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3D-templated, fully automated microfluidic input/output multiplexer for endocrine tissue culture and secretion sampling

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Abstract

A fully automated, 16-channel microfluidic input/output multiplexer (μ MUX) has been developed for interfacing to primary cells and to improve understanding of the dynamics of endocrine tissue function. The device utilizes pressure driven push-up valves for precise manipulation of nutrient input and hormone output dynamics, allowing time resolved interrogation of the cells. The ability to alternate any of the 16 channels from input to output, and vice versa, provides for high experimental flexibility without the need to alter microchannel designs. 3D-printed interface templates were custom designed to sculpt the above-channel polydimethylsiloxane (PDMS) in microdevices, creating millimeter scale reservoirs and confinement chambers to interface primary murine islets and adipose tissue explants to the μ MUX sampling channels. This μ MUX device and control system was first programmed for dynamic studies of pancreatic islet function to collect ~90 minute insulin secretion profiles from groups of ~10 islets. The automated system was also operated in temporal stimulation and cell imaging mode. Adipose tissue explants were exposed to a temporal mimic of post-prandial insulin and glucose levels, while simultaneous switching between labeled and unlabeled free fatty acid permitted fluorescent imaging of fatty acid uptake dynamics in real time over a ~2.5 hour period. Application with varying stimulation and sampling modes on multiple murine tissue types highlights the inherent flexibility of this novel, 3D-templated μ MUX device. The tissue culture reservoirs and μ MUX control components presented herein should be adaptable as individual modules in other microfluidic systems, such as organ-on-a-chip devices, and should be translatable to different tissues such as liver, heart, skeletal muscle, and others.

Graphical Abstract

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A list of materials and additional methodological details are included in electronic supporting information (ESI).

An automated 16-channel microfluidic multiplexer (μ MUX) was developed for dynamic stimulation and interrogation of islets and adipose tissue.

Introduction

The increasingly prevalent, debilitating conditions of diabetes, obesity, and metabolic syndrome are fundamentally linked to endocrine tissues such as the liver, pancreatic islets, and the various adipose subclasses. In particular, adipose tissue (fat) is now understood to be a complex, multicellular endocrine organ that has profound systemic effects, altering the function of nearly all other organ systems¹. A multitude of chronic factors result in adipose tissue expansion, which is linked to diabetes^{2, 3}, Alzheimer's disease⁴, compromised immune function⁵, and many other diseases. Despite its importance, however, there is a lack of information on the dynamic nature of adipokine secretion and nutrient uptake in adipose tissue, highlighting several unmet needs in methodology. Specifically, few techniques exist to interrogate small amounts of adipose tissue, and there is a shortage of methods to explore dynamic function of the organ. There has also been renewed interest in the dominant role of the pancreatic hormone, insulin, especially in the context of hyperglycemia and hyperinsulinemia induced by diets high in sucrose or refined carbohydrates^{6, 7}. Unfortunately, we have a limited view of the dynamic relationship between glucose, insulin, and adipose function.

Microfluidic tools offer attractive features that could help fill this knowledge gap. This potential is exemplified by the recent outpouring of organs-on-chips that nicely simulate physiology at the tissue level or even the organ level; such devices recapitulate biological functions in a manner unmatched by standard culture methods⁸. Our group^{9–11} and others^{12–21} have shown the utility of microsystems to study biological function of pancreatic islets, and we have begun applying these systems to studying primary adipose tissue function^{11, 22}. Although some studies have leveraged microfluidics to assay secretion from adipocyte cell lines^{23–25}, less has been accomplished toward studying dynamics of intact, primary adipose tissue on-chip²⁶. In terms of fluid handling, although passively controlled microdevices provide simplicity of use^{10, 11, 22, 27}, the precision in fluidic control provided by actively valved devices is virtually unmatched in glean complex functions from biological systems^{28–31}. As such, a precisely valved, customized microdevice should be a fitting analytical solution to help decipher endocrine tissue dynamics.

Herein, a customized microfluidic input/output multiplexer (μ MUX) using active microvalves is presented for generalizable dynamic control over hormones and nutrients to/from endocrine tissues. This system essentially serves as a mimic of the circulatory system and of upstream endocrine signals. The device is automated through feedback sensing of solution levels, and 3D-printed templates¹¹ are used to interface both islets and adipose tissue. High device flexibility is shown by varying from multiple fluidic outputs to primarily inputs, by operation in sampling and imaging modes, and by studying multiple murine tissues. This 3D-templated μ MUX device should be applicable to a variety of tissue types, and it could feasibly serve as a single module in devices with further integrated functionality such as on-chip bio-sensing.

