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Integrated platform for operating and interrogating organs-on-chips†

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The development of microphysiological systems, also known as organs-on-chips, has highlighted the need for improved *in vitro* models for drug discovery. However, the highly specialized skillset required to design, build, and operate these systems has hindered adoption. Here, we describe an integrated platform that enables both continuous perifusion culture and dynamic cell secretion assays. We also developed a graphical user interface that allows the user to not only control the hardware components, but also define automated programs. The system's functionality was validated by maintaining pancreatic islets in culture under continuous perifusion for 24 hours, then performing a functional glucose stimulated insulin secretion assay. We demonstrate that islets remained both viable and functional on the platform with minimal user involvement.

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Introduction

Organs-on-chips aim to recapitulate the minimal physiologically-relevant functional unit of tissues and organs.[1] This has been achieved through the convergence of advances in the fields of microfluidics, microfabrication, cellular engineering, and tissue engineering. The ability of organs-on-chips to recreate not only healthy organ-level physiology, but also dynamic pathophysiological phenomena has generated huge interest from the pharmaceutical industry. Specifically, these *in vitro* devices are viewed as a more predictive pre-clinical model for drug discovery that can potentially address the high attrition rate of promising therapeutic compounds.[2] However, a number of challenges beyond the validation of physiological and pathophysiological models has slowed the adoption of organ-on-chip technology. For pharmaceutical end users, these challenges include throughput capability, platform stability, reproducibility, and compatibility with existing laboratory equipment and processes.[3]

Automated high-throughput screening has become an important part of early drug discovery.[4] However, the complexity of operating an organ-on-chip makes integration with completely autonomous systems difficult. Further, advancements in this field have focused primarily on the development and characterization of microfluidic chips to create models of a specific organ function and/or disease state.[5] Many of these devices not only require unique methods for cell culture and functional readouts, but also implement custom fluidic controllers. Further, the diversity of existing organ-on-chip devices creates a challenge in developing platforms for validation and comparative studies. As a result, current systems, developed both in research labs and emerging start-up companies, are yet to fit seamlessly into the drug development pipeline and address the needs of the pharmaceutical industry.[6,7]

To simplify the operation of organs-on-chips, we report on a platform that automates continuous perifusion culture and dynamic cell secretion collection through the integration of programmable hardware. Key design parameters of the integrated platform include compatibility with existing cell culture equipment (*i.e.* standard CO_2 incubators), precise microfluidic control of media, and user-friendly automation. The platform was developed using in-house rapid prototyping and commercially available motors, fluidic switches, and microcontrollers. We validate platform functionality using our previously developed fluidic device for interrogating pancreatic islets.[8] Through the use of hardware that is agnostic to microfluidic chip design and development of a user-friendly interface, this platform aims to facilitate the adoption of organ-on-chip technology in research labs and companies that lack the engineering expertise to develop and operate existing organ-on-chip devices and microfluidic controllers.

Materials and methods

Hardware

A 3-channel, 8-roller peristaltic pump with independent channel control (Cole-Parmer GmbH) is used to drive media throughout the platform. A 10-position/11-port rotary shear valve (IDEX Health & Science) serves as a manifold for selection of media reservoirs and a 3-way isolation valve (NResearch Inc.)

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directs media for either recirculation or collection. Tubing and fluidic connections were obtained from Cole-Parmer GmbH.

Samples are collected in a standard 96-well plate that sits on a custom-built stage. *X*-direction and *Y*-direction movement of the stage is controlled by a NEMA-17 stepper motor (Adafruit) coupled to fast-travel, ultra-precision lead screw (McMaster-Carr). To test the system, our previously-described fluidic chip, FP-3W, was used as a model organ-on-chip device.[8] A microfluidic chip holder (Micronit) was used to seal the FP-3W fluidic chip and introduce fluidic connections. Bubble traps (Darwin Microfluidics) were placed just before the inlet of the organ-on-chip device to prevent bubbles from entering the device.

