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ARTICLE

Highly Efficient Adenoviral Transduction of Pancreatic Islets using a Microfluidic Device

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Pamuditha N. Silva[1], Zaid Atto[1], Romario Regeenes[1], Uilki Tufa[1], Yih Yang Chen[1], Warren C.W. Chan[1], Allen Volchuk[3], Dawn M. Kilkenny[1], Jonathan V. Rocheleau[1,2,4]

Tissues are challenging to genetically manipulate due to limited penetration of viral particles resulting in low transduction efficiency. We are particularly interested in expressing genetically-encoded sensors in *ex vivo* pancreatic islets to measure glucose-stimulated metabolism, however poor viral penetration biases these measurements to only a subset of cells at the periphery. To increase mass transfer of viral particles, we designed a microfluidic device that holds islets in parallel hydrodynamic traps connected by an expanding by-pass channel. We modeled viral particle flow into the tissue using fluorescently-labelled gold nanoparticles of varying sizes and showed a penetration threshold of only ~5 nm. To increase this threshold, we used EDTA to transiently reduce cell-cell adhesion and expand intercellular space. Ultimately, a combination of media flow and ETDA treatment significantly increased adenoviral transduction to the core of the islet. As proof-of-principle, we used this protocol to transduce an ER-targeted redox sensitive sensor (eroGFP), and revealed significantly greater ER redox capacity at core islet cells. Overall, these data demonstrate a robust method to enhance transduction efficiency of islets, and potentially other tissues, by using a combination of microfluidic flow and transient tissue expansion.

Introduction

Viral transduction is a powerful tool to deliver genetic material into cells yet is severely limited when used to express plasmids in tissues and other 3D cell-constructs. Viral particles are simply too large and sticky to penetrate through the intercellular space deep into a tissue [1, 2]. Our overall goal was to express genetically encoded sensors in living pancreatic islets, a microtissue ~50-150 µm in diameter [3, 4], to study the metabolic responses of individual insulin secreting beta-cells. However, we recognized that poor penetration of adenoviral particles would reduce the statistical power of our imaging, meanwhile biasing the results to a subset of beta-cells at the tissue periphery. One method to achieve high transduction efficiently is to dissociate islets into single cells prior to transduction [5, 6]. This method increases transduction efficiency and in some studies has been used to make temporal measurements to reveal cellular heterogeneity [7, 8]; however, it fails to characterize the role of islet architecture in defining the response and potentially induces other artefacts. We therefore aimed to develop a method to increase the penetration of adenoviral viral particles into intact living islets.

We previously showed that microfluidic devices can induce intercellular media flow in islets and tumour spheroids however it was unclear whether this flow could transport adenovirus (~100 nm) and adeno-associated virus (~25 nm) [2, 9-11 12]. To improve viral penetration, we redesigned our devices to ensure equal viral load to each islet held within the device. We subsequently modelled the penetration of virus particles into trapped islets using a range of fluorescently-labelled gold nanoparticles based on size. Based on our finding of a size threshold too small to allow the flow of viral particles, we consequently introduced transient EDTA treatment to expand the intercellular space [13, 14]. The combination of microfluidic flow and tissue expansion, referred to as "Highly Efficient Adenoviral Transduction (HEAT)-on-a-Chip", achieves a three-fold increase in overall transduction efficiency at the core of islets compared to standard methods. We subsequently expressed an ER-targeted redox sensor (eroGFP) using HEAT-on-Chip and confirmed enhanced viral expression to the core of the islets. More importantly, these experiments revealed a trend of greater ER redox buffering capacity towards the centre of the tissue compared to the periphery, a spatial relationship that would be impossible to discern using dissociated cells. Taken together, our data demonstrate a robust method to enhance transduction efficiency of a microtissue (the pancreatic islet), concomitantly revealing

[1]*Institute of Biomaterials and Biomedical Engineering, University of Toronto, 164 College St., Toronto, Ontario, M5S 3G9, Canada. Email: jon.rocheleau@utoronto.ca; Fax: +1 416 581 7839; Tel: +1 416 581 7839*
[2]*Department of Physiology, University of Toronto, 1 King's College Circle, Toronto, Ontario, M5S 1A8, Canada*
[3]*Keenan Research Center of the Li Ka Shing Knowledge Institute, St. Michael's Hospital, 209 Victorial St., Toronto, Ontario, M5B 1T8, Canada*
[4]*Toronto General Research Institute, University Health Network, 101 College St., Toronto, Ontario, M5G 1L7, Canada*
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heterogeneity in cellular metabolism that must be considered when interpreting maintenance of proper islet cell function.

Experimental

Pancreatic Islet Isolation and Culture

Animal procedures were approved by the Animal Care Committee of the University Health Network, Toronto, Ontario, Canada in accordance with the policies and guidelines of the Canadian Council on Animal Care (Animal Use Protocol #1531). Pancreatic islets were isolated from 10- to 20- week old C57BL6 male mice through collagenase P digestion (Roche) as previously described [2, 9, 10]. Prior to use in microfluidic devices, islets were hand-picked into non-treated culture dishes containing RPMI 1640 media (Sigma-Aldrich) supplemented with 20 mM Hepes, 11 mM glucose, 10% FBS, and 5 U/mL penicillin-streptomycin, and equilibrated in a humidified incubator at 37°C and 5% CO_2 for at least 2 hours.

Microfluidic Fluid Flow Modelling

Fluid flow through the main section of the islet holding area was modelled with COMSOL Multiphysics 4.4 (COMSOL) prior to device fabrication. This section of the device was constructed *in silico* as a three dimensional structure according to the described dimensions. Islets were modelled as solid deformable ellipsoids (i.e., porosity was omitted) with either identical geometry (120 µm radii) or variable geometry. For modelling purposes, the inlet pressure was set to 0 kPa while the exit flow rate was set to 200 µL/hr. A polyline edge that bisected the hydrodynamic traps midway through the islets was used to determine pressures and thus pressure drop across the islets. The 9 simulated models had meshes with 532624 to 668568 triangular elements and 5938 to 7161 edge elements.