Experimental Methods[‡]

Microdevice fabrication

Two layer, valved microfluidic devices (Figure 1) were fabricated using standard multilayer soft lithography methods^{32–34} with 3D-printed templating^{11, 22}. Detailed procedures are described in ESI. All experiments involving animals (mice, C57BL/6J) were performed in compliance with relevant laws and institutional guidelines and were approved under protocol number 2014-2096 by the institutional animal care and use committee (IACUC) of Auburn University.

Control interface

Pneumatic valves were actuated with solenoid switches (LHDA0531415H, The Lee Co., Westbrook, CT) controlled by a multifunction data acquisition system (USB-6002, National Instruments) and using a house nitrogen source adjusted to 20 psi with a pressure regulator. The 16 fluidic channels were addressed by 8 pneumatic control channels connected to the corresponding solenoid switches with Tygon tubing (0.02 inch ID, 0.06 inch OD, Cole-Parmer, Vernon Hills, IL). Dead-end control channels were filled with water to prevent air leakage through PDMS membranes. As a solenoid switch was activated, nitrogen gas pressurized a lower layer control channel and closed the fluidic channel in the upper layer (push-up valves). Valve actuation timing was regulated through an in-house written LabVIEW application on a PC.

Solution level sensing

Serving as the “full” sensor, two gold electrodes were placed over the reservoir and connected in a voltage divider configuration to sense solution conductivity (Figure 2A–B), using the 5 V digital output line as a source. To protect cells and minimize electrochemical gas generation, the voltage was set to off (0 V) immediately after the “full” state was reached. The “empty” sensor utilized a cooled CCD (Coolsnap HQ2; Photometrics) and an inverted microscope (Nikon Ti-E) in differential interference contrast (DIC) mode. The CCD readout was interfaced with a LabVIEW application using the Scientific Instrument ToolKit™ for real-time image analysis (R Cubed Software; Lawrenceville, New Jersey). As the reservoir was emptied, the image (focused on the reservoir bottom) was transformed to a 2D array, and a standard deviation of the region of interest (σ_{ROI}) subarray was analysed as the signal for the “empty” trigger. As the reservoir emptied, roughness emerged in its

features due to refractive index differences, and σ_{ROI} abruptly increased. Example sensor traces are shown in Figure 3B and Figure S-7.

Time and channel programs

User-defined time and channel programs were preloaded into the LabVIEW application. “Full” and “empty” sensor readouts were used to automate the device according to the preloaded program (Figure 3A).

μ MUX device characterization

Reproducibility in sampling volume was analysed by measuring the distance travelled by the leading meniscus of the sampled solution in the output tubing after each step. Measurements were repeated in triplicate (Figure S-6B).

Carry-over volumes were measured by connecting two input fluidic channels to two syringes filled with either buffer (phosphate buffered saline, PBS) or 100 nM fluorescein in PBS. Connections were made through Tygon tubing. The μ MUX time program was designed as two cycles of five buffer rinses after a single input of fluorescein solution. Each input was incubated for 10 s, and the solution in the reservoir was collected and saved in one of the output channels (one solution per channel). Solutions in output channels were collected, and fluorescence intensities were measured using a small-volume spectrofluorometer (Nanodrop 3000) (Figure S-6A).

Solution exchange times were analysed by measuring the fluorescence output from pH-responsive glass beads (~100–200 μ m) in the tissue culture reservoir as solutions of varying pH were applied (Figure 4). The pH-responsive beads were synthesized in the following manner: 100 mg glass beads (150–212 μ m, G1145-10G, Sigma-Aldrich) were placed in a centrifuge tube, washed with 1 M NaOH (1 mL, 3 $\times$), deionized H₂O (1 mL, 3 $\times$), and MeOH (1 mL, 3 $\times$), then dried at 65 °C. 5.0% (3-Aminopropyl)trimethoxysilane (281778, Sigma-Aldrich) in EtOH was added to the beads, and the tube was agitated on a rocker at room temperature overnight to introduce the amine function group on the glass surface by silanization. The beads were washed with EtOH 3 $\times$ to remove the unreacted silane. After again drying at 65 °C, the amine-glass beads were treated with FITC in DMF solution (0.1%, 1 mL) at room temperature and incubated for 2 h. The beads were then washed with DMF and EtOH, then stored in EtOH at 4 °C until use. Fluorescence images of beads during automated pH switching were measured (Nikon Ti-E), and images were analysed using ImageJ and Microsoft Excel.