An Arduino Mega 2560 microcontroller (Arduino) connected to a motor shield (Adafruit) and CoolDrive® driver circuits (NResearch Inc.) was used to control the stepper motors and 3-way isolation valves. A 24 V, 160 W power supply (Digi-Key) was used to power the entire system *via* a custom printed circuit board, comprised of 12 V and 5 V DC converters (Traco Power) and a current limiter. All electronic components are rated to function in the temperature and humidity of a standard cell culture incubator.

Design and fabrication

The entire system was designed using SolidWorks 3D computer-aided design (CAD) software (Dassault Systèmes). To ensure precise fitting and facilitate fabrication, CAD files for each hardware component were either obtained from the manufacturer or designed *de novo*. Custom parts for the platform housing and mounting hardware were also developed in SolidWorks and fabricated from clear acrylic (McMaster) using a 30 W CO_2 laser machine (Epilog). Design files for the custom parts are available on the NIH 3D Print Exchange (https://3dprint.nih.gov/discover/3dpx-011914).

Graphical user interface

A custom graphical user interface (GUI) was developed in Python, using the Tkinter package, to integrate the control of hardware components and enable user-programmed automated protocols. A USB hub (D-Link), housed within the platform, provides a central point for USB connections to each of the hardware components. As a result, only a single RS-232 serial connection is required to enable the GUI to communicate with all the hardware components.

The GUI consists of modules that allow for manual control of the platform, programming of automated procedures, and monitoring of platform status. The GUI is packaged into a single, executable file that can be installed on any computer. The complete Python code is available on GitHub (https://github.com/lievbirman/CultureFlow).

Islet isolation and culture

Pancreatic islets were procured from human organ donors by the Human Islet Cell Processing Facility at the Diabetes Research Institute (University of Miami Miller School of Medicine, Miami, FL) under IRB approval for use of human tissue for research. Islets were cultured in Prodo Islet Media (Standard) supplemented with 5% Human AB Serum and 2% glutamine/glutathione overnight at 37 °C and 5% CO_2 after the isolation procedure. All media and supplements were purchased from PRODO Laboratories.

Following the overnight static culture, islets were loaded into the fluidic chip and connected to the platform. Islets were cultured under continuous perifusion at 30 μL min^{-1} in the FP-3W fluidic chip. Media was drawn from a single reservoir, with 15 mL of media, and recirculated for all three wells during the culture period.

Dynamic glucose stimulated insulin secretion

Islet function was evaluated after 24 hours of culture on the platform using a dynamic glucose stimulated insulin secretion (dGSIS) assay. A standard perifusion buffer solution (125 mM NaCl, 5.9 mM KCl, 2.56 mM $CaCl_2$, 1.2 mM MgCl, 0.1% w/v BSA, and 25 mM HEPES, pH 7.4) was prepared for the dGSIS assay.[9] Stimulus solutions were prepared by supplementing the buffer solution with 3 mM glucose (Low G), 11 mM glucose (High G), or 25 mM KCL for low glucose, high glucose, and depolarization stimuli, respectively.

An automated perifusion protocol was loaded *via* the GUI, consisting of the following steps during perifusion at 100 μL min^{-1}: 1 hour equilibration in low glucose, 10 minutes in low glucose, 20 minutes in high glucose, 10 minutes in low glucose, 10 minutes in KCL, then 10 minutes in low glucose. After the 1 hour equilibration, which was dispensed into a waste reservoir, samples were collected in a 96-well plate with a sampling time of 2 min per well. Following the automated perifusion protocol, each well was imaged, and the number of islets was counted manually using ImageJ. Insulin content was quantified using an Insulin ELISA kit (Mercodia). Insulin secretion per islet was determined based on flow rate and number of islets per well.

