Supplementary Material For:

Collection of islets of Langerhans using an equilibrium method

Duk-Su Koh[1,2], Mark Moody[1], and Junghyo Jo[3,†]
[1]Department of Physiology and Biophysics, University of Washington, Seattle, WA, [2]Department of Physics, POSTECH, Pohang, Republic of Korea, and [3]Laboratory of Biological Modeling, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD

[†]J.J.'s current address is APCTP, POSTECH, Pohang, Republic of Korea

BioTechniques 55:34-37 (July 2013) doi 10.2144/000114053
Keywords: islets; tissue; isolation; purification; equilibrium

Supplementary Methods

Theoretical background of the equilibrium method

When islets are positioned in a vertical capillary, they will settle down slowly due to gravitation because islets (density, $\rho_I \sim 1.065$ g/mL) (1) are denser than buffer solutions such as HBSS ($\rho \sim 1$ g/mL). Their downward gravitational force is greater than the upward buoyant force (Supplementary Figure S1A). If there is a superimposed upward flow of the buffer solution, the islet can be lifted by the current. If the sum of buoyancy (F_B) and drag by the flow (F_D) equals the gravitational force (F_G), the islet stalls, trapped at one specific flow rate. Hence, we reasoned that in an angled structure where the flow rate of the solution gradually decreases, the equilibrium should be reached at a wide range of flow rates (Supplementary Figure S1B). Here, the flow condition for islets of radius (r) under relatively slow flow velocity (v) with water viscosity (η) corresponds to small Reynolds number ($\rho v r / \eta < 1$). Under this regime, the drag force exerted on islets can be described by Stokes' law ($6 \pi \eta r v$). At equilibrium, therefore, the gravitational force on an islet of radius (r) is balanced by the buoyancy and the drag forces:

$$(3/4)\,\pi r^3 \rho_I g = (3/4)\,\pi r^3 \rho g + 6\,\pi \eta r v, \qquad \text{[Eq. 1]}$$

where ρ_I and ρ are the densities of islets and water, respectively, and g is the acceleration due to gravity (Supplementary Table S1).

Supplementary Table S1

η (water viscosity)	10^{-3} Pa*s
g (gravitation constant)	9.8 m/s^2
ρ_I (islet density)	1065 kg/m^3
ρ (water density)	1000 kg/m^3
θ (tip angle)	5°

Parameters used for simulations

Considering the geometry of a tip as shown in Supplementary Figure S1B, the flow velocity (v) at the height (z) from the end of the tip is described as $v = f / (\pi a^2)$ with $a = (z + z_0) \tan(\theta / 2)$, where f is the flow rate, a is the inside radius of the tip at z, θ is the tip angle and z_0 is $a_0 / \tan(\theta/2)$ where a_0 is the radius of the tip end. For simplicity we assume that the flow rate is the same across the cross section at a specific value of z (i.e., laminar flow). Substituting this expression into Equation 1, one can solve for z:

$$z = [(9\,\eta f / 2\pi(\rho_I - \rho))^{0.5} / \tan(\theta/2)\, r] - z_0 \qquad \text{[Eq. 2]}$$

Equation 2 predicts that islets will remain suspended in the tip as a function of the square root of the flow rate and that larger islets will reside at a lower level. Supplementary Figure S1C indicates that a conical tube of a certain length can trap islets of different sizes at a broad range of flow rates.

Preparation of pancreatic islets

Islets were obtained as described previously (2). Briefly, 5–10 week old male BALB/c mice were euthanized by CO_2, following the University of Washington Animal Medicine guidelines. The pancreas was dissected, chopped into small pieces using a pair of dissecting scissors (Catalog #08–951–5; Fisher, Pittsburgh, PA), and washed twice in Hank's balanced saline solution (HBSS) containing 0.1% BSA and 10 mM HEPES buffer. The pancreatic pieces were then digested with 10 mL collagenase type P (2.5 mg/mL in HBSS) (Catalog #11 249 002 001; Roche, Indianapolis, IN) for 40 min. The pancreatic tissue was gently agitated with a homemade rotating shaker (15 rpm) inside a 37°C incubator. For some experiments, 1 mg/mL collagenase was injected into the main pancreatic ducts until the pancreas inflated significantly. After enzymatic digestion, the pancreatic digest was manually shaken 20 times, triturated 7 times with a 5 mL serological pipette, washed twice with cold HBSS, centrifuged at 500 rpm for 2 min, and transferred to a 100 mm disposable culture dish. Human islets were prepared similarly and provided by Prodo Laboratories (Irvine, CA).