The diffusion of hormones from the tissue culture region into bulk solution in the reservoir was simulated by scanning the fluorescent intensity through the depth of the tissue culture region with a confocal microscope. For devices with varying depths of the 3D-templated tissue culture region, two μ MUX input channels were filled with 100 nM or 10 nM fluorescein in PBS, and 1 waste output channel was connected to a vacuum applied syringe. The reservoir and tissue culture region were prefilled with 100 nM fluorescein. As the reservoirs emptied, the device was programmed to fill with 10 nM fluorescein and incubate. Confocal Z-scanning was initiated from the bottom to the top of the tissue culture region (Nikon A1 Multiphoton Confocal Laser Scanning Microscope; 488.0 nm laser excitation,

525/50 nm emission filter). The images were analysed using ImageJ and Microsoft Excel (Figure S-6C).

Extraction of primary islets and adipose explants

Pancreatic islets and epididymal adipose tissue pads were extracted as described previously^{10, 22} from 18–20 week old male C57BL/6J mice (Jackson Laboratories). Detailed procedures are given in ESI.

Islet secretion sampling and insulin quantification

The μ MUX device was mounted within a microscope stage-top incubator (Tokai Hit, Japan) held at 37 °C. Input channels were connected to 5 mL syringes half-filled with treatment buffers through Tygon tubing. The waste channel was attached to vacuum via a syringe. Other channels were connected to buffer tubes with vacuum applied by syringes for sample output collection (Figure S-9). The time and channel program was designed so that islets were incubated with one of the input solutions for 5 min, followed by sampling the buffer into one of the output channels with 2 rinses in between treatments. ~10 islets were loaded into the tissue culturing region on the μ MUX device (Figure S-8). After the islets were starved on-chip in BMHH buffer (3 mM glucose, 0.1% BSA) for 30 min to 1 h, the device was then set for fully automated operation by the user-defined program. Solutions in output tubing were then collected into the buffer tubes (Figure S-9). The insulin levels were measured using a homogeneous fluorescence assay (Human Insulin FRET-PINCER Assay Kit; Medimotics, St. Louis, MO) analysed with minimized background interference using our recently developed thermofluorimetric analysis protocols^{35, 36}.

Fatty acid uptake analysis

Real time fatty acid uptake by adipose tissue explants was measured either with a kit (QBT Fatty Acid Uptake Assay Kit, Molecular Devices) or with a custom fluorescence quenching assay³⁷. Each method used bodipy-labelled laurate (cell permeable fatty acid analogue, FFA*) and a cell-impermeable fluorescent quencher. The μ MUX device was mounted within a microscope stage-top incubator (Tokai Hit, Japan) held at 37 °C, and input channels were connected as before. The treatment buffer consisted of serum free DMEM, either 2 μ M of FFA* or unlabelled free fatty acid (FFA), 1 μ M quencher, and five different levels of glucose and insulin (3 mM and 50 pM, 7 mM and 0.5 nM, 11 mM and 1.0 nM, 15 mM and 1.5 nM, and 19 mM and 2 nM). The waste channel was attached to vacuum via a syringe. 3-mm diameter adipose tissue explants were removed from storage serum media, washed 3 $\times$ with fresh serum free media, and pre-treated in serum free DMEM buffer with 3 mM glucose, 0.5 nM insulin and 2.0 μ M FFA for 30 min. Each explant was then washed with serum free media and placed on a μ MUX device fabricated without 3D printed templating, where a platinum mesh was used to hold the explant in place. In this instance, the μ MUX device was semi-automatically controlled using the electrode wires for “full” sensing. During emptying, tubing connected to the waste channel was monitored by eye, since fluorescence imaging precluded the use of real-time DIC imaging of the reservoir bottom. All solutions with FFA* or FFA were held at 37 °C and alternatively pulsed onto adipose explants as fluorescent images were captured using a 10 $\times$ objective and FITC filter set every 20 second. Images were analysed using ImageJ and Microsoft Excel.

