The islets of Langerhans are regions of the pancreas containing hormone-producing cells. Within the islet, β-cells maintain glucose homeostasis by responding to and controlling moment-to-moment changes in glucose levels through the secretion of insulin. Permanent disruption of this regulatory system consequently results in the onset of diabetes, which is one of the most common metabolic diseases and afflicts millions of people worldwide.

In vitro models allow the manipulation of relevant biological and chemical cues and provide a basis for understanding β-cell physiology and pathophysiology. This research is usually carried out using conventional static wells or plates and the outcomes are analyzed by biological and biochemical approaches. However, under normal in vivo conditions, islets experience a dynamic environment in which insulin is secreted in a biphasic, often oscillatory pattern. Therefore, it would be advantageous to have a laboratory setup that more closely mimics the in vivo environment through the precise control of physiologically relevant parameters.

The goal to better understand the kinetics of insulin secretion from isolated islets in response to insulin secretagogues provides the impetus for the development of in vitro devices with a perfusion design, in which culture medium flows around the islets in a continuous-flow chamber. The term 'perfusion' is used to distinguish this method from the perfusion of medium through the vascular system of an intact pancreas. Several macro-scale perfusion systems have previously been established, with the capability to regulate temperature, pH and flow rate of the perfusate and the ability to switch between perfusate streams of various stimuli [1–4]. The main challenges that these systems face include difficult operation, limited flow control, inadequate mimicking of the in vivo microenvironment and a lack of integration with conventional analysis techniques.

Microfluidic technology is a special class of bio-microelectromechanical systems (Bio-MEMS). In recent years, this technology has emerged as a valuable tool for a wide range of analytical applications, mainly due to its versatility and enhanced efficiency. Microfluidic devices allow the consumption of minimal reagents and analytes. Their small scale also allows leveraging of microscale phenomena, such as laminar flow [5–7] and rapid diffusion [8]. This allows the implementation of new experimental modalities currently not possible with available macroscale tools. In addition, multiple tasks or analysis tools can be integrated onto a single device to improve experimental throughput [9,10].

Almost 10,000 papers have been published over the last 10 years on the topic of microfluidics [11] and the number of new publications...
**Microfluidic technology**

Microfluidic techniques entail the design, fabrication and application of microfluidic devices composed of either polydimethylsiloxane (PDMS; sometimes in combination with glass components) or Borofloat glass, only the fabrication of these devices will be briefly described.

**PDMS devices**

Due to the ease of prototyping, PDMS has now become the de facto microfluidic platform for biomolecular applications [14]. A typical PDMS fabrication workflow begins with an SU8 master, followed by PDMS curing, and ends with PDMS–glass bonding, as shown in Figure 1A [15,16].

SU8 photoresist has become the standard for PDMS molding owing to its high-aspect ratio and stiffness as a permanent, re-useable master. To construct the master, designs are first drawn in appropriate software and translated onto a photomask, either by transparency printing or by standard chrome mask lithography. Then, SU8 photoresist is spun on a dehydration-baked, polished silicon wafer and further soft baked to remove solvent impurities. After this soft baking, the photomask is placed over the photoresist and exposed to UV light (by lamp or contact aligner), irradiating the resist with the desired geometry by photo-polymerization of the SU8. A postexposure bake then further cross-links the UV exposed area and the unexposed resist is washed away by the developer solution, leaving a 'negative' image of the photomask channel structure.

After making the master, a PDMS precursor (e.g., Sylgard 184) and cross-linkers are added at a 10:1 ratio and thoroughly mixed. A vacuum chamber is used to remove the resultant bubbles and the clear PDMS mixture is poured over the SU8 master. After overnight curing at room temperature or 2 h on an 85°C hotplate, the PDMS is completely cured, retaining the channel structures from the master. After cutting the bulk PDMS into separate devices, circular access ports are punched and the device is cleaned with a piece of scotch tape to remove any dust or debris from the surface. The device and a cleaned piece of glass are then plasma treated to create hydroxyl radicals, which allow permanent chemical bonding of the PDMS to glass.

**Borofloat glass devices**

Although PDMS is used more often in the fabrication of microfluidics, glass microfluidic devices provide robust and chemically resistant microchannels. Glass devices, fabricated with anisotropic etching via hydrofluoric acid (HF), are better suited to linear microfluidics [17,18], while reactive ion etching (RIE)-based glass devices can provide a denser network of microfluidic channels [19]. Glass device fabrication typically includes lithographic transfer of an etch mask layer and bulk etching (HF or RIE), followed by direct bonding encapsulation of the finished device. An example workflow of HF-etched channels is shown in Figure 1B.

