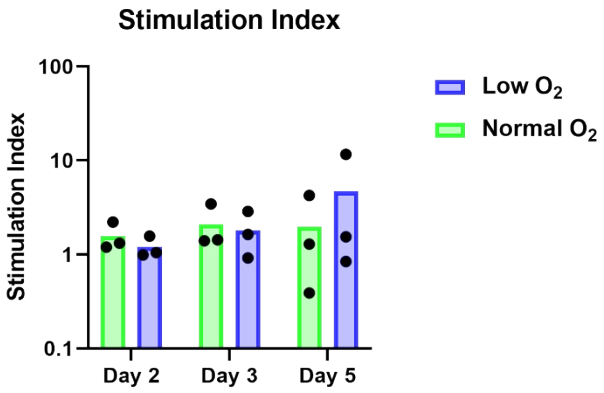
Electronic Supplementary Material (ESI) for Lab on a Chip.

SliceChip: A Benchtop Fluidic Platform for Organotypic Culture and Serial Assessment of Human and Rodent Pancreatic Slices

Supplemental Information

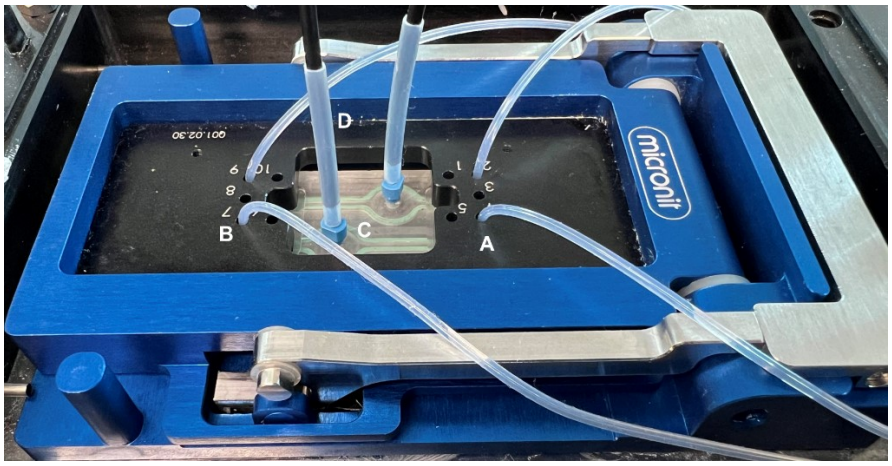
A



B

	Normal Oxygen			Low Oxygen		
	Mean	SD	N	Mean	SD	N
Day 2	1.58	0.557	3	1.203	0.319	3
Day 3	2.093	1.175	3	1.813	0.991	3
Day 5	1.977	2.020	3	4.685	6.059	3

Supplemental Figure 1. Stimulation Index data. (A) Stimulation index, taken as the ratio of the AUC of high glucose stimulation to the AUC of low glucose stimulation. Data is reported on a log scale and with individual data points reported. n = 3 on days 2, 3, and 5. (B) Mean and standard deviation data for each day of stimulation.



Supplemental Figure 2. Adapted SliceChip with oxygen sensors placed along the flow path. (A) fluid inlet (B) fluid outlet (C) PreSens oxygen sensor (D) fiber optic data-transfer cable.

Supplemental Protocol 1. Protocol for the complete assembly of the Slice Chip Platform

Materials:

Material	Quantity	Product Name	Notes
Degasser	2	DEGASi Plus Micro (Part: 0003-6352-S)	Modified channel length to 66uL (cut 18mm from each side of the tubing)
Bubble Trap	2	Diba Omnifit (Mfr # 006BT-HF, Item # UX-21940-39)	10um PTFE filter each
Bottle-CAP	10	Fluigent SKU: RES-CAP-PCK	Bottle caps for pressurization w/ 2 ports (GL-45 thread). Includes pneumatic fitting
Fingertight Conical Connector	12	IDEX Mfr # F-130X, Item # EW-02013-43	Long Thread, Natural PEEK, 1/16” OD Tubing, 10-32 Coned
T Connector	8	Product 80144	T Connector w/ 2 Female Luer Locks, 1 Male Slip
Flow Unit Connector Kit	2	Fluigent SKU: CTQ-KIT-FU2	Includes: Flow Unit S&M Adapter, FEP Tubing, Flangeless Fitting, Blue Ferrules
Flangeless PEEK Fittings	18	Flugient SKU: CTQ-KIT-XP	¼-28 to 1/16” OD
PEEK Fittings Ferrules	22	Fluigent SKU: CTQ-KIT-XP	¼-28 to 1/16” OD
Male Luer	18	Product ex) 04116	Any male luer lock w/ 1/16” Barb (1.5mm) ID Tubing

Equipment	Notes
Analytical Selector Valve	IDEX Part Number: 7060
Compressed Gas Canister	95% O2, 5% CO2
Stagetop Incubator	Tokai Hit Stagetop Incubator STXF
Keyence Microscope	BZ-X800, RRID: SCR_023617
Microfluidic Chip	
Microfluidic Chip Gaskets (x2)	
Microfluidic Chip Clamp	Product SKU: MN-FC-PRO-CH4515
Clamp Ferrules	Product SKU: MN-FC-PRO-FFKM-KIT.05
Flow Unit S (x2)	Fluigent: FLU-S-D, RRID: SCR_021144
Flow EZ (x2)	Fluigent: LU-FEX-0345, RRID: SCR_021145
EZ Low Pressure Kit	Fluigent: CTQ-KIT-LP-MFCS (Includes:

	male luer connector, luer cap, backflow filters, **1x3mm tubing**)
Bead Bath	
GL-45 Bottles (x10)	

Important note: **1x3mm tubing** from EZ Low Pressure Kit should be cut into 10 individual 10cm lengths of tubing. Later referred to as *pneumatic tubing*.

Tubing Location	Tubing Length	Quantity
Bottle to Switch	36cm	10
Switch to Degasser	26cm	2
Degasser to Debubbler	14cm	2
Debubbler to Chipp	43cm	2
Chip to Flow Unit	36cm	2
Flow Unit to Outlet	41cm	2

Tubing is cut from IDEX FEP 1/16 x .01 x 50ft roll (Part #: 1527L).
Flow Unit to Outlet tubing is a larger diameter: IDEX FEP 1/16 x .02 x 100ft roll (Part #: 1548XL).

Before Starting & Recommendations:

1. Set the heat bath to 37 degrees Celsius and the *Tokai Hit heating stage* to 40 degrees Celsius.
2. Setup the *Flow EZ* lineup system where pressure source is the *compressed gas canister.*
3. Connect the *flow units* to the *Flow EZ.*

Note: This protocol creates a network of interconnected tubing under the culture hood which must be transferred to the microscope/heat bath setup after assembly, a tray or extra set of hands can greatly assist this transfer.

Sterilization Procedure:

1. Autoclave the following components:
    - GL-45 bottles
    - Bottle-CAPs
    - Chip Clamp Ferrules
    - Male Luer Connector
    - Bubble Trap Filters
    - Microfluidic Chip Clamp
    - Backflow Filters
    - T Connectors
    - Flangeless Fittings
    - Fitting Ferrules
    - Fingertight Conical Connector

2. Spray the following components with ethanol and place inside the culture hood:
    - FEP Tubing of appropriate lengths and quantities
    - Degasser
    - Bubble Traps
    - Analytical Selector Valve (switch)
    - Microfluidic Chip
3. In the culture hood, pass ethanol, clean DI water, and air through each tube.
4. Separate and label the corresponding lengths of tubing for their correct final location (refer to materials).