Cell viability assay

Viability of islet cells was assessed using the LIVE/DEAD™ Viability/Cytotoxicity Kit (Thermo Fisher Scientific). The live/dead staining procedure was performed as an automated protocol. First, islets were washed by perfusing phosphate buffered saline (PBS) through the fluidic chip for 5 minutes. Then, the staining solution, comprised of 2 μM calcein-AM and 4 μM ethidium homodimer-1 (EtD-1) diluted in PBS, was perfused through the chip for 5 minutes. A 30 minute wait step was included to incubate the islets in the staining solution. The fluidic chip was then removed from the system and islets were imaged on a Zeiss LSM 800 confocal laser scanning microscope (Carl Zeiss AG). Quantification of islet viability was performed using ImageJ (NIH). Briefly, images from three separate field of views were thresholded and the area of live (stained with calcein-AM) and dead cells (stained with Etd-1) was determined using the 'Analyze Particles' function in ImageJ. Percent viability was calculated as the area of live cells divided by the total area of live and dead cells.

Results and discussion

Hardware overview

We have developed a platform to integrate the culture and analysis of cells on an organ-on-chip device. The platform

consists of three hardware systems interconnected in a single house: (i) microfluidic handling system, (ii) organ-on-chip, and (iii) sample collection stage (Fig. 1A). To facilitate cell culture, the footprint of the system housing is designed to fit in a standard cell culture incubator. The internal dimensions of a CO_2 incubator, which can vary by model, are approximately 470 mm × 531 mm × 607 mm (W × D × H). To ensure that the platform met the requirements to fit into an incubator, the platform design was modelled using 3D CAD software (Fig. 1B). The footprint of the fully assembled platform is just 356 mm × 432 mm (W × D), which leaves enough space for the platform to be placed in an incubator without interference from shelving mounts.

The microfluidic handling system consists of valves and a pump to deliver media to the organ-on-chip device and direct media for either recirculation or collection. A 10-position rotary shear valve serves as a manifold, which allows for up to 10 media reservoirs to be installed. The output of the manifold is split into the three channels of the peristaltic pump. Each channel of the peristaltic pump can be controlled independently which enables control and experimental groups to be run in parallel or for groups to be run in triplicate. With the current 0.051 in inner diameter tubing configuration, the pump can achieve flow rates ranging from 1.7 μL min^{-1} up to 1.7 mL min^{-1}. The wide range of flow rates and standardized connectors ensure that the microfluidic handling unit can be compatible with a variety of organ-on-chip devices. Except for the peristaltic pump tubing, fluorinated ethylene propylene (FEP) tubing is used throughout the microfluidic handling system. FEP tubing is chemically inert and gas permeable making it an ideal tubing material for use in a standard cell culture incubator.

A sample collection stage was designed to achieve sample collection into all the wells in a standard 96-well plate. The sample collection stage is comprised of two NEMA-17 stepper motors coupled to lead screws and mounted on rails. The 96-well plate sits on a custom-built stage mounted to the *X*-axis rail. The sample collection stage has a 235 mm × 160 mm range of motion. The extra space allows for an eject position, where the plate can be either loaded on to or collected from the platform.

Currently on market, there are only a few options for automated culture of organ-on-chip devices and, more specifically, for assessment of pancreatic islets. Existing platforms, such as the BioRep perifusion system, can cost upwards of $80 000. At