The fabrication of a Borofloat device also requires a photomask. Typically, a chrome mask is used to ensure clean features for etching. After obtaining the photomask, the device fabrication begins with a sulfuric acid cleaning of a blank Borofloat wafer. An etch mask layer is then deposited onto the cleaned wafer. Several types of masking layer can be used, such as the hard-baked photoresist for geometric features with resolution of 15 µm or less and the widely available chrome-evaporated substrates for geometric features with a resolution of approximately 35 µm [20,21]. However, chemical vapor deposited amorphous silicon provides the best HF etch resistance [22] and is used as the masking layer example here. After the deposition, standard photoresist is spun onto the Borofloat/silicon amalgam and exposed with the photomask in the same process as described above. The pattern on the resist is then transferred to the layer of
amorphous silicon by RIE to open up the channel areas for etching. The exposed Borofloat surface in this silicon etch mask is then submerged in a 49% HF solution for etching, forming round-bottomed microfluidic channels. In addition to the standard HF-based Borofloat micro-fabrication presented above, there are processes that use readily available chrome-coated substrates to simplify the fabrication [21]. However, the concept of a metal/silicon mask for HF etching is the same as described. Alternatively, the RIE process can form thinner (<10 µm) channels using standard photoresist as an etch mask to fabricate rectilinear, flat-bottomed micro/nanofluidic channels [23]. Specialized deep-RIE (DRIE) etching can also be used to etch even greater depths (~100 µm). After patterning the channels in the structural substrate, a blank substrate is bonded on top to seal the device. This substrate is also cleaned by sulfuric acid and drilled for access ports before bonding. Bonding is typically performed with anodic bonding or high-temperature annealing in a vacuum oven [24].

- Advantages of microfluidic technology
In general, the use of microfluidic devices offers a number of distinct advantages over conventional techniques for biological research. Because microfluidic devices inherently involve a much smaller setup, the amount of reagents and analytes used is quite small. This is especially significant for experiments requiring expensive reagents. The fabrication techniques used to construct microfluidic devices are favorable for mass production and provide a flexible substrate from which highly elaborate, multiplexed devices can be built. In addition to size and planar geometry, the transparency of PDMS and Borofloat glass microfluidics allows the easy integration of bright field and fluorescence microscopy or spectroscopy for analysis of the materials within the microchannels.

The patterns of laminar flow in microfluidics are completely predictable due to the small dimensions of the microfluidic channel. The laminar nature of the fluid flow enables the performance of unique experiments that are difficult or nearly impossible to conduct using conventional approaches [25,26]. For applications that necessitate the mixing of different reagents, two parallel streams entering a microfluidic channel can be mixed either by simple diffusion or by chaotic mixing, through the designs of microfluidic device geometry with off- and on-chip mixers and valves [27–29].

Another important feature of microfluidics is that it can provide a suitable platform for both high-throughput and comprehensive studies. Specifically, it enables the evaluation of the effects of multiple factors and parameters on a single assay. Indeed, consistent endeavors have been made to merge microfluidics with various technologies, such as microarray-based bioassay, electrochemical detection and mass spectrometry (MS) [10,30–33].

β-cell physiology & microfluidics in islet study
- Characteristics of β-cell insulin secretion
Islets, which range in size from 50–300 µm, consist of 1000–2000 cells that are made up of at least five different cell types. Approximately 65–80% of these cells are β-cells, which play a central role in the control of the body’s metabolism through their insulin secretion. The insulin release is controlled by β-cell electrical activity, metabolic events and ion signaling, which display...
the complex kinetics of biphasic and pulsatile profiles in response to the moment-to-moment changes of glucose level [34,35]. Insulin secretion is a complex and dynamic process. In short, glucose catabolism generates ATP through the mitochondrial tricarboxylic acid cycle, which consequently closes ATP-sensitive K+ (K<sub>ATP</sub>) channels, initiates plasma membrane depolarization and increases [Ca<sup>2+</sup>], through the rapid influx of calcium ions via voltage-dependent calcium channels (VDCC). This glucose-stimulated increase in [Ca<sup>2+</sup>], triggers the fusion of the insulin granules with the cell membrane and, subsequently, the exocytosis of insulin, C-peptide and proinsulin, as shown in Figure 2A [36,37]. Alternate pathways for insulin secretion, independent of K<sub>ATP</sub> and [Ca<sup>2+</sup>]<sub>i</sub>, have been described. However, the K<sub>ATP</sub> and [Ca<sup>2+</sup>]<sub>i</sub>-mediated pathways remain the primary mechanism of glucose-stimulated insulin secretion [38].

