Benchmarks

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Collection of islets of Langerhans using an equilibrium method

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Here we present a convenient method for easy hand selection of enzymatically isolated small tissues such as islets of Langerhans. Islets are continuously collected in a micropipette tip connected to a peristaltic pump. After entering the conical micropipette tip, the islets are quickly dragged up by solution flow, but this movement subsequently decreases as the flow rate decreases. Thus, the islets are trapped at a specific height where downward gravitation balances upward buoyancy and the drag provided by solution flow. Our device allows more efficient isolation of islets compared to conventional manual collection methods.

Pancreatic islets of Langerhans are clusters of endocrine cells secreting different metabolic hormones. Studies of isolated islets have enhanced our understanding of the molecular mechanisms involved in hormone secretion. Islets can be mechanically isolated from pancreatic tissue after enzymatic treatment, typically with collagenase; however, additional steps are then needed to separate islets from the more numerous acinar cells (1). For purification of large numbers of islets, density gradients, such as the Percoll gradient, exploit the high density of islets compared with other pancreatic components, including acinar cells and pancreatic ducts (2). The gradient step is usually omitted as a matter of convenience for isolation of fewer than 100 islets. Instead, the islets are identified using a stereomicroscope and hand-selected with a micropipette. In enzymatic digests of pancreatic tissues, islets are typically scattered in a large bed of acinar cell clusters. The search for each islet takes time and requires holding the push button of a micropipette for several seconds. This collection step is repeated until the target number of islets are collected. To improve this hand picking procedure, we have developed a simple method using a peristaltic pump whereby islets can be collected continuously (Figure 1).

A 10 µl micropipette tip is connected to a plastic adaptor (red tube in Figure 1A). We use part of the rubber aspirator delivered with Drummond disposable micropipettes (VWR Cat. No. 53432–604, Radnor, PA), which fits many disposable 10 µl micropipette tips. The adaptor is further connected to silicone tubing (i.d. 3 mm, o.d. 5 mm, blue tubing) that serves as a handhold while islets are collected. It is partially flexible so that islets collected in the micropipette tip can be expelled into a new dish when squeezed with the holding fingers. Finally, the adaptor tubing is connected to a peristaltic pump (Rabbit, Rainin Instrument, Oakland, CA) to drive the solution flow at a constant speed. Using polyvinyl chloride tubing with a small diameter (i.d. 0.76 mm, Rainin Instrument, Cat. No. 39–624), flow rates of 1–300 µl/min can be controlled.

Since the pump drains solution at a constant volume, the flow rate is high at the narrow tip end, allowing islets to be sucked into the tip efficiently (Figure 1B). After entering the pipette tip, the islets move upward by dragging flow and buoyancy, while gravitation pulls the islets downward. The flow rate gradually decreases along the tip as the cross-sectional area increases, resulting in less drag. Since buoyancy and gravitation remain constant, upward movement gradually decreases and the islets eventually stall at an equilibrium height (see Supplementary Methods). As shown in Figure 1C, islets moved up as flow rate increased and moved down as flow rate decreased again in a vertically positioned micropipette. The equilibrium positions of islets match well with theoretical predictions (Figure 2A). Since some islet isolation protocols include serum in Hank's balanced salt solution to improve viability, we tested the effect of 10% fetal bovine serum (FBS) on the equilibrium height. In spite of the expected increased viscosity, islets were collected at similar positions (Figure 2B). To collect islets manually under a stereomicroscope, the tip needs to be held an angle of 45–55° (Figure 1A). In the tilted tip, islets behaved similar to when in the vertical position (Figure 2C). Note that in the tilted tip, the vertical component of the drag force is still balanced with the gravitational and buoyancy forces (Supplementary Methods, Equation S1).

If enzymatic digestion is insufficient, some islets remain attached to acinar cells and ductal structures such as blood vessels. Those islets tend to

Method summary:

This method enables efficient collection of islets of Langerhans following enzymatic digestion of mouse or human pancreas tissue. Based on a simple physical principle, islets may be collected continuously using this procedure, which requires less effort and time than the standard approach. Resulting islet collections retain function, making them suitable for downstream studies.