Microfluidic Device Fabrication

Microfluidic devices were fabricated by soft lithography with PDMS (Dow-Corning) as previously described [2, 9, 10]. Our designs used hydrodynamic traps to hold islets as they have previously been used to shelter both single cells and larger objects (e.g., bubbles) [15-23]. Device dimensions were first modelled by finite element analysis on COMSOL Multiphysics software. Device masters imprinted on chrome glass masks were used to expose SU-8 2100 negative photoresist (Micro-Chem) spin-coated onto 6" silicon wafers (Mech grade; University Wafer). Individual devices were fabricated by mixing a PDMS elastomer base with curing agent at a 1:10 ratio that was poured over the master. After heating for 1 hour at 40°C, the PDMS was peeled off the master, and inlet and outlet ports were hole-punched with the bevelled barrel of a 22G needle. PDMS sections were plasma bonded (Harrick Plasma Cleaner) to no. 1 coverglass slides (24 × 50 mm; VWR Scientific) or to each other (in the case of the bubble trap and reservoir ceiling).

The entire microfluidic device is composed of three units bonded to a slide: bubble trap, islet holding area with hydrodynamic traps, and dampening section. This modular design eases the transitions between loading islets into the device and exchanging media during live cell imaging. The bubble trap adapted from Zheng et al. [24] features a single channel with a three-pass serpentine section (300 µm wide and 150 µm tall) followed by a cylindrical reservoir (4 mm wide and 5 mm tall) covered by a second bonded layer of PDMS. The reservoir features a single exit channel connected to the inlet of the islet holding area with Tygon tubing (0.6" inner diameter and 5 cm length; Cole-Palmer). The channels were made 150 µm tall and feature 8 hydrodynamic traps (each 300 µm wide and 311 µm long) spaced 847 µm apart in curved channel length. Each hydrodynamic trap connects to the main channel with a 25 µm tall and 25 µm wide nozzle at its base. The main channel (modeled as "Spiral 3" Fig. S1A) is 330 µm wide until it reaches the nozzle of the first hydrodynamic trap. At this point, the main channel width progressively increases to a final fixed width of 632 µm after the final trap. The exit channel of the islet holding area is connected to the dampening section by a short length of Tygon tubing (1 cm; 0.6" inner diameter). The dampening section features a single 330 µm wide by 150 µm tall serpentine channel with three passes. The outlet to the dampening channel is connected to the syringe pump (New Era) with Tygon tubing (0.6" inner diameter; 60 cm in length).

Transduction Procedure

Transduction was carried out in either a culture dish or device (as indicated) on islets two hours post-isolation. Adenovirus was diluted to 5×10^9 pfu/mL in 300 µL RPMI media (described in Pancreatic Islet Isolation and Culture section above). For dish treatments, transduction media was placed on a 35 mm diameter non-treated petri dish prior to islet loading; islets were subsequently left undisturbed to equilibrate. For device treatments, islets were gravity loaded directly into the hydrodynamic traps before the inlet tubing was connected to the bubble trap, which was previously primed with transduction media. EDTA, when used, was diluted to 2 mM in media. Transductions were conducted at room temperature. After 75 minutes, islets were either handpicked by micropipette from the dish or ejected from the device by gravity flow into an intermediate dish containing islet media. The islets were consequently transferred from this dish into a fresh 35 mm non-treated dish containing 2 mL of islet media and placed into a CO_2 incubator at 37°C and 5% CO_2 for at least 24 hours. The identical protocol, in the absence of adenovirus, was used for experiments testing islet physiological properties.

Nanoparticle Fabrication and Imaging

Fluorescent nanoparticles (NPs) were produced as previously described [25-27]. First the gold NP cores with different diameters (5, 15 and 100 nm) were synthesized, followed by surface modification with polyethylene glycol and subsequent conjugation to Cyto633-NHS (Cytodiagnostics). *For more details, refer to Supplemental Experimental*. Imaging media supplemented with single-size NPs were flown into islet-

loaded devices at a flow rate of 200 µL/hr. The 5, 15 and 100 nm NPs were diluted to 25 nM, 5 nM and 0.03 nM final concentrations, *respectively*, in imaging media supplemented with 100 µg/mL TRITC-labelled dextran (average molecular weight ~20 kDa) and EDTA (0 or 2 mM; as indicated). NPs were excited using a 633 nm laser of an LSM710 confocal microscope (Zeiss) and fluorescence emission was collected using a 638-747 nm bandpass filter. TRITC was excited with a 543 nm laser and emission was collected using a 548-626 nm bandpass filter. Z-stack images were collected at Time 0 and after 30 minutes of 2 mM EDTA exposure. A custom built macro for ImageJ (National Institute of Health) was designed to measure NP accumulation as the mean fluorescence intensity in a region of interest (ROI) defined by the islet cross-section divided by the mean fluorescence intensity in the main channel of the device (averaged over four slices 1.7 µm apart starting 10 µm from the base of the islet). Depth-dependent NP accumulation was measured for concentric ROIs each established as 20% of the total area of the islet cross-section.

Fluorescence microscopy with dextran

FITC-conjugated dextran (i.e., "Fluorescent Dextran"; 70 kDa) was diluted to 100 µg/mL in imaging media with salt composition comparable to RPMI media (125 mM NaCl, 5.7 mM KCl, 0.42 mM $CaCl_2$, 0.38 mM $MgCl_2$, 10 mM HEPES, 11 mM Glucose, and 0.1% BSA; pH 7.4) containing equivalent Ca^{2+} and Mg^{2+} concentrations. The diluted fluorescent dextran was used to either highlight the islet intercellular space or report on media exchange through the tissue. To image intercellular space, fluorescent dextran was flown through islet-loaded devices with increasing amounts of EDTA (0, 0.5, 1, 2, 5, 10 and 20 mM) for 10 minutes at 400 µL/hr prior to imaging. Post-image acquisition, devices were flushed with media for an additional 10 minutes prior to delivery of the next EDTA treatment. Images were collected using the 20×/0.8 NA air objective of a LSM710 confocal microscope (Carl Zeiss), 488 nm excitation, emission bandpass between 493-538 nm, and z-slices at 1 µm intervals. The z-stack images were processed and analyzed with a custom-built macro in ImageJ (National Institutes of Health). This macro inverts and filters the images (enhanced contrast, gamma stretch, Gaussian blur) to define the intercellular space and characterise its volume and surface area. The change in intercellular space was quantified as 'changes in intercellular space surface area to islet volume ratio'.