Rapid and sustained stimulation of β-cells with glucose induces biphasic insulin secretion. The first phase corresponds to a prompt, marked increase in the secretory rate that is transient (4–8 min). This is followed by a decrease to nadir and a sustained flat, or gradually increasing second phase that lasts as long as the glucose simulation is applied (Figure 2A). The pulsatility of insulin secretion is reflected in oscillations of plasma insulin concentration. Simultaneous measurement of [Ca<sup>2+</sup>]<sub>i</sub> and insulin secretion have shown that [Ca<sup>2+</sup>]<sub>i</sub> oscillations are synchronous in all islet regions and that each oscillation is accompanied by an oscillation in insulin secretion. The oscillations consist of fast oscillations, with a period of 1 min or slow oscillation, with a period of 2–7 min, or a combination of the two [39]. Ca<sup>2+</sup> is the direct regulator of insulin pulsatility. Dissociations between the two phenomena have been reported, suggesting that other glucose-induced metabolic signals can also drive oscillations of insulin secretion. However, their efficacy has been demonstrated to be less than that of [Ca<sup>2+</sup>]<sub>i</sub> oscillations [40].

Microfluidics design for β-cell study

The use of perfusion systems to understand β-cell kinetics in vitro has been a key factor in the elucidation of islet physiology and pathophysiology. Microfluidic devices can be considered miniaturized versions of perfusion apparatus with the additional benefit of the ability to simultaneously conduct other assays, such as fluorescent assays or electrophoretic analysis of secreted products. Furthermore, the micro-dimension of the channels and chambers, which range in size from a few micrometers to hundreds of micrometers, are compatible with islet size (50–300 µm in diameter) and also well-matched to in vitro flow volume of the pancreatic vascular system. Therefore, microfluidic devices could provide an ideal platform for mimicking the in vivo microenvironment to perform islet immobilization, perfusion and analysis in vitro.

The basic principles of designing such miniaturized microfluidic devices for islet studies rely on the following important goals:

- The successful immobilization of islets and optimal maintenance of microenvironments;
- The capability to continuously assess β-cell physiological events via perfusion flow dynamics, in order to resolve the rapid secretory and metabolic waveforms intrinsic to β-cells;
- The easy integration of single or multiple analytical tools;
- A high degree of spatio-temporal resolution of the analytical tools.

Islet immobilization

A suitable design for the immobilization of islets is one of most important requirements and also one of the biggest challenges for the success of these devices, owing to the unique cellular composition and 3D cytoarchitecture of islets.

In their earlier work, the Kennedy group designed a Borofloat mini-scale perifusion chamber (300-mm diameter) containing a single islet with an 80-ml fluid reservoir above [41,42]. In this device, pressure-driven fluid bathed the confined islet via perifusion channel, and released insulin was sampled by electro-osmotic flow (EOF). Later, the group developed a consisting of four individual channel networks. A single islet per channel was housed and immobilized using a PDMS plug in the channel. The islet was continuously perfused and insulin perfusates were pulled into the reaction channel where they mixed with fluorescein isothiocyanate-labeled insulin and anti-insulin antibody for insulin quantification [17]. In 2009, a similar device was scaled-up to accommodate 15 channels with improved throughput and automated online insulin assay (Figure 3A) [21]. The Piston group designed a 6-port PDMS device for a single islet study. The islet was loaded manually into a channel and the top wall of the channel was actuated for immobilization (Figure 3B) [43].
The clear advantage of this plug/wall approach is that the islet can be precisely located, as governed by design and is favorable for single islet study, however, precautions must be taken to prevent and minimize mechanical stress. Islets are vulnerable to mechanical stress, even minimal stress can cause islet damage and insulin release, especially within a device equipped with pressure-driven flow control.

As an alternative, others have developed an islet-trapping method \cite{44,45}. This involves an array of small circular wells (pockets), which are 150-µm deep and have a diameter of 500 µm, located at the bottom of a perfusion chamber (Figure 3C). This design allows the islets to passively sit and react to medium in a manner that is analogous to the in vivo scenario and interface with the perfusing
medium. In addition to avoiding unnecessary mechanical stress and sheer force, this method is simple to use. Multiple islets can be analyzed simultaneously, which increases analytical throughput. However, this device pools the secreted products together, which can either be an advantage or a disadvantage, depending on the intended application. Another approach is an open-channel device, in which islets are cultured on a glass-bottom dish or coverslip and the apparatus is placed over the islets and held in place by surface tension, as shown in Figure 3d [46]. This device and its network also involve the generation of fluid plugs that both stimulate and serve to concentrate the islet secreted factors. These fluid plugs can then be split off and multiple analysis methods can be performed simultaneously. This eliminates the need for islet loading, which is an additional advantage that the open-channel device has over closed-channel devices.

Fluid control

It is necessary to consider the integration of stable, flexible and desired fluid control into a microfluidic network for β-cell applications in order to study hormone kinetics in conjunction with metabolic and biochemical events in the β-cell.

Actuation of liquid flow can be implemented by external pressure forces (external and integrated pumps or micropumps) or by the combination of capillary forces and electrokinetic mechanisms. Microfluidic flow, driven by pressure, either continuous or stepwise, is a standard method that has proven adequate for many well-defined islet applications [21,44–46]. However, one of the drawbacks in using a syringe pump is the generation of significant flow oscillations due to perturbation of the setup, especially at low flow rates.