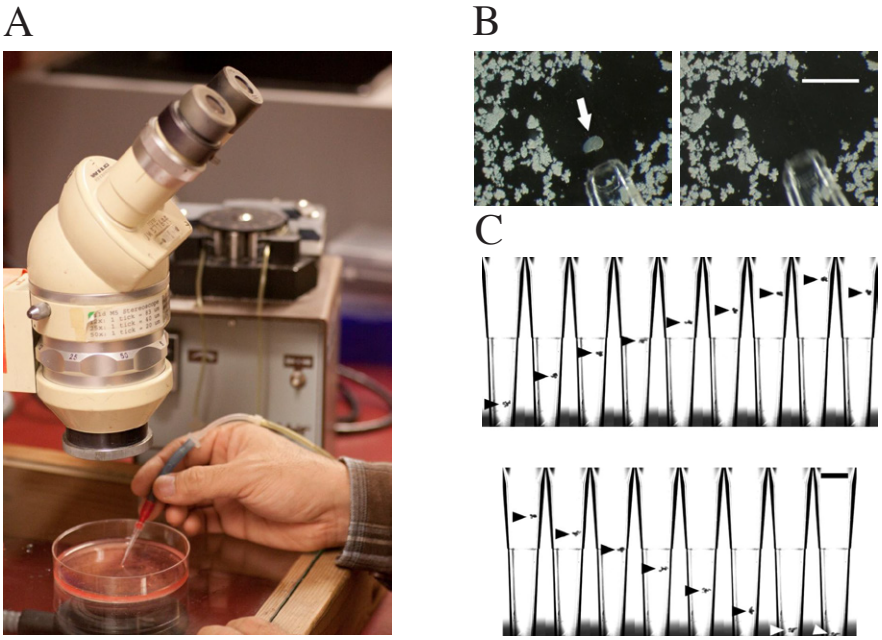

FIgure 1. Setup and demonstration of the equilibrium method. (A) Photograph of a 100 mm dish containing an islet-acinar cell mixture on a trans-illumination box. The collection device consists of a micropipette tip connected to an adaptor (red) and flexible tubing (blue) that is attached to a peristaltic pump. Mouse pancreas was treated with collagenase as described in the Supplementary Methods. (B) Islet collection. An islet (marked with an arrow) surrounded by acinar cells before (left micrograph) and after (right micrograph) selection. Note that the tip was advanced toward the islet for collection. Scale bar represents 1 mm. (C) Determination of equilibrium height at different flow rates. Micrographs show the heights of islets in a micropipette tip. Flow rate was increased stepwise from 46 (1st image) to 184 µl/min, and then decreased to 32 µl/min (last image in the second row). Each picture was taken after the islets reached their equilibrium position. Four islets were included in this measurement with the micropipette tip in a vertical position. Photographs were taken with a horizontally aligned stereomicroscope. Scale bar represents 2 mm.