To study media exchange within the islet, confocal images were collected every 1.56 s as imaging media supplemented with 100 µg/mL fluorescent dextran (i.e., "fluorescent media") was flown in the device. As fluorescent dextran reached the hydrodynamic trap, non-fluorescent media (i.e., "clear media") in the islet was pushed out, appearing as a clear tail. The islet clearance time was defined as the arrival of fluorescent media at the hydrodynamic trap to the disappearance of the tail, as done previously [2, 10]. Post-image acquisition, fluorescent media was flushed out of the device by flowing clear media at a rate of 1000 µL/hr. When studying the effect of a new flow rate, clear media was flown at the same flow rate for 5 minutes prior to the introduction of fresh fluorescent media.

Transduction Efficiency and Depth of Penetration Quantification

Following transduction with GFP adenovirus, islets were cultured for a minimum of 24 hours. Islets were subsequently fixed with 2% paraformaldehyde for one hour prior to nuclear staining with DRAQ5 diluted 1:1000 in BMHH imaging media (125 mM NaCl, 5.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM HEPES, 11 mM Glucose, and 0.1% BSA; pH 7.4) supplemented with 0.1% Triton X-100. Confocal z-stack images were collected at 1 µm intervals using 488 nm and 633 nm laser lines to excite GFP and DRAQ5, *respectively*. To analyze the depth of viral penetration and transduction efficiency, images acquired at 25 µm from the glass surface were processed using a custom-built macro in ImageJ. This macro enumerates the total number of nuclei as well as the number of nuclei co-localized with viral GFP signal (i.e., "GFP-positive"). Transduction efficiency was defined as the percentage of GFP-positive cells compared to total nuclei. Depth of penetration was assessed as the fraction of GFP-positive nuclei compared to total nuclei present in two radial segments (with equal number of total nuclei) of the islet in cross section.

Cell Viability

Freshly isolated islets were flowed in the device with culture media at 200 µL/hr in the presence or absence of 2 mM EDTA for 75 min. Another set of islets were similarly treated with 0.3% H_2O_2 for 1 hour to induce apoptosis. After overnight culture, these islets were incubated for an hour at 37°C in BMHH imaging media with 2 mM glucose. The islets were subsequently stained in imaging media with 5 mg/mL fluorescein diacetate (FDA) and 2 mg/mL propidium iodide (PI) in 2 mM glucose for 30 minutes at RT with orbital shaking. Z-stack images of islets were collected with an LSM710 confocal microscope using 488 nm (FDA) and 543 nm (PI) excitation with 493-565 nm (FDA) and 566-719 nm (PI) emission bandpasses. Viability for each islet was calculated as the percentage of FDA-positive cell out of the total FDA-positive and PI-positive cells on an image plane 20 µm above the base of the islet.

NAD(P)H autofluorescence

Freshly isolated islets were flowed with culture media at 200 µL/hr for 75 min in the absence or presence of 2 mM EDTA. After overnight culture, islets were equilibrated in a dish at 2 mM glucose in BMHH imaging media (37°C for 40 min). The islets were subsequently loaded into the device, which was mounted in a stage-top microscope incubator (Okolabs) set to 37°C. NAD(P)H autofluorescence was imaged as done previously described using a Ti:Saph two-photon laser tuned to 705 nm and attenuated to 2.5 mW, and a 40× 1.3 NA oil immersion lens [9]. NAD(P)H images were acquired sequentially on islets in 2 mM, 10 mM (10 min), and 20 mM (15 min) glucose. The average intensity was determined for 10 regions of interest that were randomly selected in each islet cross

section. The fold change in NAD(P)H autofluorescence intensity was reported as the mean autofluorescence intensity of the 10 regions relative to 2 mM glucose stimulation.

Ca^{2+}-oscillations

Islets used for NAD(P)H autofluorescence measurements were recovered from devices by gravity flow and equilibrated in 2 mM glucose (1 hr, 37°C) prior to dye loading in imaging media containing 2.5 µM Fluo-4 AM (Life Technologies) and 2 mM glucose (30 min; RT with orbital shaking). Islets were then re-loaded into the device mounted on a heated stage set to 37°C. The islets were subsequently stimulated with 10 mM glucose and Fluo-4 images were acquired at 1 s intervals for 2.5 minutes (total time). The fluorescence intensity of randomly-selected ROIs was normalized by the mean intensity of the time series.

Insulin Secretion Assay

Freshly isolated islets were flowed in media with and without 2 mM EDTA for 75 minutes at 200 µL/hr. After removal from the device and overnight culture, islets were incubated in a 35 mm non-tissue culture treated petri dish with 2 mM glucose in KRB media (128.8 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$ 2.5 mM $CaCl_2$, 5 mM $NAHCO_3$, 10 mM HEPES, 0.1% BSA) for 1 hour at 37°C. 6 to 8 islets per treatment group were transferred to a 1.5 mL tube into a final 50 µL volume of KRB media containing 2 mM glucose. The islets were incubated for 30 minutes at 37°C before the supernatant was collected. Islets were then re-suspended in 50 µL of KRB media with 10 mM glucose and incubated at 37°C for 30 minutes. After the supernatant was collected, the islets were subjected to a final stimulation with 20 mM glucose in KRB media in a similar fashion. Total insulin was collected by mixing the islets with 50 µL of lysis buffer (1% Triton-X-100, 100 mM NaCl, 50 mM HEPES, 5% glycerol) and incubating on ice for 50 minutes. Insulin content in the supernatants and lysate were measured using a mouse insulin ELISA kit (Crystal Chem).