Results and discussion

Microfluidic Multiplexer (μ MUX) Device Design

Pneumatic microvalve-based microfluidic multiplexers were introduced soon after the invention of PDMS valving³⁸. As in analogous electronic components, the microfluidic multiplexer can address large numbers (n) of fluid channels with a smaller number ($2 \log_2 n$) of pneumatic controls and is normally used as one utility component of larger, integrated microfluidic frameworks³⁹. More recently, others have demonstrated that the microfluidic multiplexer is valuable as a stand-alone component for automated handling of fluids and cell culture substrates^{40–42}. Based on this concept, we designed our microfluidic multiplexer (μ MUX) chip for delivering treatments and sampling secretions from tissues, serving as a kind of mimic of the circulation in the endocrine system.

The 16 fluidic channels, which were addressed by 8 pneumatic control channels ($2 \log_2 16 = 8$), served as either inputs or outputs for automated perfusion and sampling of endocrine tissue. For more economical space management, the standard multiplexer design was modified into a radially symmetric design surrounding a centralized tissue culture region (Figure 1A). This approach reduced the channel footprint and minimized channel lengths, thus minimizing dead volume between stimulants or samplings. Since the fluidic channel cross-section was measured to be $7580 \pm 150 \mu\text{m}^2$ (Figure S-2), channel volumes could be accurately calculated. Switching between two different inputs (see Video S-3) for varying stimulation to cells, the carry-over volume from the first stimulant to the next would range from 31 to 68 nL (Figure S-3). Even without wash steps included, this dead volume represented a negligible fraction, merely 0.15% to 0.34%, of the typically transferred reservoir volume of 20 μL . Note that reservoir carry-over was addressed later by including wash steps (ESI and Figure S-6A). The design also allowed higher density arrangement of fluidic reservoirs and/or tubing interfaces (filled black circles in Figure 1A; smaller ports in Figure 1B).

Another important design component in the μ MUX was the macro-to-micro interfacing at the central tissue culture reservoir, sculpted using 3D-printed templates as recently described¹¹. By changing the design of 3D-printed templates, various reservoir geometries that were fit to different cell types could be directly molded into PDMS by the templates, as further described in Figures S-1 and S-12. This interface design was constrained by needs for adequate temporal resolution as well as sufficient sample volume collection for hormone assays. In contrast to continuous sampling systems^{9–13, 15–18}, the μ MUX operated through discrete sampling defined by switching of solution every few minutes. To avoid known issues with sheer stress on pancreatic islets¹² during rapid switching, a tissue culture/trapping region was separated from the fluidic exchange interface. As shown by the 3D CAD rendering in Figure 1C and the device cross-section in Figure 1D, the solution in the central reservoir could be quickly drained and replaced—through an exchange via to microchannels—without significantly disturbing islets (or other tissues) sitting in the culture/trapping region. An added advantage of this 3D-templated interface is the ability to customize to the tissue or cells of interest.

This 3D-templated, μ MUX device was thus customized for interfacing to a centralized tissue culture reservoir with minimal sampling or stimulation dead volume from 16 input/output channels. As discussed below, this system could be automated for interrogation of tissue to serve as a highly flexible mimic of the circulation in the endocrine system.

System Automation

The μ MUX was operated through discrete sampling defined by drainage and replacement of the reservoir's solution every few minutes, as noted above (see Video S-1 for an example of operation). The tissue of interest was bathed in one solution introduced from a μ MUX input channel, and following the designated time, all solution in the reservoir was removed and collected in tubing for downstream assays using one of the μ MUX channels as an output. It is important to note that islets were never exposed directly to air, and adipose tissue explants were only briefly exposed to air and were maintained in a wetted state throughout experiments. With pneumatic micro-valving operated by computer controlled solenoids, cell media could be digitally controlled with minimal dead volume in a highly flexible manner. However, to fully automate this system, it was necessary to include real-time sensing of the solution level in the central reservoir. Peristaltic pumping with valves (3-valve pump) was not suitable to meet the flow rate requirement ($\sim 20 \mu\text{L}$ in <10 s), and our discrete sampling method is less compatible with constant-flow syringe pumps. By clearly defining starting and ending reservoir volumes (i.e. "empty" or "full"), the μ MUX could be fully automated using the same software that was used for valve control.