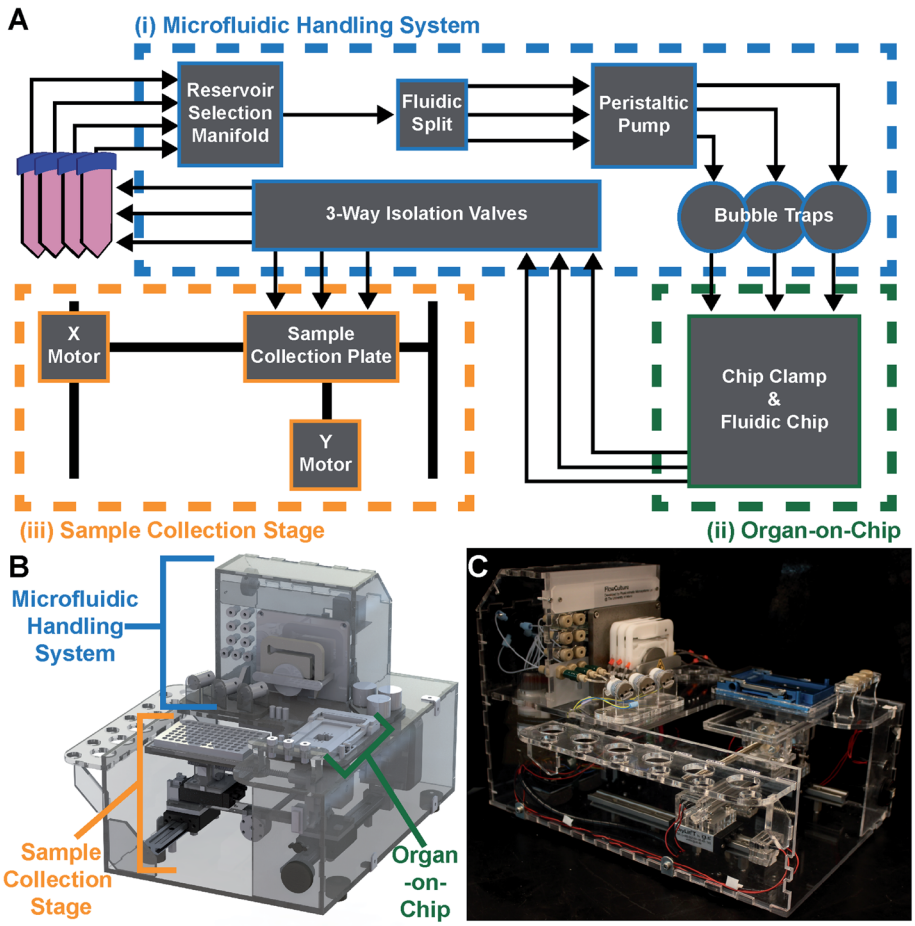

Fig. 1 Design and development of integrated platform. (A) Schematic representation of integrated platform comprised of three major systems: (i) microfluidic handling system; blue, (ii) organ-on-chip system; green, and (iii) sample collection stage; orange. (B) Three-dimensional CAD render of platform design. Bubble traps not pictured (C) fully assembled platform with all components included. Motors are blocked by other components.

this price point, the BioRep system is specifically for high-throughput perifusion.[9] Additionally, this system is not designed for cell culture. While the system does provide precise temperature control, there is no option for gas or humidity control. The ability to only provide the proper temperature control, limits the BioRep system capability for long-term culture.

In its current configuration the entire integrated platform we have developed costs approximately $7000 (Suppl. Table 1†). However, nearly half of this cost is due to the peristaltic pump and the microfluidic chip holder. Low-cost peristaltic pumps have been previously developed, but require much larger tubing which would increase the overall in system volume and lead to increased delay times for sample collection.[10] The cost of the microfluidic chip holder can be avoided through the use of other organ-on-chip devices, which can be connected to the integrated platform through the standardize fluidic connections. The overall cost of these components is justified by the ease of programming, which also reduced the complexity of the GUI, and the overall end user experience from setup to operation.

Software overview

To simplify user control of the platform hardware, software has been developed to allow for automated protocols to be programmed and run from a single Graphical User Interface (GUI). The lean back-end code, written in Python, uses a modular system architecture which is amenable to expanding the hardware components and software features. Each hardware component is controlled *via* modules that translate Python code into serial command outputs.

To minimize the connections running out of the incubator, communication for hardware components was centralized through a USB hub. The USB hub provides a single RS-232 serial connection to the computer running the software (Fig. 2A). The compact, user-friendly GUI software facilitates operation of the system through either a manual or automatic mode (Fig. 2B). Manual mode allows the user to control selection of reservoirs, set sampling rate, move the collection stage, and independently set the flow rate for each pump channel. Thus, manual mode enables the full operating range of the system to either perform short experiments or optimize conditions. The automatic mode offers the same flexibility of choice as the manual mode but allows the user to define experimental protocols *via* a step-by-step input form. This allows for longer experiments that require multiple media changes and/or switching between culture and sample collection.