Endoplasmic Reticulum Redox Assay

An adenovirus exhibiting targeted ER expression and a redox-sensitive GFP (eroGFP) was transduced into islets as described (5×10^9 pfu/mL). Thirty-six hours post-transfection, islets were suspended in imaging media and loaded into devices for imaging. Confocal image series were collected under dual excitation (458 and 514 nm laser lines) and 516-598 nm band pass emission collection. Imaging media supplemented with 5 mM dithiothreitol (DTT) was flown into the device at 400 µL/hr (i.e., the slowest flow rate required to achieve maximal media clearance) for 20 minutes, and consequently flushed with fresh imaging media by continuous flow for 20 minutes. The change in media was indicated by a 2 sec transient stream of fluorescent dextran (70 kDa; 100 µg/mL). For each islet, 20 regions of interest were selected, representing cells in both the outer rim and the inner core. The change in fluorescence intensity ratio (514:458) versus time was modelled as an exponential decay function:

$$y(t) = A_o e^{-kt}$$

The exponential decay constants, k, of 276 cells from 8 islets were plotted against the normalized distance from the center of the islet cross-section. These data points were fitted to a parabolic function:

$$y(x) = \beta_0 + \beta_1 x^2$$

where coefficients $\beta_0 = 6.39 \times 10^{-3}$ and $\beta_1 = -4.49 \times 10^{-7}$. Both coefficients have p-values <0.0001 by t-test as analyzed on the statistical analysis software, JMP.

Results

Design of a microfluidic device with parallel hydrodynamic traps.

We previously showed microfluidic devices can induce media flow to the center of islets, with our most recent design holding islets in hydrodynamic traps [10]. We first considered the arrangement of these traps to determine the feasibility of using this flow to transport adenoviral particles (Fig. 1). Our previous device held islets in series along a microfluidic channel, which induces laminar flow streams either through the first islet (*red path*) or down the bypass channel (*blue path*) (Fig 1A). Due to the serial placement of the traps, the media flowing through the first islet (*red*) continues to flow through subsequent islets trapped downstream in the device. We were concerned that this arrangement would result in decreasing viral load at each subsequent islet along the series (*red* → *pink* → *white path*). To ensure an even viral load to all islets held in a device, we subsequently re-designed the device to have parallel hydrodynamic traps (measuring 300 µm wide by 311 µm tall) connected by a spiralling bypass channel (Fig. 1B). This parallel arrangement allows for each islet to receive fresh viral media that has not passed through another islet. Individual islets filled the traps due to a stepped decrease in flow once the nozzle was blocked by tissue (see Supplmental Movie S1), although occasionally multiple very small islets were found in a single trap. To evaluate the viral load on each islet, we first used finite element analysis to measure the expected pressure drop (ΔP) across each islet in the device (Fig. 1C &D). The goal was to achieve a consistent ΔP across all islets to ultimately flow the same amount of virus into each tissue. Our first design, "Spiral 1" (*black* fill), featured a main channel with a fixed width (Fig. 1C). Subsequent designs, "Spiral 2" (*black* + *green* fill) and "Spiral 3" (*black* + *green* + *red* fill), featured gradually widening outer channels (Fig. 1C). Spiral 1 modelling showed a gradual increase in the ΔP across each subsequent islet in the device (Fig. 1D, *black* squares) suggesting islets along the device would experience progressively greater flow. In contrast, Spiral 2 (*green* circles) and 3 (*red* triangles) showed a flatter dependence on position, with Spiral 3 showing the smallest variance in ΔP across all islets (Fig. 1D). Spiral 3 also out-performed the other designs when modelled with islets of non-uniform geometry and with the devices non-uniformly loaded with islets (Fig. S1). We

further validated our *in silico* results by fabricating all three designs and measuring the average particle counts entering each hydrodynamic trap and the average speed across all traps using particle image velocimetry (PIV) (Fig. S2). These data showed that islets would experience the most consistent viral loads using Spiral 3. The final design also includes a bubble trap and serpentine channels to mitigate effects of sudden pressure changes and the entire PDMS-based device fits onto a single glass coverslip less than 6 cm in length (Fig. 1E and F). Overall, this device places 8 islets in parallel hydrodynamic traps designed to induce similar media flow-rates, and ultimately similar viral load, at each islet.

Simulating particle flow through intercellular space

Our objective was to flow viral particles through the intercellular space of *ex vivo* pancreatic islets. To evaluate the potential of our microfluidic device to induce viral particle penetration, fluorescently-labelled gold nanoparticles (NPs) were flowed through the device and islet penetration was measured using live cell confocal microscopy (Fig. 2). We used NPs of varying core diameter to mimic viruses on the order of adenovirus (100 nm) and adeno-associated virus (15 nm), and smaller (5 nm) (Fig. 2A). Of all NP sizes examined, only the 5 nm NPs flowed into the islets, specifically within crevices along the rim of the tissue (Fig. 2B; *red arrows*). Image analysis confirmed that accumulation of 5 nm NPs was greater than the larger particles, but was also unable to fully penetrate the tissue (Fig. 2C&D). These data reveal a fundamental threshold blocking the flow of particles larger than 5 nm, suggesting microfluidic flow alone cannot transport virus into the islet intercellular space.

Using EDTA to reveal islet intercellular space

Beta-cell to beta-cell adhesion is mainly a consequence of the Ca^{2+}-dependent cell adhesion molecules E-cadherin and N-cadherin, and the Ca^{2+}/Mg^{2+} chelator EDTA is commonly used in combination with trypsin to dissociate islet tissue into individual cells [13, 14]. To examine whether Ca^{2+} chelation could be used to increase viral particle penetration by releasing beta-cell adhesion, we first determined in a dose-dependent response that 2 mM EDTA induces significant expansion of islet intercellular volume (Fig. S3; Supplemental Results). Increasing concentrations of EDTA caused a visible increase in the intensity of fluorescent dextran within the islet, with saturation above 2 mM EDTA (Fig. S3B&C). Islets were subsequently found to contract within ~30 min of EDTA washout suggesting the response was reversible within the device (Movie S2). We empirically characterized the flow of media through the tissue in the presence and absence of 2 mM EDTA, to determine that flow rates greater than 200 µL/hr induced full islet media exchange within 30 s (Fig. S4; Supplemental Results). Consequently, we imaged the accumulation of NPs with core diameters similar to adenovirus (100 nm) (Fig. 3). As cellular adhesion weakened, the intercellular space expanded sufficiently to permit the flow of 100 nm NPs to the core of the tissue (Movie S2; Fig. 3A&B). In addition, NP accumulation was evenly distributed across the tissue (Fig. 3C). Finally, as islets contracted with washout of EDTA, some NPs remain trapped within the intercellular space (Movie S2). These data predicted that adenoviral particles would similarly penetrate EDTA-expanded tissue and subsequently become trapped upon washout to uniformly infect the tissue.