As shown in Figure 2, a customized "full" sensor was fabricated based on solution conductivity⁴³. Two gold electrodes were positioned onto the PDMS substrate and into the central device reservoir. A 5 V digital output (DO) line from a USB DAQ device (USB-6008, National Instruments) was applied to one electrode, and the other electrode was attached to a 1 M Ω resistor. Upon filling of the reservoir to the point of contact with both electrodes, the circuit (Figure 2B) was completed, placing the buffer solution in series with a 1 M Ω resistor in a voltage divider configuration. By measuring the voltage drop across the fixed resistor using an analog input (AI) line, a simple thresholding algorithm could be used to electrically detect the "full" state of the reservoir in a straightforward and robust manner. In initial testing, an "empty" sensor was validated with aqueous buffer using the same voltage divider concept, but with an electrode at the bottom of the reservoir (Figure S-11). Unfortunately, the BSA-containing cell media exhibited surfactant effects that resulted in irreproducible wetting of the electrode surface, making consistent "empty" sensing less desirable with this approach.

The final "empty" sensor design employed optical imaging, taking advantage of the patterned reservoir bottom, which was moulded with a 3D-printed template. Details in the surface pattern of the reservoir bottom were nearly invisible when solution was present, due to the similarities in refractive index (η) between PDMS ($\eta_{\text{PDMS}} = 1.4$) and water ($\eta_{\text{water}} = 1.33$). Upon emptying the reservoir, however, PDMS-to-air interfaces were easily detected by imaging in differential interference contrast (DIC) mode ($\eta_{\text{air}} = 1.00$). One of various feature edges could be imaged, and the standard deviation of the region of interest (ROI) was

used as a robust, real-time indicator of the “empty” state of the reservoir, as shown by the images and data in Figure 2C–E.

The in-house written control program (LabVIEW) and hardware consisted of six main sections: (1) A software front panel with parameter inputs for assigning each microchannel as a fluidic input or output, setting the cut-off values for voltage or ROI standard deviation, and selecting the ROI for image analysis; (2) a time and channel program input array for stepwise chip operation; (3) valve control hardware for operating the fluidic channels through the DAQ system; (4) the conductivity sensor for the “full” signal voltage readout; (5) a camera interface for real-time image grabbing as the “empty” sensor; and (6) a software logic loop (flowchart in Figure 3A) to interpret the various inputs and sensors for automation of the μ MUX system. As shown by representative automation data in Figure 3B, real-time measurements from the “full” sensor (voltage drop; orange trace) and the “empty” sensor (σ_{ROI} ; green trace) were the primary drivers of system automation according to the programmed logic in Figure 3A. Data from ~3 min of an automated test run—in temporal sampling mode—are shown in Figure 3B. Channel numbers are represented as blue diamonds; channels 0 and 15 were set as input fluidic lines, channels 1 through 4 were set as output fluidic sampling lines, and “channel” 16 was used as a code for closing all valves. This test run automatically switched channels in the following channel order: 15, 1, 15, 2, 15, 3, 0, 4, 0. Complete data sets from this control system collected during endocrine tissue sampling or imaging are included ESI (Figure S-7).

μ MUX Device Optimization

Continuous flow microsystems^{10–13, 15–18, 20} for sampling endocrine cells require a relatively slow flow rate to protect cells from shear force induced damage. By contrast, in this μ MUX design (Figure 1D), cells resting at the bottom of the culture/trapping region were shielded from shear stress, even at much higher flow rates. However, the design imposed an upper limit on the depth of the culture/trapping region. In order for stimulants to reach the cells quickly and for secreted hormones to be sampled with adequate temporal resolution, this region should be as shallow as possible, yet without washing away the cells or creating shear stress problems.