Design verification

Verification testing was performed to ensure to that the platform met design specifications for flow rate accuracy, valve function, and leak-free operation. To verify that the power supply and GUI control for the commercial pump were functioning as intended, flow rate accuracy tests were performed. The system was first calibrated by dispensing 100 μL of water in 1 minute using a function built into the peristaltic pump. Volume was determined by measuring the mass of water collected. Using the GUI, the pump was then programmed to run for 1 minute at flow rates ranging from 10 μL min^{-1} to 100 μL min^{-1}. For all flow rates tested, the average volume collected at the collection stage from each channel never varied by more than 5% from the expected volume. Further, the standard deviation for each tested flow rate was less than 1.2 μL (Table 1). Across all flow rates, the average volume difference from the expected value was just 1.1 μL. Together, these results

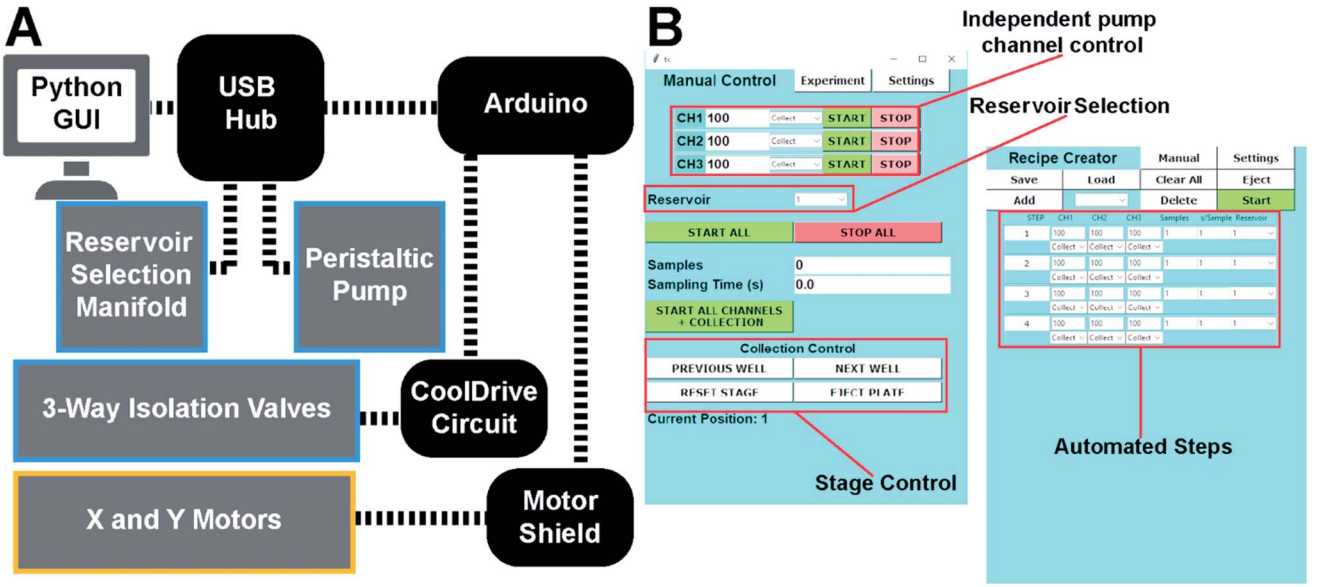

Fig. 2 Integration of hardware control *via* Python GUI. (A) Communication network to enable GUI control of hardware *via* a single USB hub. An Arduino microcontroller is used to provide control to the 3-way valves and motors. (B) Screenshots of the main page and recipe creator page of the GUI. From the main page, the user can manually control most aspects of the platform. From the recipe creator page, the user can set a series of steps to run automatically.

Table 1 Flow rate verification testing results. The volume dispensed at each flow rate was collected for 1 minute. Volume collected displayed as average ± standard deviation ($n = 3$)

Flow rate (μL min^{-1})	Volume collected (μL)	Percent error
10	10.5 ± 0.08	5.0%
25	26.0 ± 1.15	3.9%
50	51.0 ± 1.18	2.0%
75	75.8 ± 1.19	1.1%
100	102.1 ± 1.06	2.1%

demonstrate that the integrated platform maintains a level of high accuracy and precision.