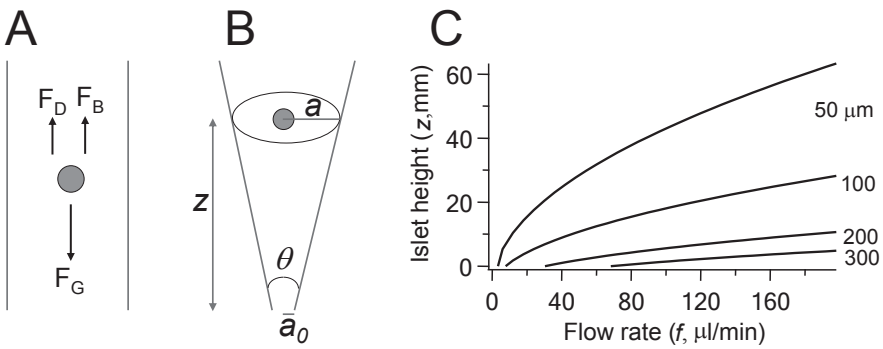
Imaging islets

Islets in a micropipette tip were visualized under a stereomicroscope (Wild M7A; Wild Heerbrugg, Heerbrugg, Switzerland) at 60–500× magnification. Under transillumination (fiber optic ringlight illuminator 100 W; Volpi Co., Auburn, NY), islets appear transparent and acinar cells white, enabling visual identification of islets (Figure 3A and Supplementary Figure S2A). Images were obtained using a Canon EOS 50D adapted to the ocular tube of the stereomicroscope. Islet movement was determined by measuring islet location using ImageJ (http://imagej.nih.gov/ij/). The saline solution used for imaging experiments was composed of (in mM) 150 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 10 glucose (pH 7.4 adjusted with NaOH).

To measure intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$), islets were loaded with Fluo-4 AM Ca^{2+} indicator dye for 30 min at 37°C, incubated without the dye for 30 min, and monitored with a confocal microscope (Zeiss 710 with a 40× oil lens, Thornwood, NY). The dye was excited at 488 nm and fluorescence emission was detected at 510–540 nm. Fluorescence intensity in single islet cells was measured using ImageJ. For constant osmolarity of saline solutions containing either 2 or 16 mM glucose, NaCl concentration was adjusted. The confocal Ca^{2+} imaging was performed with a local solution perfusion system to maintain the temperature of perfusates at 35–37°C (1).