To optimize the depth of the cell culture/trapping region, devices with depths between 0.47 to 1.24 mm were fabricated using 3D-printed templates (Fig. S-4 and S-5) and tested by operation with trapped murine islets. Devices with the shallowest trapping regions (<500 μ m) were non-functional due to intermittent loss of islets during flow, but regions at or above a depth of 0.57 mm showed consistent retention of the cells. Next, to ensure acceptable temporal resolution during sampling, a novel method to mimic islets was devised. pH-responsive beads with a diameter distribution very similar to islets (100–200 μ m) were synthesized by reacting glass beads with (3-Aminopropyl)triethoxysilane to introduce amine groups, followed by a reaction with FITC to create a thiourea linkage to a fluorescein moiety (Fig. 4A–D). Since the phenolic pKa of fluorescein exhibits acid-base equilibrium, its fluorescence intensity is dramatically reduced at acidic pH⁴⁴. By programming the μ MUX device to switch between buffers of pH 5 and 9, this pH dependence was exploited to mimic the timing of islet stimulation (see Video S-4). At various culture/trapping region depths, the

bead fluorescence intensities were increased to a maximum value following introduction of pH 9 buffer, indicating complete solution exchange (Fig. 4E). Relatively rapid exchange (~30 s) was observed with depths of 0.48 and 0.57 mm, while deeper reservoirs caused proportionally longer solution exchange times. As the 0.57 mm depth was shown to alleviate islet loss during flow and to allow ~30 s solution exchange, this depth was chosen for all experiments to follow.

Sampling volume consistency and solution carry-over were also optimized. The sampling volume was defined largely by the difference in solution levels marked by triggering of the “empty” and “full” sensors, but there was some dependence on the pressures and vacuum applied to input and output channels, respectively. As long as vacuum and pressure levels were relatively unchanged during the experiment, however, the variance in sampled volume was <1% on a given μ MUX device (Figure S-6B). To avoid carry-over of treatments and samplings, particularly within the culture/trapping region, reservoirs were quickly emptied and refilled three times with the next treatment solution during μ MUX operation, i.e. two rinsing steps and an incubation step. This approach was confirmed to decrease carry-over to <1% between treatments after the second rinse step (Figure S-6A).

Temporal Sampling Mode of μ MUX: Dynamic Islet Function

The islets of Langerhans are composed of five different endocrine cells (α , β , δ , PP, and ϵ cells), each of which secrete characteristic hormones in response to metabolic changes, as well as other cells including vascular cells, resident immune cells, neurons, and glial cells⁴⁵. β cells secrete one of the more dominant endocrine hormones, insulin; defects in either secretory mechanisms or peripheral tissue responses to insulin are primary causes and/or symptoms of diabetes. With renewed interest in the blood glucose abnormalities induced by diets high in sucrose or refined carbohydrates^{6, 7} there is a need for robust, programmable tools to study dynamic islet function. The input/output μ MUX device presented herein was specifically tailored as a tool capable of not only introducing customized temporal patterns to the cells but also temporally sampling cellular secretions. The high flexibility and programmability of this digitally-operated device is a key advantage compared to other microdevices previously used to study islets^{9–13, 15–18, 20}. The 16 fluidic channels could be assigned as either inputs for treatments or outputs for sampling, allowing—for example—accurate mimics of postprandial blood glucose and gut hormone (incretin) levels.

To demonstrate the temporal sampling mode of the μ MUX device, dynamic insulin secretion was first measured during treatment of islets with square waveforms of glucose at physiologically relevant concentrations (Figure 5A). In this instance, the system was programmed with 2 input channels (3.5 mM and 19.5 mM glucose), 13 timed outputs for temporal secretion sampling, and 1 waste channel. The μ MUX was operated under a square wave glucose program in automated fashion to collect ~90 minutes insulin secretion profiles from groups of ~10 islets. Islets responded with glucose-stimulated insulin secretion profiles that mirrored the input glucose waveforms, increasing to approximately 30 pg islet⁻¹ min⁻¹ then decreasing again within a few minutes of the glucose changes (Figure 5A). To confirm the device's flexibility in temporal sampling mode, the program was then modified to 3 input channels (3.5 mM glucose, 19.5 mM glucose, and 19.5 mM glucose with 25 mM KCl),

12 timed outputs, and 1 waste channel. In this way, glucose concentration could be increased and held at the high level while adding KCl (Figure 5B). Again, for all groups of islets, the increased glucose levels resulted in similar levels of secreted insulin (17 to 32 pg islet⁻¹ min⁻¹). When 25 mM KCl was added, insulin secretion was increased even further to as high as 65 ± 5 pg islet⁻¹ min⁻¹ due to exaggerated depolarization of the cell membranes, which enhances insulin vesicle recruitment. These results confirmed the μ MUX device to be suitable for automated, microfluidic studies of insulin secretion dynamics.