Key features of the platform include efficient switching between media reservoirs and accurate sample collection. The low dead volume of the selection valve ensures there is essentially no mixing of media during reservoir changes. The dispensing nozzles were positioned such that the tip of the nozzle grazes the top of the collection plate to collect the final drop of media and mitigate the chance of media being dispensed outside of the designated well. To verify these design specifications, a test protocol requiring two reservoirs and switching between sample collection and waste dispensing was developed. A "snake" pattern was implemented for collection in the 96-well plate to minimize travel distance between well columns (Fig. 3A). The automated protocol set a flow rate of 1 mL min^{-1} and a sampling rate of 15 seconds per well. First, 10 samples were collected from reservoir one, the system then switched to reservoir two and dispensed to a waste reservoir for 3 minutes. In the last step, the system switched back to sample collection and 10 samples were collected from reservoir two (Fig. 3B). Upon completion of the programmed steps, the system automatically ejected the collection plate for the user to retrieve (Suppl. Video S1†). Leaks were rarely observed. The primary cause of the minor leaks that did occur was improperly connected tubing and was easily resolved by tightening the connections.

Automated dynamic culture and viability assay

To validate that the integrated platform is compatible with organ-on-chip systems, human pancreatic islets were loaded into the FP-3W fluidic chip and cultured on the platform for 24 hours and assessed for cell viability. An automated culture protocol, to recirculate islet culture media at 30 μL min^{-1} for 24 hours, was programmed and performed using the GUI developed for the system. The geometric features in the flow path of the FP-3W fluidic chip are designed to minimize shear stress on islets while providing a sufficient supply of nutrients. After the 24 hour culture protocol, islets could be visualized in the culture wells with minimal deviation from their original position (Fig. 4A). Additionally, no bubbles were observed in the fluidic chip during or after the culture the period.

To demonstrate the versatility of the platform and that cells remained viable, an automated live/dead staining protocol was performed. The automated protocol successfully labelled islet cells with either calcein-AM or EtD-1, which qualitatively demonstrated that islets were still highly viable after the 24 hour culture period (Fig. 4B). Quantitative image analysis was also performed. Islet viability, as a percentage of total islet area, was calculated as the live cell area divided by the sum of both live and dead cell area of each islet. Compared to the post-isolation viability (88.9%) reported by The Human Islet Cell Processing Facility, which performed the islet isolation, islet viability was unchanged (89.1 ± 5.4%) after 24 hours of dynamic culture on the integrated platform (Fig. 4C).

Functional assessment of human pancreatic islets

The ability to perform on chip functional assessments is an important feature for organs-on-chips. The integrated platform aims to facilitate the switch from culture to assessment. To demonstrate this capability, a dGSIS assay was performed on human pancreatic islets that were in culture on the platform. Approximately 40 islets were loaded into each well of the fluidic chip and cultured for 24 hours on the integrated platform. After the culture protocol was completed, solutions of low glucose (3 mM), high glucose (11 mM), and KCL (25 mM) were loaded on to the platform. Next an automated dGSIS protocol was loaded and run from the Python GUI. Islets were exposed to low glucose for 1 h to acclimate to the perifusion buffer solution. Then, samples were collected every 2 min while islets were exposed to low glucose, high