To mitigate this general problem in other applications, efforts have been made to develop micropumps (reciprocating and continuous flow) to control fluid flow. Recently, the Roper group has applied the approach to a β-cell study, with a significant benefit for chemical gradient creation and maintenance [47,48]. EOF also plays an important role in the manipulation of liquid flow in microfluidic contexts. Since the electroosmotic velocities can be independent of channel size, whereas flow in pressure-driven devices is much more significant with large channels, the technique is beneficial when microchannels are small. For instance, this is often applied to chemical separation and capillary electrophoresis of secreted products [49,50]. The Kennedy group pioneered the application of EOF to microfluidics for perfusate insulin sampling and this will be discussed later.

Droplet-based microfluidic networks have also been used in creative ways for various chemical and biological applications, ranging from protein crystallization to compartmentalization of single cells [51–53]. The principal idea behind droplet-based microfluidics is the use of a single microscale fluid plug as a confinement chamber for biological assays and chemical reactions via precise droplet generation and spatial stabilization. In general, there are two approaches to droplet creation; pressure-driven and passive droplet generation. Each of these approaches has a specific set of advantages and disadvantages. Beyond the general advantages, such as reducing both reagent and sample consumption and enabling high-throughput analysis, the small liquid volume of the droplets has significantly enhanced the temporal resolution of secreted insulin samples in a way that is impossible to achieve under a continuous flow setup. The Piston group recently reported a novel vacuum-driven method for passive-droplet sampling to quantify pancreatic islet zinc secretion using a fluorescent indicator (FluoZin-3), with significantly improved
temporal resolution [54]. Under 11 mM glucose stimulation, this device has the capability to measure zinc secretion with a resolution as high as approximately 800 fg islet \(^{-1}\) min\(^{-1}\). The quantitative nature of this approach provides a useful model for understanding zinc/insulin cosecretion. Furthermore, the sampling technique may be applicable to a wide variety of biological systems where time-resolved cellular secretion must be measured in real-time. However, one disadvantage of the system is that the islets must be analyzed in a serial fashion and, therefore, a potential scale-up would be needed to increase its analytical throughput.

In 2008, the Ismagilov group developed the chemistrode, which is a microfluidic device incorporating droplet-based flow [46]. Instead of directly measuring β-cell electrical activity, this droplet-based microfluidic device can manipulate and observe molecular signals with high spatial and temporal resolution within droplets that continuously encapsulate, then separate from an islet. These droplets encapsulate and separate at a rate of 1.5 s for both stimuli pulse and secreted insulin. The ability to deliver molecular stimuli at a specific location and then record, store and analyze the pulses of molecules released from an islet could potentially revolutionize our understanding of β-cell physiology. It would also enable truly fascinating experiments in chemistry and biology.

It is worth mentioning that closed-channel systems are inherently limited by the physical walls of the device. Others have developed open systems with fluid-driven flow, which are generally referred to as digital microfluidic systems. These systems allow independent control of discrete fluid droplets that can be created and manipulated using electrowetting techniques [55,56]. While potentially useful, no digital microfluidic devices have been applied to islets or β-cells to date.

The integration of various mixers (off- and on-chip) can be attractive for a broad range of potential applications in islet studies. Despite the importance of chemical gradient generation in understanding β-cell kinetics, there are only a few existing microfluidic setups capable of generating and maintaining suitable chemical microenvironments. The perfusion system from the Oberholzer and Eddington groups has a simple design with a high degree of control over the shape of the glucose stimulation profile modulated through off-chip mixer and syringe pumps. It works via automated control of Labview software. Furthermore, this system demonstrates a flexible capability in chemical gradient creation with a high level of consistency (Figure 4A) [44,45]. Despite the use of a large chamber volume, it is still possible to measure characteristic biphasic insulin secretory patterns with adequate resolution of the kinetics to draw clinical conclusions. However, the large chamber volume limits rapid fluid exchange and both temporal and spatial resolution. In another approach, a microfluidic device is equipped with two on-chip diaphragm pumps and off-chip valves and is well characterized by comparing...
the amplitudes of a series of sine, triangle and sinusoidal waves to theoretical waveforms, as shown in Figure 4B [47,48]. This type of flow control is well suited for experiments that use small scales of volumes. However, this type of mixer has significant delay times for mixing and longitudinal diffusion due to the nature of valve-controlled pumps.

Integration of analytical tools & spatiotemporal resolution
Microfluidic devices offer tremendous potential for the on- and off-chip integration of electrical, optical and spectroscopic high-performance analytical tools. The ability to integrate multiple operations greatly enhances the capabilities of microfluidic tools in the fields of biomedical research, diagnostics and drug development. The complete and full review of various integrated analytical tools has been conducted elsewhere [27,57,58]. In this review, we will evaluate current existing integration techniques used for islet studies.