Temporal Stimulation and Imaging Mode of μ MUX: Dynamic Adipose Tissue Function

Although the principle function of white adipose tissue was traditionally assigned to energy storage, it is now understood that the tissue is a complex, multicellular endocrine organ with hormonal effects that modify the function of nearly all other organ systems¹. In comparison to the case of pancreatic islets, however, there have been relatively few studies focused on the dynamic function of white adipose tissue. We have recently shown that the microfluidic platform is well suited for interrogating this tissue^{11, 22}, yet limited dynamic information was collected due to device limitations. Insulin is a dominant endocrine hormone, and its secretion by pancreatic islets is dynamically modified based on nutrient levels in the bloodstream, as evidenced from data shown in Figure 5. To improve our understanding of the dynamic response of adipose tissue to this hormone, the μ MUX device was programmed to mimic postprandial glucose and insulin levels and to apply these solutions to small samples (3-mm biopsies) of adipose tissue explants extracted from mice. At the same time, the system was programmed to facilitate functional imaging of the explants.

Recent work has shown that fatty acid uptake can be visualized with fluorescence imaging by using bodipy-modified free fatty acids (FFA)⁴⁶. Exploiting the inherent flexibility of the μ MUX device, an improved version of FFA uptake analysis was developed which permits real-time measurement of uptake rates. By alternatively pulsing labelled and unlabelled free fatty acids, uptake and release rates could be directly imaged as a function of treatments. An additional benefit of the μ MUX system was revealed here, i.e. that the tissue is only minimally disturbed during automated operation, allowing high resolution fluorescence imaging in real time.

As such, the μ MUX was operated in temporal stimulation and imaging mode to mimic postprandial glucose and insulin levels during real-time FFA uptake imaging (Video S-5). This mode required at least 10 of the channels to serve as inputs comprised of five different concentrations of insulin (0.05 – 2.0 nM) and glucose (3.0 – 19.0 mM), each with either labelled (FFA*; bodipy-laurate) or unlabelled (sodium laurate) free fatty acids. The sequence of programmed treatments and input channel numbers during explant imaging is shown in Figure 6A. In other words, the 16-channel μ MUX permitted a well-resolved, dynamic input program to be applied to cells with up to 4-bit resolution during real-time imaging. This program was designed to closely mimic the timing and magnitudes of serum glucose and insulin levels following a meal. As shown in Figure 6B, the adipose explant began with an initial rapid uptake of available FFA followed by insulin-dependent exchange rates throughout the treatment program. The fluorescence intensity data (purple) was processed by measuring the initial uptake or release rate as a linear slope over a five-minute window,

and the absolute values of these slopes were plotted over time (blue). These absolute slopes represent relative FFA exchange rates (uptake or release) between the cells and surrounding solution. Example explant images are shown in Figure 6C, and an insulin dependence plot is shown in Figure 6D. Interestingly, both uptake and release rates were observed to follow the pattern of glucose and insulin, in agreement with initial tests of the methodology (Figure S-10). These results suggest that FFA exchange machinery, e.g. FFA transport protein function, is temporally dependent upon insulin and glucose magnitudes with dynamic fluctuations even at the ~10-min time scale. Enabled by the microfluidic platform, these measurements represent the first observation of real-time FFA exchange as a function of programmable, dynamic glucose and insulin inputs.

Conclusions

A microfluidic multiplexer device (μ MUX) with a customized, 3D-templated tissue culture interface was developed and proven feasible for dynamic and quantitative measurements of both hormone secretion and nutrient sensing/uptake from two types of primary murine tissues. The device not only permitted confirmation of the dynamic function of pancreatic islets, but it also allowed new information to be gathered on temporally-resolved free fatty acid exchange in adipose tissue. These results reinforce the generalizability of the μ MUX device, which should be translatable to other tissues such as liver, heart, skeletal muscle, etc. The device could also be envisioned as a single module in future integrated devices with, for example, on-chip bio-sensing or in-line separations.