Evaluating Transduction Efficiency and Depth of Viral Penetration

To assess the effect of microfluidic flow and transient tissue expansion on transduction efficiency, we compared adenoviral transduction of GFP using traditional incubation ("Static" media in a culture dish; 75 min) and microfluidic flow (Fig. 4). Twenty-four hours post-transduction, GFP expression and cell nuclei were imaged using dual-colour confocal microscopy (multiple z-dimensions; Fig. 4A). As expected, islets statically transduced exhibited robust GFP expression only at the tissue periphery; expression at the core (either 15 or 25 µm into the islet) was minimal or undetected (Fig. 4A; top panels). Similar GFP expression was observed with static transduction in the presence of EDTA (Fig. 4A), suggesting that simply increasing intercellular space is not sufficient to enhance transduction efficiency. Notably, it was difficult to find intact islets after static-EDTA treatment and overnight culture, consistent with the tissue dissolving to single cells without the structural support of a microfluidic channel. Microfluidic flow alone did not improve transduction efficiency (Fig. 4A; consistent with exclusion of NPs of diameters > 5 nm). In contrast to all the other conditions tested, islets exposed to 'Flow + EDTA' showed robust GFP expression throughout the tissue (Fig. 4A). Consistent with the walls of the device supporting the dissociated tissue, all of the islets remained intact after overnight culture, and there was no visual evidence of dissolution. Transduction efficiency was quantitatively assessed in a number of islets as the percentage of GFP-positive (transduced) cells compared to the total number of cells (DRAQ5-positive) per image (Fig. 4B-E). The total transduction efficiency was significantly higher with combined 'Flow + EDTA' compared to the other methods (Fig. 4B; ~75% compared to 30-47%, *respectively*). To quantitatively assess the depth of penetration into the tissue, confocal images (cross-sections of transduced islets) were spatially partitioned into the rim (outer) and core (inner) regions with an equal number of cells defined in each region (Fig. 4C). The core of 'Flow + EDTA' islets showed significantly higher transduction efficiency compared to the other treatments (Fig. 4D). All of the treatments resulted in expression in cells on the islet rim, however somewhat surprisingly 'Flow + EDTA' produced significantly greater transduction efficiency in this region as well (Fig. 4E). Taken together, these data demonstrate that the combination of microfluidic flow and EDTA-induced tissue expansion significantly enhanced delivery of adenovirus beyond the peripheral layer of cells, subsequently increasing transduction efficiency throughout the entire islet. Our protocol, hereby referred to as HEAT-on-a-chip, sequentially includes: 1) loading islets into a parallel hydrodynamic trap

microfluidic device; 2) flowing media containing adenovirus and 2 mM EDTA for ~75 min to allow penetration of viral particles; and, 3) flowing in fresh media (~30 min) and culturing the islets (overnight) to allow tissue contraction and gene expression, *respectively* (Fig. 4F).

Assessing effects of HEAT-on-a-Chip treatment on islet physiology

We considered the tissue expansion by EDTA transient and reversible since islets in the device condensed immediately with fresh media (Movie S1). We aimed to determine whether the EDTA treatment had any long-term islet function by comparing multiple physiological responses after one day of recovery. First, the EDTA treatment had no effect on cell viability after one day of recovery (Fig. 5A-C). EDTA-treated islets after one day of recovery also maintained glucose-stimulated two-photon NAD(P)H response consistent with normal beta-cell metabolism (Fig. 5D&E). Furthermore, EDTA-treated islets after one day of recovery showed glucose-stimulated Ca^{2+}-influx characteristic of first phase insulin secretion (Fig S5). However, beta-cell electrical connectivity through gap junctions ensures normal baseline and pulsatile insulin secretion, and critically depends on cell-cell contact [28, 29]. To assess whether tissue reconnected to allow beta-cell electrical connectivity, glucose-stimulated Ca^{2+} oscillations from individual cells were measured one-day post-EDTA treatment (Fig. 5F). Representative traces show synchronous Ca^{2+}-oscillations at regular intervals across islets that are offset by a similar phase in both control and HEAT-on-a-chip islets (Fig. 5G). Synchrony was observed in both EDTA-treated and control islets, which contrasted the uncoupled Ca^{2+} oscillations of dispersed islet cells (Fig 5H&I). Finally, we measured glucose-stimulated insulin secretion, which showed no difference between control and EDTA-treated islets (Fig 5J). These data suggest islets fully recover one day post-expansion with normal cell metabolism, synchronous tissue electrical activity, and glucose-stimulated insulin secretion.

Measuring islet spatial redox buffer capacity

The ER lumen maintains an oxidizing redox state to promote secretory protein disulphide bond formation [30, 31]. This oxidizing state is largely due to Ero1 activity, an ER resident protein that is regulated by multiple signals including hypoxia, the unfolded-protein response (UPR), and flavin adenine dinucleotide (FAD) [32, 33]. Due to the wide range of mechanisms controlling Ero1 activity, we postulated that a population of islet beta-cells would exhibit greatly varying ER redox capacity, thus affecting the ability to fold insulin and resist ER stress. To characterize islet beta-cell heterogeneity in ER redox buffering capacity, we expressed the ER-localized redox-sensitive GFP construct eroGFP using HEAT-on-a-chip (Fig. 6). We measured the ER redox capacity of individual cells in each islet by ratiometric confocal microscopy (458:514 nm) of the response to dithiothreitol (DTT) treatment (Fig. 6A) [5, 34, 35]. It was apparent particularly during DTT-washout that cells at the periphery (rim) of the islet responded more slowly than those closer to the core of the islet, consistent with lower Ero1 activity (Fig 6A&B, red box). To quantitatively characterize this variation, DTT-washout curves for multiple cells across multiple islets were fit to a first-order exponential decay function (Fig 6B). The decay constants for these fits, which are proportional to the ER redox capacity, were plotted against the corresponding radial distance of each cell from the core of the islet (Fig 6C). These data consistently showed cells at the periphery of the islet require longer periods of time to re-oxidize the ER compared to cells deeper in the core of the tissue. It is well established that alpha-cells are located on the outer rim of mouse islets [36]; however, this architecture cannot fully account for the phenomena since a consistent trend was found through multiple layers of the tissue. These data therefore suggest that beta-cells at the islet core have higher redox buffering capacity than more peripheral beta-cells, and further demonstrate the utility of HEAT-on-Chip in intact living islets.