Real-time sensing and monitoring of electrical, biochemical and ion-signalling activities are critical for in vitro ß-cell research. The most common off-chip macroscale system is the use of optical detection because it provides a range of applications (absorbance, fluorescence and chemiluminescence) with low background noise and detection limits. The transparency and planar nature of Borofloat glass and PDMS provides an optimal platform for this integration. Of those, fluorescence detection – using fluorescence or confocal microscopy – is still the most widely used optical method for islet study due to its superior selectivity and sensitivity.

Many ß-cell cellular processes, including proliferation, exocytosis, metabolism, protein biosynthesis and gene expression are regulated by highly coordinated, spatio-temporal changes in [Ca\(^{2+}\)]\(_c\). ß-cell Ca\(^{2+}\) homeostasis and signal transduction are precisely controlled by ion transport mechanisms that facilitate the influx and efflux of Ca\(^{2+}\) into and out of the cytoplasm [59]. Glucose-stimulated calcium influx occurs in a biphasic fashion followed by two types of oscillation, slow (0.2–0.5/min) and fast (3–5/min). The shape and frequency of calcium oscillations mainly depend on the intrinsic characteristics of ß-cells and the nature or concentration of the extracellular signals [39,40]. In both the microfluidic and microfluidic environments, it is often a significant challenge to obtain a good spatio-temporal resolution of calcium signaling. With the use of calcium indicator dyes such as Fura-2 AM or Fluo-4 AM in a microfluidic device, the spatial resolution is often not a major concern due to the localized spatial proximity of the micro-device and the high selectivity and sensitivity of the fluorescence probes. On the other hand, temporal resolution of calcium signaling can be influenced or controlled by the flow dynamics of a specific microfluidic device. In microfluidics, the control of temporal resolution can be achieved by designing a microfluidic system with selective methods of medium delivery, valves and compartmentalization. Currently, most contemporary microfluidic devices sufficiently resolve the spatio-temporal resolution of calcium signaling, including the capability to detect fast and slow calcium oscillations (Figure 5A) [21,43,60], although, some do have limitations.

Another example of the use of a microfluidic environment to study calcium signaling was conducted by the Piston group to determine the role of gap junction-coupled K\(_{ATP}\) channel activity in the regulation of ß-cell insulin release. This experiment was conducted in a transgenic mouse model that expresses zero functional K\(_{ATP}\) channels in approximately 70% of ß-cells, but the residual 30% of ß-cells has normal K\(_{ATP}\) channel density. It was demonstrated that glucose-induced membrane potential, calcium influx and insulin release critically depend on the integrity of gap junctional coupling of K\(_{ATP}\) channel activity across neighboring ß-cells [61,62].

Zinc ions are co-secreted with insulin in the ß-cell and, therefore, the measurement of zinc secretion in response to insulin secretagogues can also be used as an assay for insulin secretion. Although the relationship between insulin and zinc is complex, zinc plays a clear role in the synthesis, storage and secretion of insulin, as well as in the conformational integrity of insulin in its hexameric form. Recent studies have demonstrated that zinc has a profound autocrine regulatory influence on insulin secretion via ATP-gated and zinc-modulated P2XR channels [63] and the deletion of the mouse Slk30a8 gene, encoding zinc transporter-8, results in impaired insulin secretion [64]. Using laser-scanning confocal microscopy combined with various fluorescence probes such as FluoZin-3 and Zinquin, the Kennedy group successfully identified that zinc efflux corresponds to the exocytosis of insulin/zinc-containing granules, and that glucose-induced VDCC activation is localized to specific membrane domains where exocytotic insulin release also occurs [65,66]. More importantly, it
was found that the temporal and spatial kinetics of zinc are controlled in agreement with insulin release profile [67]. A microfluidic droplet-based device with a volume of approximately 0.47 nl and a higher capability of temporal resolution was conducted by the Piston group to study zinc release from \( \beta \)-cells. The study successfully identified two classes of secreted zinc oscillatory waveforms from a single islet: fast (~20–40 s) and slow (~5–10 min), as shown in Figure 5B. Furthermore, it was demonstrated that zinc oscillatory waveforms closely coincide with intra-islet calcium oscillations and secreted insulin pulses [54]. The device platforms described here will have a significant impact on future studies aimed at elucidating the relationship between insulin and zinc secretion.

The secreted insulin from either macroscale or microscale perfusion apparatus is typically collected using a fraction collector and then the amount is measured off-chip, using either the ELISA or radioimmunoassay (RIA) [44,68,69]. In general, these measurements are performed at relatively low resolution at minute increments on multiple islets due to the volume and amount of secreted insulin needed for detection via standard ELISA kits. In an attempt to overcome this obstacle, Kennedy and his associates pioneered a microfluidic platform that incorporated continuous islet perfusion, perifusate sampling using EOF, and an online electrophoresis immunoassay that allows quantitative monitoring of biphasic and oscillatory insulin secretion from a single islet with temporal resolution.