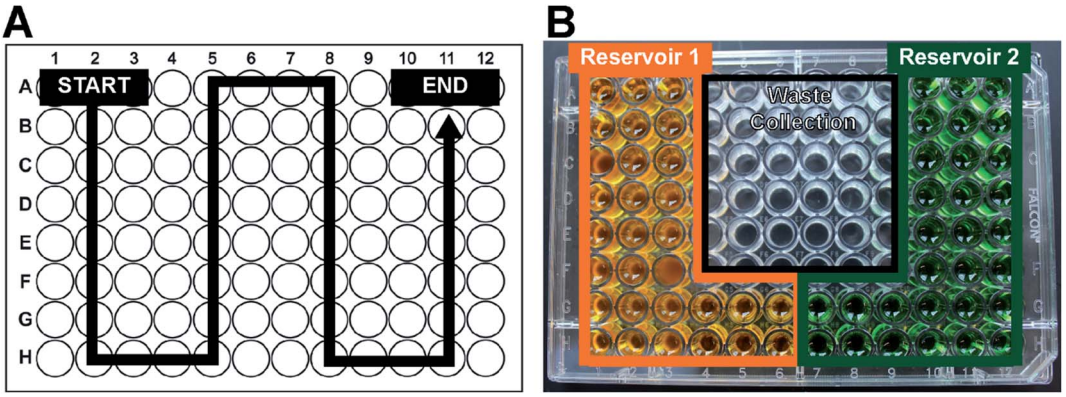

Fig. 3 Validation of microfluidic handling system and sample collection stage (A) "Snake" pattern programmed for sample collection to minimize travel distance when a column of wells was filled. (B) Validation of platform functionality *via* a programmed protocol set to collect from reservoir 1, dispense to waste, then collect from reservoir 2.

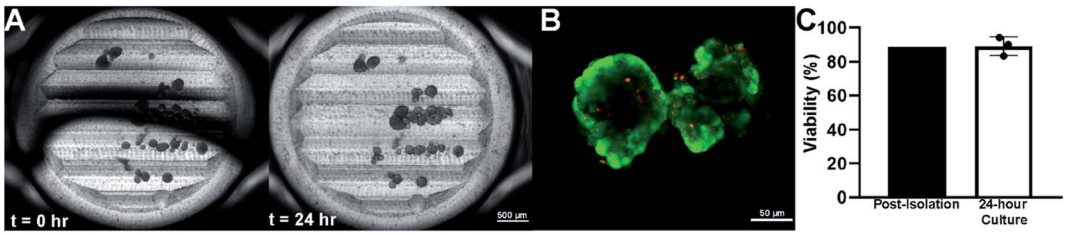

Fig. 4 Islet viability in FP-3W after 24 hour culture on integrated platform. (A) Brightfield images after loading islets into FP-3W ($t = 0$ h) and after 24 hours of continuous perifusion on the integrated platform. At $t = 0$ h, the media used to transfer islets in the chip does not completely fill the well, which causes refraction of the light. After 24 hours, the well is filled with media and islets are in approximately the same location. (B) Representative live/dead stain of islets using ethidium homodimer-1 (red, dead) and calcein-AM (green, live). (C) Quantification of islet viability from three field of views after 24 hour culture and automated staining procedure. Islets viability remained unchanged compared to the viability obtained by Human Islet Cell Processing Facility.

glucose, and KCL solutions in series. Upon completion of the automate dGSIS assay, each well was imaged, and the number of islets was manually counted using ImageJ (Fig. 5A).

It is well-established that insulin secretion from islets *in vivo* is characterized by a "first phase" spike, followed by a delayed "second phase" of sustained secretion.[11] Further, this response is observed specifically when glucose is metabolized within an islet.[12] Stimulation by KCL, which causes membrane depolarization, only results a first phase spike of insulin secretion before returning to basal levels. Insulin secretion, normalized to the flow rate and number of islets per well, was collected during the automated dGSIS protocol (Fig. 5B). There is about a 6 minute delay from the time high glucose or KCL stimulation is applied to the time a significant increase in insulin secretion is observed. This is partly a result of delays in media delivery and sample collection due to system volume. Overall, the functional assessment of pancreatic islets demonstrated that the integrated platform can not only provide stimuli to cultured cells, but also can resolve the biphasic insulin secretion profile through sample collection.

