composition was also a key parameter regarding robustness; methanol as an organic modifier was associated with column clogging and poor performance when chromatographing the organoid medium, while acetonitrile provided significantly improved performance (see Figure 4C for illustration of the retention time repeatability (RSD between 2 and 5%) for the system).

Temperature Controlled Drug Delivery Ensures Improved Robustness. Heroin can spontaneously decompose into its metabolite 6-acetylmorphine. However, we found that heroin could also be converted to morphine nonenzymatically. At 37 °C, approximately 5% conversion of heroin to morphine was observed after 24 h and 20% over 120 h (see Supporting Information SI2). Hence, at physiological temperatures required for metabolic functional cells, heroin can decompose to 6-acetylmorphine and morphine in the absence of liver organoids. However, when cooled to 4 °C, less than 1% morphine was formed in the absence of liver organoids (see Supporting Information SI2). To avoid the formation of morphine prior to organoid exposure, a 3D-printed syringe cooler was designed and implemented in the system (Figure 1).

"Organ-in-a-Column"-LC-MS Drug Metabolism Studies on iHLC. Our next step was to evaluate the system's functionality for tracking drugs and metabolites over time with cooled organoid medium supplemented with 10 μM heroin and iHLC organoids coloaded with glass beads. Figure 5 shows the degradation of heroin and the corresponding generation of metabolites 6-acetylmorphine and morphine for individual "organ-in-a-column" units, as documented by three experiments performed with iHLC organoids generated from the WTC-11 cell line, on different days and columns.

Controls (i.e., columns loaded with beads but not loaded with organoids) generated nondetectable amounts of morphine during the 12 h experiment, establishing that the precolumn cooling system efficiently prevented nonenzymatic degradation. As expected, a gradual conversion of heroin to 6-acetylmorphine was detected in columns without organoids. In contrast, in organoid-loaded columns, heroin levels decreased more rapidly, while morphine levels increased over time. The data presented in Figure 5 were generated over 12 h of continuous and fully automated/unsupervised analysis, suggesting that on-line coupling of organoids and MS via commercial LC hardware is feasible.

The patterns observed for heroin metabolism were similar to that obtained with standard, off-line methods for studying liver organoids,[11] i.e., a drop in heroin levels in parallel with morphine levels increasing and plateauing on an hour-scale basis, compared to substantially more rapid metabolism rates when employing microsomes and S9 fractions.[14]

Next, heroin metabolism was compared in columns using iHLC organoids independently differentiated from three iPSCs. As expected, the biological variation in these experiments resulted in larger standard deviation but still identified significant levels of morphine when compared to the control (n = 3) (see Supporting Information SI3).

CONCLUDING REMARKS

In this proof-of-concept study, liver organoids have been loaded in liquid chromatography column housings ("organ-in-a-column") and coupled on-line with mass spectrometry for direct analysis of drug metabolism. Features of the here described setting include a substantial degree of automation compared to our previous manual efforts,[11] selective measurements through multiple reaction monitoring, and an increased degree of robustness through the use of standard LC parts and fittings, compared to noncommercial chips previously employed.[15] The setup could be used for directly identifying liver organoid-induced drug metabolism and subsequent hour-scale monitoring of metabolism. Future qualitative identifications of metabolites will include further standardization of column packing and inclusion of internal standards to reduce ESI-MS signal variations.

The system will be further explored for additional drugs and configurations. Improvements will include an autosampler for multidrug analysis, a valve for diversion of salts, and on-line sample clean-up setups. This encourages the next steps to include expanded drug metabolism studies for mapping enzyme activity (e.g., drugs such as phenacetin, tolbutamide, and fluoxetine, metabolized by CYP2D6, CYP2C9, and CYP1A2, respectively), which can have clear benefits in e.g., personalized drug development when assessing organoids grown from individual patients/patient groups.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.2c04530.

Additional experimental details, materials, and methods, regarding live/dead staining (S1); heroin stability tests (S2); proteomics (S3); and mRNA studies (S4) (PDF)

3D-files (ZIP)

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Notes

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■ REFERENCES

(1) Olson, H.; Betton, G.; Robinson, D.; Thomas, K.; Monro, A.; Kolaja, G.; Lilly, P.; Sanders, J.; Sipes, G.; Bracken, W.; et al. *Regul. Toxicol. Pharmacol.* **2000**, *32*, 56−67.

(2) Skardal, A.; Shupe, T.; Atala, A. *Drug Discovery Today* **2016**, *21*, 1399−1411.

(3) Bhatia, S. N.; Ingber, D. E. *Nat. Biotechnol.* **2014**, *32*, 760−772.

(4) Lancaster, M. A.; Knoblich, J. A. *Science* **2014**, *345*, No. 1247125.