**Figure 5. Analytical resolutions of calcium influx, zinc signaling and insulin release.**

(A) Glucose-stimulated slow and fast calcium oscillations detected in the microfluidic environment (mouse 6 displays slow oscillations and mouse 5 displays fast oscillations).

(B) Quantitative glucose-stimulated Zn\(^{2+}\) secretion using microfluidic droplet sampler from basal level of approximately 40 fg islet\(^{-1}\) min\(^{-1}\) to approximately 90 fg islet\(^{-1}\) min\(^{-1}\). Observed slow oscillation (9.9 ± 2.0 min spacing, 8.7 ± 2.3 min plateau width) resembled well-characterized slow insulin pulses.

(C) Calcium and insulin secretion patterns showing mouse-to-mouse imprinting of three different mice. Displayed oscillation frequency averages are 9 min (Mouse 1), 4.5 min (Mouse 2) and 15 s (Mouse 6).

(D) Traces of insulin secretion from off-line analysis using Chemodroplet (plugs), showing the fluorescence of Alexa Fluor 594 marker and the calculated insulin secretion rate.

(A) Adapted with permission from [60] © Public Library of Science (2009).

(B) Adapted with permission from [54] © American Chemical Society (2009).

(C) Adapted with permission from [60] © Public Library of Science (2009).

(D) Adapted with permission from [46] © National Academy of Science (2008).

**Key term**

**Islet perfusion:** A laboratory setup used to study islet or \( \beta \)-cell physiology in vitro, in which islets are exposed to flow.
resolution at approximately 10 s [41]. Later, they further improved the assay throughput (≤ 15 islets) and insulin detection sensitivity by tenfold with the capability to characterize fast and slow oscillation patterns (Figure 5C) [21]. To date, the ability to rapidly quantify temporally resolved insulin secretion at the single islet level using this type of microfluidics has been successfully demonstrated in several islet studies [60,70–73]. In addition, the chemistrome device from the Ismagilov group enables the stimulation, recording and analysis of molecular signals with a high spatio-temporal resolution. The stimulation pulse can achieve a temporal resolution of 1.5 s and the insulin secreted from a single islet can be measured at a frequency of 0.67 Hz, as shown in Figure 5D [46].

In contrast to the single islet assay, the Oberholzer and Eddington groups have developed a device that enables simultaneous fluorescent imaging of mitochondrial metabolic activity and intracellular calcium, as well as the collection of secreted insulin [44,45]. The device was designed for the purpose of integration into standard clinical islet isolation protocols, with simplicity in mind. With simple design geometry and an off-chip mixer, the device allows simultaneous imaging of intracellular calcium signaling and mitochondrial potential with a high signal-to-noise ratio and moderate spatio-temporal resolution (Figure 6). In addition, this device enables efficient mixing, uniform distribution of rapidly alternating solutions, and the generation of user-defined chemical gradients in time. This device was originally conceived to develop a functional evaluation of the isolated human islets prior to transplantation. Therefore, this device was used to evaluate a statistically representative sampling of the islet population, instead of a single islet, in order to increase assay throughput and provide a more global picture of the pretransplant islet preparation. This microfluidic device uses a relatively large chamber volume (100 µl) capable of characterizing the biphasic insulin secretory pattern of 20–30 islets without losing spatio-temporal resolution.

**Environmental control in microfluidic device for islet study**

In addition to the dependence of insulin release on the intrinsic characteristics of β-cells and applied stimuli, it can also be influenced by other environmental factors such as local oxygen tension, pH and temperature [74,75].

Pancreatic islets are highly oxygenated in vivo by an extensive vascular system. The β-cells located in nonvascularized isolated

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**Figure 6. Simultaneous islet perfusion and multiple-channel fluorescence imaging.**

Representative record of temporal insulin secretion, calcium influx (Fura-2 ratio of fluorescence) and mitochondrial potential changes (Rhodamin 123) in mouse islets stimulated with 14 mM glucose. Data generated from the Oberholzer and Eddington groups.
islets are supplied with oxygen solely by diffusion from the surrounding environment. Previous studies in perfusion and static culture setups have demonstrated that insulin secretion is altered and abolished under hypoxic conditions [74,76]. One of the reasons PDMS-type microfluidic devices are advantageous for cell-based research is due to high diffusivity oxygen in PDMS (D = 4.1 × 10^{-5} \text{ cm}^2/\text{s}) [77] and high-oxygen solubility (0.18 cm^3 (STP)/cm^3 atm) compared with other polymers and materials used for microfluidic device fabrication [78]. In general, passive permeation of oxygen through PDMS is assumed to be sufficient for short-term islet studies and, therefore, researchers do not typically measure oxygen concentration in microfluidic device setups. However, careful consideration of factors in the device design, such as the thickness of PDMS wall and the precise control of oxygen in the microfluidic device, such as the integration of on- or off-chip oxygen generators, is necessary to limit the potential impact of oxygen concentration on β-cell signaling, insulin secretion and survival, especially in long-term studies. So far, no systemic studies have been reported to this end.