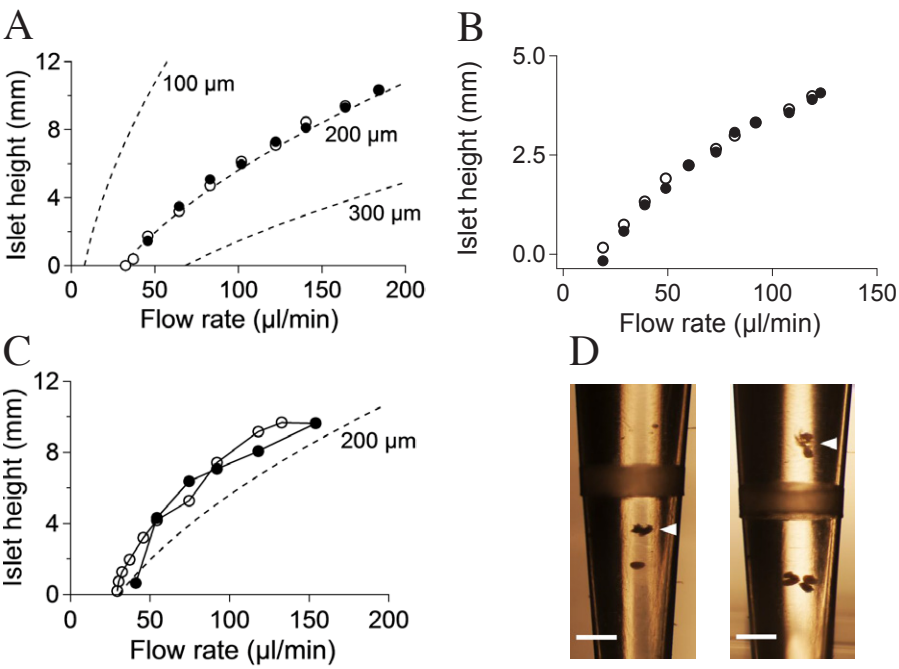

Figure 2. Movement of islets in the micropipette. (A) Heights of islets in the micropipette tip from the experiment in Figure 1B. Open and closed symbols represent islet height during gradually increasing and decreasing flow rates, respectively. Broken lines indicate the theoretical prediction of islet heights for different islet diameters using Supplementary Equation 2 (Supplementary Methods). Contrary to the predictions, islets of different sizes were not separated, since intact islets tend to form a clump. The final height of an islet clump seems to depend on the average size of the islets (208 µm in this experiment); theoretical predictions for the heights of 100, 200, and 300 µm islets are shown as dashed lines**.** (B) The experiment in (A) was repeated with a saline solution containing 10% fetal bovine serum (FBS) (open circles). Islet heights were also measured in control saline solution (closed circles). (C) Measurements with a tilted micropipette tip (51° from horizontal). Islet heights were measured as the vertical height from the horizon (d * $\sin\theta_t$, where d is the distance of islets from the tip and θ_t is the tilted angle). (D) Behavior of islets attached to other tissues. Islets tethered to acinar cells (arrowhead, left micrograph) and ductal structures (arrowhead, right micrograph). Scale bars represent 0.5 mm.

reach higher equilibrium heights due to the lower density of acinar cells (1.015–1.045 g/mL versus 1.065–1.070 g/mL for islets) and the larger surface area of ductal structures (i.e., more drag) compared with round islets of similar sizes (Figure 2D).

Another advantage in using a micropipette tip with a small opening (i.d. 0.6 mm) is that suction is strongest at the opening. Therefore, with a wide range of flow rates (0.5–5 µl/s), only the islets targeted by the tip are picked up, minimizing contamination with surrounding acinar cells (Figure 1B). To remove acinar cells captured with the islets, we performed a second round of selection. With some practice, more than 100 islets can be picked up within 20 min from a single pancreatic digest. As expected, our method can be used to collect human islets and other tissues such as pancreatic ducts (Supplementary Figure S2). Human and rodent islets were trapped at similar heights in the micropipette tip, suggesting comparable size and density for islets from the two species.

Next, we examined the purity and functionality of islet cells collected with our method. Visual inspection of islets did not show any deterioration of islet morphology during purification