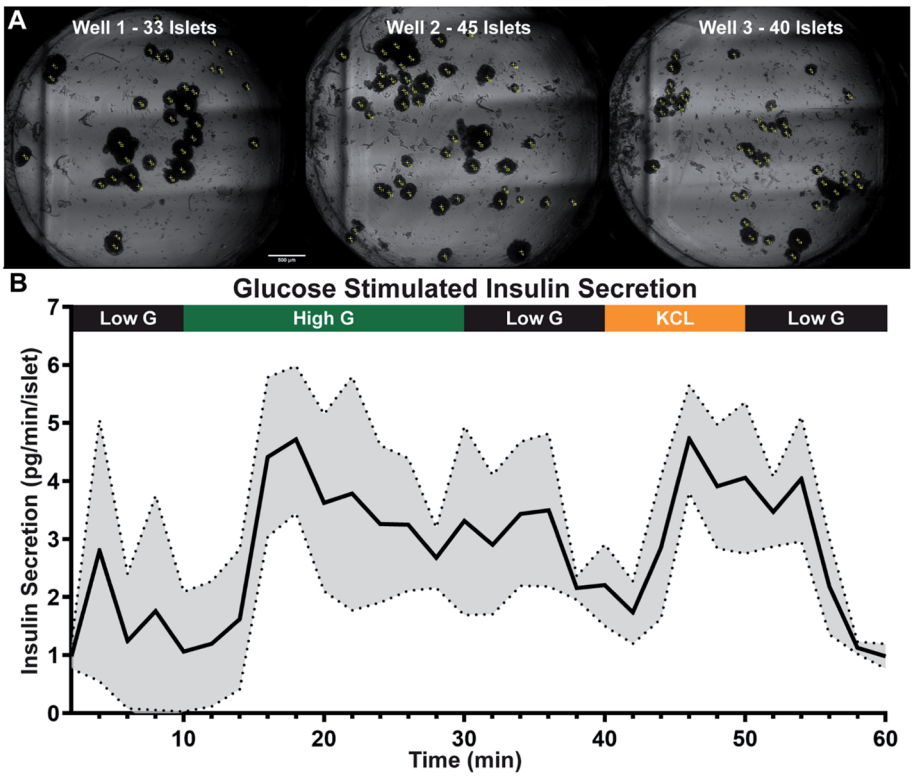

Fig. 5 Quantification of islets and insulin secretion profile obtained from automated dGSIS. (A) Screenshot of counted islets in each well of the FP-3W fluidic chip after 24 hours of dynamic culture. (B) In response to high glucose stimulus (11 mM; High G), insulin secretion follows a biphasic response with a first phase spike followed by a delayed second phase, which runs into the second low glucose exposure (3 mM; Low G). This is followed by a drop toward the baseline insulin secretion before a first phase secretion peak is achieved by exposure to 25 mM KCL. The observed delay between stimulus exposure and peak insulin secretion is due to system volume. The shaded region of the dGSIS profile represents the standard deviation from the mean insulin secretion ($n = 3$).

Conclusions and future directions

The platform designed, built, and tested here demonstrates the ability to integrate continuous perifusion culture and cellular assessment assays for use with organ-on-chip devices. We were able to culture pancreatic islets and perform both a dGSIS and a live/dead assay using automated protocols through a GUI designed to operate the platform. Further, the results presented demonstrate that the use of our previously-developed FP-3W fluidic chip can be expanded to include continuous perifusion culture, in addition to the previously described interrogation methods. The fluidic and mechanical components implemented are sufficiently precise to capture dynamic cell secretion phenomenon, such as the biphasic insulin secretion profile of pancreatic islets. Compared to commercially available automated perifusion systems, this integrated platform provides a wider range of capabilities at lower price point. Overall, this platform and approach for automated operation of organs-on-chips are an important advancement for transitioning organ-on-chip technology to a broader end user population.

Future work will aim to develop protocols for culture and assessment of other 3D cell constructs. While the current system relies heavily on plastic parts and prototyping-grade electronics, the design requires little modification to be manufactured with robust, high-quality components. Ultimately, we hope to develop a polished system that can be competitive in the organ-on-chip market as a platform for a wide range of experiments involving culture and assessment using any of the currently available organ-on-chip devices.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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