Discussion

An attractive feature of *ex vivo* pancreatic islets is that they represent a primary tissue composed of multiple interacting cell types on the microscale. Islets also show coordinated glucose-stimulated electrical activity and insulin secretion due to gap junction coupling of beta-cells. To dissect mechanisms of insulin secretion, many labs genetically modify beta-cells using viral constructs, however highly efficient transduction is only possible in islets dispersed to single cells. This dispersion process disrupts cell-cell communication, thereby prohibiting accurate measurement of coordinated cellular activities, such as electrical connectivity. Consequently, beta-cell responses may no longer represent normal islet physiology and may show increased cell-to-cell variability as an artefact.

To increase adenoviral transduction efficiency of islet islets and potentially other tissues, we re-designed our microfluidic device to treat each islet in the device with similar viral load using parallel hydrodynamic traps connected by a gradually expanding bypass channel. Using gold NPs, we subsequently showed a fundamental size threshold that is too small (~5 nm) to allow entry of adenovirus (~125 nm) or smaller adeno-associated virus (~25 nm) into the tissue even when combined with fluid flow. This small threshold is in contrast to the ~100 nm threshold generally found in tumours [12], but entirely consistent with the tight cell-cell connectivity required for islet electrical connectivity and pulsatile insulin secretion [13, 14]. We subsequently explored temporary disengagement of beta-cell adhesion molecules by removing free Ca^{2+} from the media using EDTA. EDTA dose-dependently expanded the intercellular space to allow gold NPs as large as 100 nm in diameter to flow into islets. We showed that this tissue expansion was reversible within the time frame of transduction (60-90 min), with islets showing normal beta-cell metabolism, synchronous electrical activity across the tissue, and glucose-stimulated insulin secretion. Finally, using the HEAT-on-a-chip protocol, we expressed a fluorescent protein throughout islets and achieved ~75% efficiency of beta-cell

expression, including cells located in the tissue core. Other groups have previously explored methods to transgenically modify *ex vivo* islets, either virally [37-40], or with DNA-functionalized nanoparticles [41, 42]. A major disadvantage of these methods is the long vector transduction and incubation time (between 24 and 48 hours) required for efficient gene expression. Our much faster procedure (75 minutes) results in islets being ready for use in under 24 hours of isolation.

Using the HEAT-on-Chip protocol to express eroGFP throughout islets, we were able to discern a depth-dependent increase in cellular ER redox potential. To our knowledge, this study is the first to successfully follow the response of a genetically encoded sensor at a significant depth within this particular tissue. Our data suggest that there are multiple mechanisms regulating ER redox potential that vary within the structure of the pancreatic islet. ER redox potential heterogeneity has previously been reported in yeast possibly due to asymmetric segregation of ER tubules during replication [34]. Peroxireductin 4 (Prdx4) is crucial for improving insulin biosynthesis by metabolising hydrogen peroxide generated by Ero1 activity [43, 44]. Islets express a pancreas-specific Ero1 isoform, Ero1β, required for insulin biosynthesis [45, 46], and develop a hypoxic core in culture [47, 48]. We anticipate that Ero1 activity of this isoform under these conditions (i.e., fully oxidized FAD) would reduce the overall ER redox potential [31-33, 49]. Furthermore, hypoxia triggers Hif-1α expression, which in turn increases Ero1α expression [49-51]. Therefore, cells deeper in the islet may be compensating for a decrease in Ero1 catalytic activity by increasing Ero1α abundance; however, cells on the islet periphery may not receive a similar cue to modulate protein expression. These findings support the notion that islet cells exhibit a high degree of plasticity and can adjust to different stresses, and further validates the utility of the HEAT-on-a-chip protocol.

HEAT-on-a-chip will allow us to further explore the consequences of beta-cell metabolic heterogeneity using other genetically encoded sensors such as those that report on insulin secretion [52] and glucose metabolism [53]. These sensors can be monitored with high temporal and spatial resolution to reveal molecular dynamics at the subcellular level. We are particularly interested in expressing a genetically encoded $NADP^+$-sensor (called Apollo-$NADP^+$) to determine the role of the NADPH/$NADP^+$ redox state in glucose-stimulated insulin secretion [53]. HEAT-on-a-chip will also improve biochemical assays that require high transduction efficiency. For example, we previously showed fibroblast growth factor receptor-like 1 (FGFRL1) over-expression increases insulin production and insulin secretion in a murine beta-cell line [54]. We now plan to extend our findings to intact primary human islets to perform higher sensitivity insulin ELISA or conduct western immunoblot analysis on critical signalling pathways, given that both of these biochemical assays require high transduction efficiency to discern significant biological effects. We could also modify the expression of key ER regulatory proteins, such as Ero1, in islets via HEAT-on-a-chip and perform biochemical studies to explore beta-cell heterogeneity as it relates to insulin processing. Further investigation into beta-cell function may lead to the development of new therapeutics and/or improvements to existing treatments for diabetes.