Based on Fick’s laws of diffusion, the time needed for heat transfer is proportional to the second power of the length. Consequently, the efficiency of heat exchange in microchannels and chambers has been calculated to be higher than that in conventional tubes and dishes. In fact, this property of microfluidic devices has proven useful for the application of microfluidics to thermodynamically driven processes such as in reaction chambers for PCR [79,80] and capillary electrophoresis [81]. However, PDMS or other elastomers often used in microfluidics have poor thermal conductivity, which makes it difficult to design efficient and fast external temperature controls (heating stage/tape, incubator and water bath). Internal thermal control is also challenging, since it requires microsize design, high reliability, ease of operation and low-cost fabrication [82,83]. In addition, greater care should be taken for air bubble generation and the destabilization of flow caused by either external or internal temperature control. Several measurements of fluid temperature in the microscale apparatus have been reported through the measurement of [84–87]:

- The flow outside of device;
- The electrical conductivity of the solution;
- Thermochromic solution and beads;
- NMR thermometry;
- Raman spectroscopy;
- Fluorescence probes.

Each approach has pros and cons and is useful for specific applications. In the existing microfluidic devices used for islet study, temperature control using external means is most commonly used and is sufficient in most applications, with the aforementioned limitations. In the future, the optimization of temperature control via device design and characterization is warranted.

**Summary of microfluidic application used in islet study**

Table 1 summarizes some of the most recent microfluidic devices developed for use in islet studies, highlighting their key strengths, limitations and suitability for certain applications. Despite the number of interesting devices, many of them have proven most valuable when used for a specific experimental goal or purpose and cannot be generalized to application in all islet studies.

**Future perspective**

For the past 30 years, the perifusion system has been an important laboratory setup to understand islet physiology. Yet, within the last 5–10 years, the development of miniaturized perifusion systems using microfluidic technology has innovated the way researchers approach β-cell research. Although still in an early stage of development, the principles governing the design, fabrication and application of microfluidics in β-cell research have been introduced. However, it is clear that further optimization and standardization is necessary. In works published previously, most of the devices were built for specific experiments, with customized technologies that are not widely available. To realize the great promise of microfluidic technology, it is essential to bridge the gap among engineers, biologists and clinicians in a spirit of cooperation to deliver novel yet user-friendly systems with standardized procedures for common applications.

The plethora of microfluidic devices that have been created have and must continue to be categorized into two classes:

- Basic science platforms designed to better understand β-cell physiology;
Clinical platforms aimed at improving existing treatment options with the ultimate goal of curing diabetes.

In the near future, the true measure of whether microfluidics has made a substantial impact on diabetes research will be the significance of discoveries generated from microfluidic-based studies, the improved time, efficiency and cost over macroscale assays and, most importantly, the willingness of researchers to accept the platform as a promising alternative to the macroscale format. Furthermore, integration and automation still remains a considerable hurdle for higher accuracy, reproducibility and throughput. Future integrated systems should be able to gather precise data, not only at the microscale level of the islet and single cell, but also at the molecular level. In the first decade’s endeavor, we have seen the remarkable innovations that researchers have developed using microfluidic technology and their potential applications. Looking toward the future, we can be assured that these innovations are just the beginning and more discoveries are yet to come.

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No writing assistance was utilized in the production of this manuscript.