From an analytical standpoint, modest increases in the number of pneumatic valves should dramatically improve μ MUX resolution in the future. 32 fluidic channels could be controlled by 10 pneumatic channels ($2 \log_2 32 = 10$), 64 controlled with 12 valves ($2 \log_2 64 = 12$), and so on. This would allow even finer stimulant gradients (e.g. post-prandial insulin/glucose) to be introduced to cells and would also promote increased temporal resolution on cell secretion sampling, all while maintaining the precision and flexibility of a digitally-controlled device. A reduction in tissue culture reservoir volume would also help improve input/output resolution. Nonetheless, as presented herein, the 16-channel μ MUX is well-poised for a variety of novel dynamic studies on endocrine tissues.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. μ MUX device design. **(A)** Schematic of the μ MUX channel layouts, with fluidic channels shown in black and pneumatic control channels in red. **(B)** Photo of assembled devices with and without 3D-templated interfaces. **(C)** CAD rendering of the 3D-printed template and **(D)** example device cross-section with customized, 3D-templated tissue culture reservoir and channel interface.

Figure 2.

Automated solution level sensing. **(A)** Photo of working device in the microscope stagetop incubator. Tubing was connected to both control channels and valved fluidic channels. Two gold wires were positioned near the top of the 3D-templated reservoir to serve as the “full” sensor. **(B)** Circuit diagram of voltage divider for conductivity sensing as the “full” signal trigger. The source wire was connected to a digital output line of the NI-DAQ for voltage supply, and the sensing wire was connected to a 1 MΩ resistor across the differential analog input for voltage readout. **(C)** Microscope (bottom view) images of the μMUX tissue culture reservoir. Two possible ROIs (red and yellow regions) were analysed in real time for the “empty” signal (see Video S-2). **(D)** Corresponding edge detection images used for ROI analysis. **(E)** Representative data shows the standard deviation of pixel intensities within the example ROIs (red and yellow), and the “empty” trigger cutoff value (gray dotted line) is shown for the ROI with the most obvious change (red).

Figure 3. μ MUX system automation. **(A)** Programmatic flow chart LabVIEW application used for automation. Each of the 16 channels was assigned as input or output by a user-defined program. Depending on the input assignments and incubation/wait times, the controller followed the depicted logic flow until completion of a run. **(B)** Automation data from a test run of the μ MUX. The imaging-based “empty” sensor (green) and the conductivity-based “full” sensor (orange) readouts were used to trigger sequential steps in the programming. With channels 0 and 15 as inputs, channels 1 to 4 outputs, and channel 16 as a “close-all-valves” code, this test run automatically switched channels (blue diamonds) in the following order: 15, 1, 15, 2, 15, 3, 0, 4, 0. Complete automation data sets are shown in ESI, Figure S-7.

Figure 4.

Islet mimics for solution exchange experiments. **(A)** Scheme of pH-responsive bead synthesis. **(B)** A DIC image, **(C)** fluorescent image, and **(D)** combined image of modified beads mixed with untreated beads. (Scale bar is 100 μm). **(E)** Representative fluorescence intensities from pH-responsive beads placed in the tissue culture region of the μMUX device during automated operation. Solution exchange times were optimal (~ 30 s) for devices with culture region depths of 0.48 mm and 0.57 mm. Deeper culture regions resulted in unacceptably long exchange times.

Figure 5.

Temporal sampling mode of the μ MUX. **(A)** Dynamic insulin secretion sampling with 13 timed output channels was accomplished with a programmed glucose square wave treatment from 2 input channels. **(B)** Dynamic insulin secretion sampling with 12 timed output channels using programmed glucose and KCl treatments from 3 input channels. In both **(A)** and **(B)**, upper right images show the channel assignments, while lower right box plots show aggregate cellular responses.

Figure 6.

Temporal stimulation and imaging mode of the μ MUX. **(A)** Stimulation of adipose tissue explants with a mimic of post-prandial insulin and glucose at 5 different magnitudes was accomplished while sequentially pulsing labelled (FFA*) and unlabeled free fatty acids (10 timed input channels). **(B)** Adipose tissue responded with insulin-dependent FFA exchange rates that closely followed the input pattern, with dynamic fluctuations even at the ~10-min time scale. **(C)** Representative fluorescent images of the tissue during FFA* uptake. **(D)** Insulin dependence plot using compiled data. **(E)** Assignment map showing the required 10 timed input channels and 1 waste channel.