Our data and HEAT-on-a-Chip protocol are also both relevant to islet transplantation, a promising strategy to permanently restore blood glucose regulation via hepatic portal vein infusion of donor islets [55, 56]. One of the biggest hurdles to islet viability and transplantation success is that newly transplanted islets fail to graft into the recipient tissue due to a variety of factors including poor re-vascularization and a strong immune response [47, 57-59]. Our data suggest that beta-cells in the core of the islet have the greatest potential to produce functional insulin after successfully grafting with the host tissue, due to increased ER redox potential. However, the *ex vivo* hypoxic state would persist if the donor and host vasculature fail to integrate, leaving this beta-cell population at risk of both apoptosis and necrosis. Alternatively, gene therapy of islets prior to transplantation could be used to promote beta-cell survival and angiogenesis to improve islet survival and transplantation success. Gene therapy has previously been investigated to improve islet survival post-transplantation, including *in vivo* IL-10 expression in mice [60] and hepatic growth factor (HGF) expression of *ex vivo* rat islets via static culture [61, 62]. The viral transduction procedure (infecting the donor prior to islet isolation) of the former study is not translatable to a clinical setting while the latter only showed moderate effects. Alternatively, fibroblast growth factor-2 (FGF2) is known to promote angiogenesis in pancreatic islets, but its application in gene therapy has yet to be explored [63]. Our HEAT-on-a-chip protocol could be used to improve expression of this ligand, effectively priming islets for greater transplantation success. As an example, enhanced expression of a combination of different pro-survival, anti-inflammatory and pro-angiogenic genes, including HGF, IL-10 and FGF2, is predicted to improve islet grafting. By increasing transduction efficiency, it is hypothesized that a greater number of beta-cells would survive post-transplantation thereby reducing the required islet equivalent units per patient, making valuable donor tissue available to a greater number of patients.

To validate our protocol in a rodent model or translate to a clinically relevant scale, the current device would need to be re-designed to accommodate ~100 and ~100 000 islets, *respectively*. Additional hydrodynamic traps could be added to the main channel to hold more islets per device. Joining an array of islet holding areas in series would provide the most efficient high-throughput arrangement if the bypassed media stream from each individual unit is segregated from islet effluent. Once this platform has been scaled to accommodate more islets, it will be possible to revisit the therapies shown to have moderate success to re-assess whether increased gene expression further improves transplantation success.

Our data also demonstrate the utility of microfluidic devices to screen the targeting of NPs to islets and other tissues based on the idea that penetration would increase label contrast. Using controlled mass transfer in the device, we explored the permeability of islets to different sized particles. Our findings have significant implications for the design of targeting NPs to measure beta-cell mass [64, 65] and to deliver drugs to beta-cells

[25]. Both will require NPs to exit the vasculature via heavily fenestrated endothelial cells of islets. However, our data suggest there is a fundamental size barrier to penetrate further into the islet intercellular space. In the islet, capillary diameter is approximately 5 µm and each endothelial cell is surrounded by one or more beta-cells [66]. However, capillary fenestrations accounted for only 5% of the beta cell surface area [67], and these fenestrations are only ~50 nm in diameter [66]. If NPs are designed to penetrate the intercellular space in addition to the capillary fenestrations, we estimate a 200-fold increase in beta-cell surface exposure. However, this small size threshold presents a trade off between increased tissue penetration and reduced NP surface area for targeting agent functionalization. The balance of this trade-off could ultimately be explored using our device.

Conclusions

The pancreatic islet is a model *ex vivo* microtissue for studying biological processes but it pose physical challenges to genetic engineering. To increase adenoviral transduction efficiency of islets and potentially other tissues, we re-designed our microfluidic device to treat each islet in the device with similar viral load using parallel hydrodynamic traps connected by a gradually expanding bypass channel. Using gold NPs, we subsequently showed a fundamental size threshold that is too small (~5 nm) to allow entry of adenovirus (~125 nm) or smaller adeno-associated virus (~25 nm) into the tissue even when combined with fluid flow. We subsequently explored temporary disengagement of beta-cell adhesion molecules by removing free Ca^{2+} from the media using EDTA. EDTA dose-dependently expanded the intercellular space to allow gold NPs as large as 100 nm in diameter to flow into islets. We showed that this tissue expansion was reversible within the time frame of transduction (60-90 min), with islets showing normal beta-cell metabolism and synchronous electrical activity across the tissue. Finally, using the HEAT-on-a-chip protocol, we expressed a fluorescent protein throughout islets and achieved ~75% efficiency of beta-cell expression, including cells located in the tissue core. Using the HEAT-on-Chip protocol to express eroGFP throughout islets, we were able to discern a depth-dependent increase in cellular ER redox potential.

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Figure Legends

Figure 1: A microfluidic device to enhance viral transduction of pancreatic islets. (**A**) Schematic showing a section of a microfluidic device where islets are held in a series of hydrodynamic traps connected by bypass channels. Laminar flow segregates the media into streams that pass either through the islet (*red*) or exclusively through the bypass channel (*blue*). If used for transduction protocols, adenoviral titre would dilute for each subsequent islet due to the serial arrangement of this device. (**B**) The islet holding area composed of eight hydrodynamic traps re-arranged into a

parallel configuration. Media flows through either a single islet in a hydrodynamic trap (*red*) or through the main channel (*blue*). Islet effluent exits through the nozzle of the hydrodynamic trap and effluent collects in the outer arm. The effluent streams are directed away from tissue samples in consequent nozzles. Dimensions of hydrodynamic trap and nozzle as shown. (**C**) Overlayed schematic of three islet holding area designs with islets (in *yellow*) in numbered hydrodynamic traps. Spiral 1 features a fixed channel width (*black*) whereas Spiral 2 gradually widens to span the *black* and *green* regions, and Spiral 3 gradually widens to span the *black, green* and *red* regions. (**D**) Simulated pressure drop (ΔP) across islets along the channel normalized to the first islet. Spiral 3 (*red*) shows lower ΔP variability compared to Spiral 1 (*black*) and Spiral 2 (*green*) designs. (**E**) Three-dimensional schematic of the microfluidic device with channels formed from PDMS. A bubble trap featuring a tall reservoir with a PDMS cover is positioned prior to the inlet and supplies media into the islet holding area. Serpentine channels connect to the outlet of the islet holding area and precede the bubble trap, providing additional resistance to dampen sudden pressure changes. (**F**) Photograph of a microfluidic device. Red dye in the channels allows easy visualization of all major device components. Ruler gradations indicated in centimeters.