**Table 1. Summary of microfluidic devices recently used in pancreatic islet studies.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Type of device</th>
<th>Strength</th>
<th>Limitation</th>
<th>Suitable application</th>
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<tr>
<td>Kennedy</td>
<td>Borofloat glass Single-layer Single/multiple channel (&lt;15) Pressure flow control EOF insulin sampling</td>
<td>Single or multiple islets analysis (&lt;15) Low- to high-throughput High sensitive online insulin immunoassay Improved temporal resolution of insulin and calcium signal Real-time capability</td>
<td>Design complexity Operator dependence Potential mechanical or shear force stresses Limited fluid control</td>
<td>Islet physiology Drug screen</td>
<td>[17,21,42,60]</td>
</tr>
<tr>
<td>Piston</td>
<td>PDMS-glass Single-layer Pressure or Vacuum flow control</td>
<td>Single islet analysis Easy operation High temporal resolution of zinc and calcium signals Passive-droplet sampling Real-time capability</td>
<td>Low throughput Operator dependence Potential mechanical and shear stresses Limited fluid control</td>
<td>Islet physiology Cell-to-cell communication</td>
<td>[43,54,88]</td>
</tr>
<tr>
<td>Ismagilov</td>
<td>PDMS and culture dish Open system Multilayer Pressure-driven droplet Chemistrode</td>
<td>Single islet analysis High resolution of stimulus: 1.5 s High resolution of insulin sampling: 1.5 s Multiplex analysis</td>
<td>Low throughput Not real-time</td>
<td>Islet physiology Molecular signaling in whole islet</td>
<td>[46]</td>
</tr>
<tr>
<td>Roper</td>
<td>PDMS + glass Three-layer On-chip pumps off-chip valves On-chip mixer</td>
<td>Single islet analysis Input and output waveform formation Real-time capability Gradient creation</td>
<td>Complexity of design Low throughput Moderate resolution</td>
<td>Islet physiology Drug screen</td>
<td>[47,48]</td>
</tr>
<tr>
<td>Oberholzer and Eddington</td>
<td>PDMS + glass Three-layer Off-chip mixer</td>
<td>Single or multiple islet analysis Simple design geometry Easy gradient generation High-throughput Real-time capability Multimodal functional assay Moderate resolution Off-chip mixer Larger-chamber volume</td>
<td>Islet physiology Human islet function evaluation Drug screen</td>
<td>[44,45]</td>
<td></td>
</tr>
</tbody>
</table>

PDMS: Polydimethylsiloxane.
Executive summary

- In vitro research models are very important for understanding islet and β-cell physiology.
- Perifusion setup mimics islet in vivo environment by precisely controlling the relevant parameters.
- Microfluidic devices can be developed as a miniaturized perifusion system with unique advantages.
- The application of microfluidics in islet research has been limited in the past.

Microfluidic technology

- Polydimethylsiloxane and Borofloat glass are two common materials used for microfluidic fabrication in islet study.
- Microfluidic fabrication is relatively simple and easy.
- Microfluidic technology offers these distinct advantages: small volume, inexpensive, amenable to elaboration and multiplexed devices and easy integration of analytical tools.

β-cell physiology & microfluidics in islet study

- Islets have unique cell populations and the insulin secretion from β-cells plays a key role in regulating blood glucose.
- In response to environmental stimuli, insulin secretion is a biphasic, often oscillatory pattern.
- Stable, flexible and desirable fluid control in microfluidics enables the study of insulin kinetics in conjunction with metabolic and biochemical events in β-cells.
- Droplet techniques have significant potential to enhance the spatio-temporal resolutions of the stimuli and samplings and observe molecular signals.
- Off- and on-chip mixers help the generation and maintenance of suitable microenvironments.
- Microfluidic integration increases analytical throughput and allows sensing and monitoring of β-cell electrical, biochemical and ion-signalling activities.

Summary of microfluidic application used in islet study

- Each of the existing microfluidic devices used in islet studies have their own unique features.
- Microfluidic device application proves to be most valuable when used for a specific experimental goal and purpose.

Bibliography

Papers of special note have been highlighted as:
- of interest
- of considerable interest

**Reported the second generation microfluidic chip based on [17].** The device improved assay throughputs by simultaneous 15-channel analysis of online insulin secretion in parallel and showed a decent temporal insulin resolution from a single islet. This device offered a novel research platform to study the dynamics of islet insulin secretion.


**Described an optimized microfluidics based on [42] that enabled perfusion of islets, improved temporal resolution of monitoring insulin release and easy control of the cellular environment.**


**Reported the development and evaluation of one of earliest microfluidic devices used for islet study with the capability of online electrophoresis insulin assay. The device demonstrated the feasibility of using micro-scale system for monitoring β-cells, but with some limitations.**


**Developed a six-port microfluidic device for the purpose of studying the communication of calcium signaling across the islet and demonstrated that calcium communication depends on a delicate interaction between the degree of coupling and KATP activation. This device provides a unique experimental setup and offers advantages over conventional approaches for the study of islet physiology.**


**Reported a second generation microfluidics based on the device developed in [45]. With simple design geometry, it improved flow distribution within the device and enabled the creation of user’s defined chemical gradient with decent spatial-temporal analytical resolutions.**


**Described a three-layer microfluidic with a unique islet immobilization approach. The device is particularly useful for real-time, intact human islet evaluation prior to islet transplantation and drug screen.**


**Developed a ‘chemistrod’, a plug-based microfluidic device that enables the stimulation, recording and analysis of molecular signals with high spatial and temporal resolution. This device provides a creative research platform for the understanding of β-cell physiology.**

Described a three-layer microfluidic that is capable of delivering stimulant waveforms to islet cells using on-chip pumps and mixing channels. This perfusion system is suitable for studying islet physiology.