Setup Assembly – Inside the Hood:

1. Gather tubing for the ***Bottle to Switch.*** For each tubing:
    a. Add a *flangeless fitting* with the threaded side facing towards the end of the tubing.
    b. Add a *blue fitting ferrule* with the conical side facing towards the threading and slide it along the tubing to meet the *flangeless fitting* to create a *flangeless fitting/ferrule complex.*
    c. Adjust this complex so that there are approximately 4" of excess tubing below the *fitting ferrule* (adjust the length of tubing as needed for the GL-45 bottle size, the opening of the tubing should sit close to the bottom of the bottle ~1mm).
    d. Screw this complex into the *Bottle-CAP.*
2. Attach a *fingertight conical connector* to the opposite end of each tubing.
3. Screw each of these *fingertight conical connectors* into the *analytical selector valve (switch).* There should be 5 tubing complexes attached to each valve (occupying slots 1-5).
4. For each valve/bottle cap assembly:
    a. Connect the *pneumatic fittings* into the other *Bottle-CAP* port.
    b. In 4 of the caps, place a *T connector* with the male slip insert into the *pneumatic fitting* and the female openings exposed.
    c. Place a *male luer connector* onto each of the female openings and attached a *pneumatic tubing* between each piece.
5. Gather the tubing for ***Switch to Degasser.*** For each tubing:
    a. Attach a *fingertight conical connector* on the end of the tubing and screw it into the center of the *selector valve.*
    b. On the other end of the tube, attached a *flangeless fitting/ferrule complex* by adding a *flangeless fitting* with the treads facing the end of the tubing. Then place a *blue fitting ferrule* with the conical side facing toward the *flangeless fitting*.
6. Gather the tubing for ***Degasser to Debubbler.*** For each tubing:
    a. On each end of the tubing attach a *flangeless fitting/ferrule complex* (Described in Step 5b).

    b. Assemble a *bubble trap (debubbler)* by placing a filter between the two halves and using an Allen wrench to screw the *debubbler* together. The assembly should be tightened by loose enough that you can spin the bottom half of the *debubbler.*
    c. Set one side of the tubing into the assembled *debubbler* by screwing the *flangeless fitting* into one of the *debubbler's* ports.
7. Gather the ***Debubbler to Chip*** tubing. For each tubing:
    a. On one side of the tubing attach a *flangeless fitting/ferrule complex.*
    b. Screw the *flangeless fitting* into the *debubbler's* other port. Leave the other end of the tubing free to eventually connect to the chip.
8. Gather the ***Chip to Flow Unit*** tubing. Place the *flow unit connector kit* on one end of the tubing by following manufacturing instructions. Leave one end of the tubing free to connect to the chip later.
9. Gather the ***Flow Unit to Outlet*** tubing (*reminder: larger ID tubing).* Attach one end of the tubing to the *flow unit connector kit* and leave the other end free for collection of media.
10. Attach the *clamp ferrules* into all the *microfluidic chip clamp* inlet and outlet ports.
11. Gather the ***Chip to Flow Unit*** tubing. To create the outlets, insert the free end of the first tubing into port 7 of the *microfluidic chip clamp* so that the end of the tubing is flush with the end of the ferrule inside the clamp. Repeat with the free end of the second tubing into port 9 of the *microfluidic chip clamp.*
12. Gather the ***Debubbler to Chip*** tubing. To create inlets, insert the free end of the first tubing into port 2 of the *microfluidic chip clamp* so that the end of the tubing is flush with the end of the ferrule inside the clamp. Repeat with the free end of the second tubing into port 4 of the *microfluidic chip clamp.*
13. Leave the clamp open, sperate the top and bottom of the chip, space out each of the other preassembled components under the hood. UV the entire setup for 20 minutes.