Figure 2: Fundamental size barrier prevents viral particle infiltration through islet intercellular space. (**A**) Schematic of fluorescently-labelled gold nanoparticles (NPs) with core diameters on the order of adenovirus (100 nm), adeno-associated virus (15 nm) and smaller particles (5 nm). (**B**) Representative confocal images of different sized NPs (*left* panel) flowing through device with islets in hydrodynamic traps (DIC image; *right* panel). Red arrows indicate 5 nm NPs accumulation within intercellular regions at the islet rim. Scale bar represents 50 µm. (**C**) Average accumulation of different sized NPs represented by measured fluorescence intensity in representative image cross-sections acquired 10 µm the from base of each islet. (**D**) Average accumulation of NPs according to segmentation from the core to the rim of each islet. * Indicates $p<0.05$ by one-way ANOVA. N = 9 – 15 islets harvested from two mice on separate days.

Figure 3: Expansion of islet intercellular space enhances nanoparticle penetration. (**A**) Representative confocal images of fluorescent nanoparticles (NPs) with 100 nm core diameter in the absence or presence of 2 mM EDTA (*left* panels; as indicated) after 75 minutes of flow at 200 µL/hr. EDTA allows NPs to penetrate deeper into the islet core due to dissociation of cell-cell junctions and expansion of intercellular space (DIC images at *right*; scale bar represents 50 µm). (**B**) Average NP accumulation in cross-sectional images acquired 10 µm from the base of each islet in the absence or presence of EDTA. (**C**) Average accumulation of NPs in the absence and presence EDTA according to cross-section segmentation. * Indicates $p<0.05$ by one-way ANOVA. N = 9 – 15 islets harvested from two mice on separate days.

Figure 4: Viral transduction efficiency and particle penetration are optimal for islets exposed to EDTA and microfluidic flow. Islets were transduced with 5×10^9 pfu/mL of adenovirus carrying a green fluorescent protein (GFP) gene cassette. Transduction was performed in a standard culture dish without flow ("Static") or in the device ("Flow") in the absence (-) or presence (+) of EDTA for 75 minutes. Twenty-four hours post-transduction, islets were fixed and nuclei were stained with DRAQ5 (*blue*). GFP expression was determined by confocal microscopy. (**A**) Dual colour confocal z-slice images acquired 15 and 25 µm from the base of each islet reveal that the combination of islet exposure to 2 mM EDTA and media flow visibly enhanced GFP expression deeper within the islet tissue. Scale bar represents 50 µm. (**B**) Transduction efficiency measured as percentage of GFP-positive cells compared to total cell number (total DRAQ5-positive nuclei) in z-slices acquired 25 µm from the bottom of each islet. (**C**) Map of cell x-y position from a representative islet that underwent transduction with combined flow and 2 mM EDTA exposure. GFP-positive cells are represented by blue dots surrounded by a green halo while non-expressing cells are represented as blue dots without halos. Each islet cross-section was segmented into two regions: core and rim, and the boundary set such that each region contains the same number of cells. (**D**) Cells located in the core of islets treated with 2 mM EDTA under flow exhibited significantly higher transduction efficiency than all other treatment regimes. (**E**) Cells at the islet rim exhibited higher transduction efficiencies overall compared to the core, with EDTA/flow islets exhibiting the highest efficiency overall. * Indicates $p<0.05$ by one-way ANOVA. N = 16 – 29 islets from a total of five mice. (**F**) Schematic illustration of highly efficient adenoviral transduction (HEAT)-on-a-Chip protocol.

Figure 5: EDTA treated islets maintain function. (**A**) Islets were treated with microfluidic flow in the absence (-) or presence (+) of 2 mM EDTA for 75 min and subsequently allowed to recover for 24 hr in normal culture. Representative images show the islets after labelling with fluorescein diacetate (FDA; live cells; *green*) and propidium iodide (PI; dead cells; *red*). Scale bar represents 50 µm. (**B**) Islet stained with FDA/PI after 1 hour incubation in 0.3% H_2O_2 to induce cell death. Scale bar represents 50 µm. (**C**) Percentage cell viability calculated for N=13 (-EDTA) and N=15 (+EDTA) islets. (**D**) Representative NAD(P)H autofluorescence images of islets treated for 75 min with (+) or without (-) 2 mM EDTA under flow and subsequently cultured for 24 hours prior to stimulation with 2, 10, or 20 mM glucose, as indicated. NAD(P)H intensity increases with glucose dose, independent of exposure to EDTA. Scale bar represents 20 µm. (**E**) Fold changes in NAD(P)H autofluorescence exhibited no significant variation between islets treated with (+) or without (-) EDTA. N = 25 total islets for each treatment from three mice. (**F**) Representative fluo-4 images of islets recovered 24 hrs after being treated in media with (+) or without (-) 2 mM EDTA. Data from three regions of interest (ROIs) in each image are shown. Scale bar represents 50 µm. (**G**) Fluorescence intensity traces on three ROIs of each islet demonstrate synchrony of Ca^{2+}-oscillation peaks (and/or) troughs, regardless of prior EDTA exposure, over a 2.5 minute data acquisition period. Scale bars represent 50 seconds. (**H**) Dispersed islet cells stained with fluo-4 and stimulated with 10

mM glucose. Scale bar represents 50 µm. (**I**) Fluorescence intensity traces on three ROIs within the field of view demonstrate a lack of synchrony in Ca^{2+} oscillations. Scale bar represents 50 seconds. (**J**) Islets treated with (+) or without (-) 2 mM EDTA under flow for 75 minutes one day prior to static stimulation at 2, 10 and 20 mM glucose. N=10 runs from between 6-8 islets per run from five mice.

Figure 6. Islets exhibit radial distance-dependent heterogeneity in ER redox buffering capacity. (**A**) Representative ratiometric time course images of an islet excited with 458 nm and 514 nm laser lines. A shift from green to red indicates a change from oxidized to reduced state. Scale bar represents 50 µm. (**B**) The intensity ratio increased upon treatment with DTT (reducing agent) revealing the kinetics of the change in ER redox state at the periphery (ROI 1) and the center (ROI 2) of the islet. When DTT was removed ("washed out"), cells within each of these defined ROIs exhibited differential recovery rates. Recovery profiles were fitted to exponential decay functions. (**C**) Plotting ROI decay constants against normalized distances reveals a negative correlation between decay constant value and distance from the center of the islet. Yellow shading represents 95% prediction bands. The data indicate that cells in the core of an islet exhibit greater ER redox buffering capacity than those at the periphery.