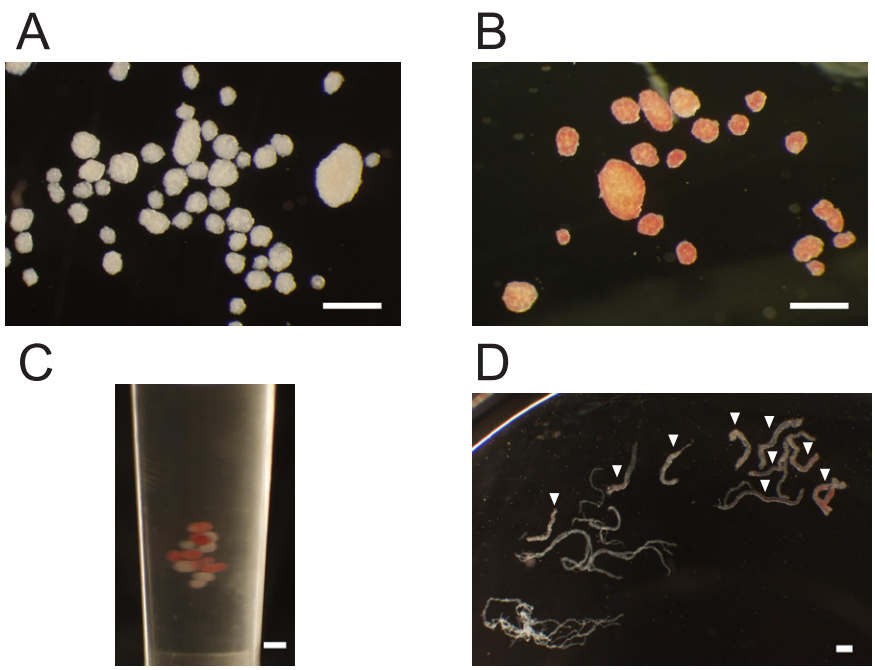
Insulin secretion

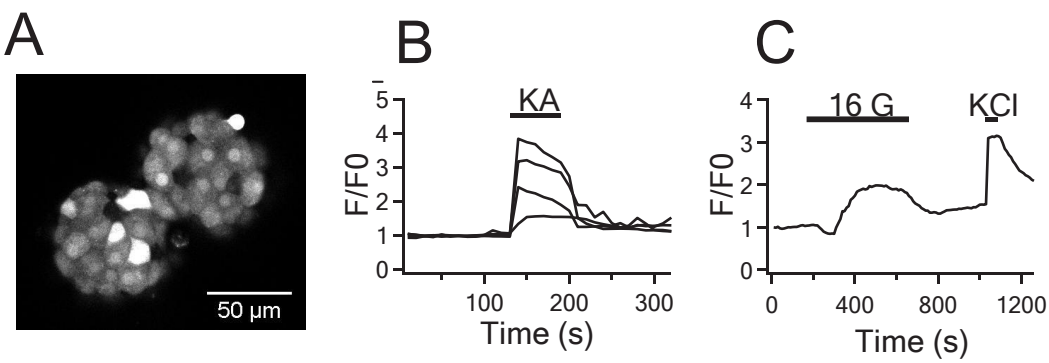
Islets were incubated in 5 mM glucose-containing RPMI culture medium in 96-well culture plates overnight. The next day, the islets adhering to the plastic surface were washed twice with a saline solution containing 2 mM glucose and incubated in the same solution for 1 h at 37°C. The supernatant was discarded and the islets were washed twice again. Islets were sequentially incubated with 2 and 16 mM glucose for 1 h each. The supernatants were sampled, centrifuged at 1200× g for 3 min, and frozen. Insulin content was measured by ELISA according to the manufacturer's instructions (Mercodia, Winston Salem, NC).



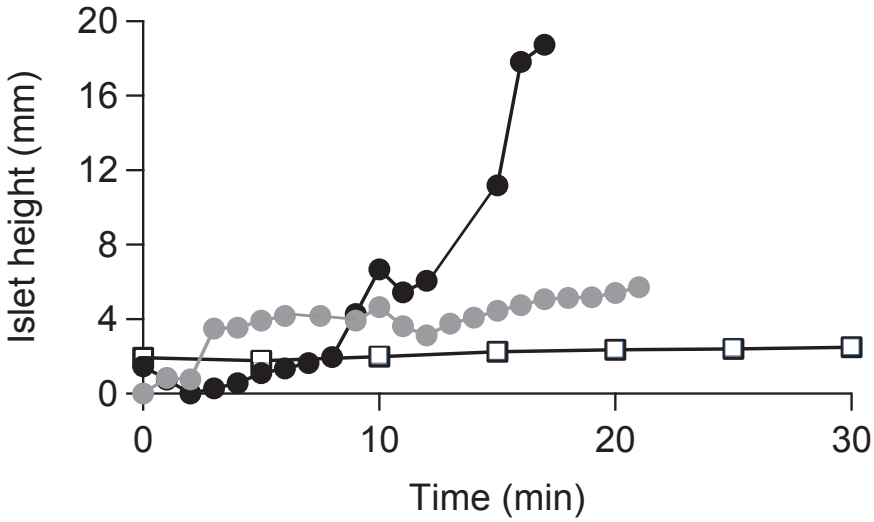
Supplementary Figure S1. Theoretical background for the equilibrium method. (A) Three forces determining the movement of objects such as islets with a constant upward flow; Drag force (F_D), buoyant force (F_B), and gravitational force (F_G). (B) Physical dimensions of a gradually widening tube such as a micropipette tip. (C) Predicted equilibrium height (*z*) of differently sized islets in a micropipette tip at different flow rates (*f*). Islet height (*z*) was calculated using Equation 2 and the values in Supplementary Table 1.