(5) Shinozawa, T.; Kimura, M.; Cai, Y.; Saiki, N.; Yoneyama, Y.; Ouchi, R.; Koike, H.; Maezawa, M.; Zhang, R.-R.; Dunn, A.; et al. *Gastroenterology* **2021**, *160*, 831−846.e810.

(6) Qosa, H.; Ribeiro, A. J.; Hartman, N. R.; Volpe, D. A. *J. Pharmacol. Toxicol. Methods* **2021**, *110*, No. 107083.

(7) Dorrell, C.; Tarlow, B.; Wang, Y.; Canaday, P. S.; Haft, A.; Schug, J.; Streeter, P. R.; Finegold, M. J.; Shenje, L. T.; Kaestner, K. H.; Grompe, M. *Stem Cell Res.* **2014**, *13*, 275−283.

(8) Tauro, B. J.; Greening, D. W.; Mathias, R. A.; Mathivanan, S.; Ji, H.; Simpson, R. J. *Mol. Cell. Proteomics* **2013**, *12*, 587−598.

(9) Boj, S. F.; Hwang, C.-I.; Baker, L. A.; Chio, I. I. C.; Engle, D. D.; Corbo, V.; Jager, M.; Ponz-Sarvise, M.; Tiriac, H.; Spector, M. S.; et al. *Cell* **2015**, *160*, 324−338.

(10) Lin, A.; Sved Skottvoll, F.; Rayner, S.; Pedersen-Bjergaard, S.; Sullivan, G.; Krauss, S.; Ray Wilson, S.; Harrison, S. *Electrophoresis* **2020**, *41*, 56−64.

(11) Harrison, S. P.; Siller, R.; Tanaka, Y.; Xiang, Y.; Patterson, B.; Kempf, H.; Melum, E.; Åsrud, K. S.; Chollet, M. E.; Andersen, E.; et al. *bioRxiv* **2020**, DOI: 10.1101/2020.12.02.406835.

(12) van Midwoud, P. M.; Janssen, J.; Merema, M. T.; de Graaf, I. A.; Groothuis, G. M.; Verpoorte, E. *Anal. Chem.* **2011**, *83*, 84−91.

(13) De Haan, P.; Santbergen, M. J.; van der Zande, M.; Bouwmeester, H.; Nielen, M. W.; Verpoorte, E. *Sci. Rep.* **2021**, *11*, No. 4920.

(14) Skottvoll, F. S.; Hansen, F. A.; Harrison, S.; Boger, I. S.; Mrsa, A.; Restan, M. S.; Stein, M.; Lundanes, E.; Pedersen-Bjergaard, S.; Aizenshtadt, A.; et al. *Anal. Chem.* **2021**, *93*, 3576−3585.

(15) Skottvoll, F. S.; Aizenshtadt, A.; Hansen, F.; Martinez, M. A.; Andersen, J. M.; Bogen, I. L.; Kutter, J. P.; Pedersen-Bjergaard, S.; Lundanes, E.; Krauss, S. *Analysis & Sensing* **2021**, *2*, 1−5.

(16) Kogler, S. On-line "Organ-in-a-Column" Liquid Chromatography Electrospray Ionization Mass Spectrometry for Drug Metabolism Studie. Master's Thesis, University of Oslo, 2019. http://urn.nb.no/URN:NBN:no-75955.

(17) Mathapati, S.; Siller, R.; Impellizzeri, A. A.; Lycke, M.; Vegheim, K.; Almaas, R.; Sullivan, G. J. *Curr. Protoc. Stem Cell Biol.* **2016**, *38*, 1G.6.1−1G.6.18.

(18) Ang, L. T.; Tan, A. K. Y.; Autio, M. I.; Goh, S. H.; Choo, S. H.; Lee, K. L.; Tan, J.; Pan, B.; Lee, J. J. H.; Lum, J. J.; et al. *Cell Rep.* **2018**, *22*, 2190−2205.

(19) Bell, C. C.; Hendriks, D. F.; Moro, S. M.; Ellis, E.; Walsh, J.; Renblom, A.; Fredriksson Puigvert, L.; Dankers, A. C.; Jacobs, F.; Snoeys, J.; et al. *Sci. Rep.* **2016**, *6*, No. 25187.

(20) Doellinger, J.; Schneider, A.; Hoeller, M.; Lasch, P. *Mol. Cell. Proteomics* **2020**, *19*, 209−222.

(21) Moravcová, D.; Planeta, J.; Wiedmer, S. K. *J. Chromatogr. A* **2013**, *1317*, 159−166.

(22) Stoll, D. R.; Carr, P. W. *Two-Dimensional Liquid Chromatography: A State of the Art Tutorial*; ACS Publications, 2017.