Experiment Prep – Inside the Hood:

1. After the 20 minute UV exposure period, with clean gloves and extreme care, use a paintbrush to transfer a pancreatic slice into the well on the bottom half of the chip, smoothing out any folds. With forceps place an anchor atop the slice. Repeat for the second well.
2. Place the bottom half of the chip into the clamp following the alignment posts. Then align the top half of the chip and place it down maintain gentle pressure.
3. Place your forefinger through the upper viewing window of the *microfluidic chip clamp* from the top surface in. Maintain gentle pressure as you close the *microfluidic chip clamp.*
4. Bring media and other solutions into the hood and replace caps with each of the *bottle-CAPs* taking care to maintain the same bottle order for each side of the chip.

Experimental Setup:

1. Carefully move the clamp/tubing complex into the *Tokai Hit stage warmer* on the *Keyence microscope.* Place the outlet tubings on the left side exit holes from the stage warmer. Place the inlet tubings on the right-side exit holes from the stage warmer.
2. Take the free end of the ***Switch to Degasser*** tubing and attach the *flangeless fitting* to the left side of the *degasser.* Repeat for the second set of ***Switch to Degasser*** tubing.
3. Take the free end of the ***Chip to Flow Unit*** tubing and attach the *connector kit* to the *flow unit.* Repeat for the second set of ***Chip to Flow Unit*** tubing.
4. Bring the bottle/switch complex from the hood to the heat bath.
    a. Place the bottles in the heat bath, assuring the liquid line of the bottles are fully submerged in the beads.
    b. Attach the free end of the ***Degasser to Debubbler*** tubing to the right side of the *degasser.* Repeat for the second set of ***Degasser to Debubbler*** tubing.
5. Attach the ***Flow to Outlet*** tubing to the flow unit via the connector.
6. Connect the bottles to the *Flow EZ* via the pneumatic tubing outlets from the *Flow EZ* placing it into the open female end of the first bottle in the bottle assembly.
7. Set the flow rate of the *Flow EZ* to 80ul/min.

Assembly Breakdown:

1. Carefully unclamp the *microfluidic chip clamp* and remove the slice from the chip.
2. Unscrew each component of the assembly. For each component, flush with ethanol and DI water. Then blow clean dry air through each piece.

Oxygen Consumption Model		
Parameter	Value	Unit
T	37.0	°C
η_{media}	0.6922	mPa·s
η_{tissue}	.700	mPa·s
ρ_{media}	993.357	kg/m^3
ρ_{tissue}	1000	kg/m^3
$\alpha \cdot D_{oxymedia}$	3.54e-12	mol/(m·mmHg·s)
$\alpha \cdot D_{oxytissue}$	1.24e-12	mol/(m·mmHg·s)
U_{inlet}	80.0	µl/min
P_{ref}	1.0	atm
P_{outlet}	0.0	atm
C_{mmO_2}	0.001	M
C_{cr}	0.0001	M
R_{maxO_2}	-0.034	mol/(m^3·s)
$C_{O_2media(initial)}$	120 or 60	mmHg
$C_{O_2tissue(initial)}$	100	mmHg

Glucose Washout Model		
T	37.0	°C
η_{media}	0.6922	mPa·s
ρ_{media}	993.357	Kg/m^3
U_{inlet}	20.0—80.0 (10.0 increments)	µl/min
P_{ref}	1.0	atm
P_{outlet}	0.0	atm
$D_{glucosemedia}$	3.0e-9	m^2/s
$C_{glucose(initial)}$	0	mM
$C_{glucose(end)}$	5	mM

Supplemental Table 1. COMSOL Parameters for Oxygen Consumption modeling and Glucose Washout Modeling

Sample ID	Diabetes Duration (Years)	Age (Years)	Sex	Ethnicity	BMI	Date used
HP-22278	n/a	37	M	Hispanic	29.1	10/9/2022
nPod6584	n/a	22	M	Caucasian	21.1	2/6/2023
HP-23159	n/a	37	M	Hispanic	27.2	6/8/2023

Supplemental Table 2. Pancreatic Donor Demographics