Supplementary Figure S2. Other tissues collected using the equilibrium method. (A) Human islets obtained from Prodo Laboratories were mixed with rodent acinar cells, and picked up using our method. (B) Human islets stained with dithiazone (DTZ) as in Figure 3B. (C) DTZ-stained human islets (red) and unstained rodent islets were collected at the same height in the micropipette tip. (D) Mouse pancreatic ducts and blood vessels. For pancreatic ductal structures, 10% fetal bovine serum was used to reduce their adherence to dish and micropipette tip during collection. Ductal structures with enclosed red blood cells (arrowheads) are identified as blood vessels. Others may be pancreatic ducts with ductal aborizations. Scale bars are 0.5 mm.





Supplementary Figure S3. Intracellular Ca^{2+} signal evoked by activation of glutamate receptors and high glucose. (A) Ca^{2+} imaging of two islets loaded with Fluo-4 Ca^{2+} sensitive fluorescent dye. A cell layer near the islet surface was focused in the confocal microscope experiment. The image shown was obtained during application of 0.5 mM kainate, an activator of AMPA-type glutamate receptor channels. (B) Fluorescence measurements in individual cells before, during, and after kainate treatment. Fluorescence level (F) was normalized to the initial fluorescence at time zero (F0). Intracellular Ca^{2+} rose in four cells (lines), while the majority of cells were not responsive (see Supplementary Movie 1). Glucose concentration was low (2 mM) for this measurement. (C) The same islets were challenged with high glucose (16 mM). Many cells responded to high glucose in a synchronized manner (Supplementary Movie 2). Average fluorescence of whole islets is shown. Note the significant delay of Ca^{2+} rise after onset of glucose stimulation due to metabolism of glucose to generate ATP. ATP blocks ATP-sensitive K^+ channels and therefore depolarizes the membrane, leading to opening of voltage-gated Ca^{2+} channels and Ca^{2+} influx (1). At the end of the experiment, a saline solution with high KCl (150 mM) was applied to depolarize the cell membrane up to 0 mV. Solutions were applied to the islets using a local perfusion system with the capacity to control temperature at 35–36°C (1). A representative recording of one of six experiments.



Supplementary Figure S4. Loose islets floating higher in a pipette. Islet heights were measured over >15 min at a flow rate of 32 µl/min either in a control saline solution (open rectangles) or in saline containing 0.1% trypsin (closed symbols, 2 different experiments). Islets digested with trypsin moved up gradually, while control islets stayed at the same position over the observation period. Measurements were taken at room temperature.

Additional Legends

Supplementary Movie 1. Confocal Ca^{2+} imaging experiment shown in Supplementary Figure S3A. The label in the upper right corner indicates the period of 0.5 mM kainate treatment.

Supplementary Movie 2. Confocal Ca^{2+} imaging experiment shown in Supplementary Figure S3B. The labels in the upper right corner indicate the period of 16 mM glucose and 150 mM KCl treatments.

References

1. **Koh, D.S., L. Chen, C.A. Ufret-Vincenty, and S.R. Jung.** 2011. A fast solution switching system with temperature control for single cell measurements. J. Neurosci. Methods *199*:35-42.
2. **Smith, P.A., P. Rorsman, and F.M. Ashcroft.** 1989. Modulation of dihydropyridine-sensitive Ca^{2+} channels by glucose metabolism in mouse pancreatic beta-cells. Nature *342*:550-553.