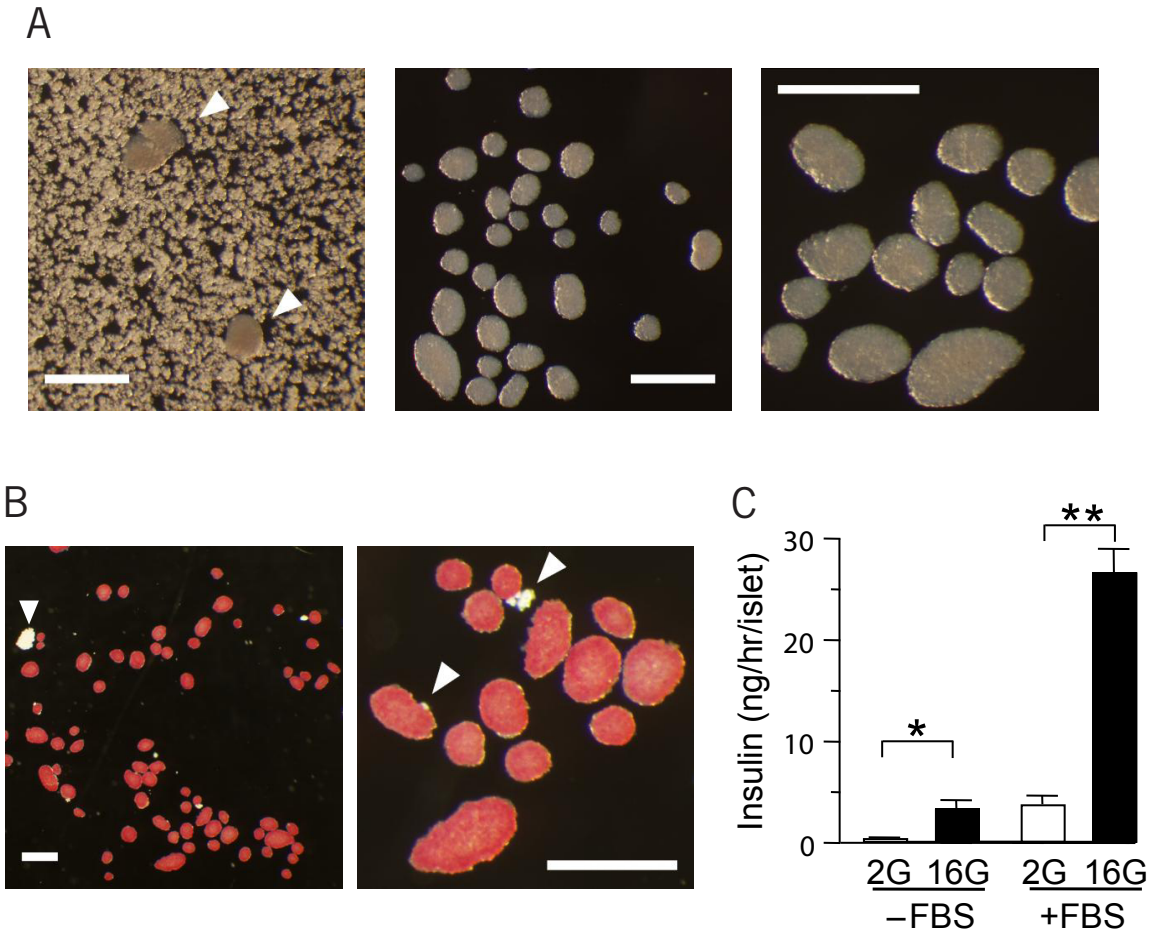

Figure 3. Morphology and insulin secretion from islets harvested by the equilibrium method. (A) Photographs of islets embedded in pancreatic acinar cells (left) and after collection (middle). A few islets are shown at a higher magnification, demonstrating clear surfaces (right). (B) Islets were stained with 0.5 μg/mL dithiazone solution for 10 min and then washed with dye-free saline (left). An acinar lobule marked with an arrowhead was included. Islets with attached acinar tissues are shown at a higher magnification (right). Scale bars in (A) and (B) represent 0.5 mm. (C) Glucose-stimulated insulin secretion in the absence and presence of 10% fetal bovine serum (FBS). Insulin secretion in 2 mM (2G) and 16 mM (16G) glucose-containing saline was measured using ELISA as described in the Supplementary Methods. Duplicate measurements were obtained for each condition from 3–5 experiments. Significance level for Student's *t*-test, $P < 0.05$ (*) and $P < 0.01$(**).

(Figure 3A). Unlike the Percoll gradient method, we can pick up the healthy islets and leave behind the damaged ones. Dithizone staining (3) clearly demonstrates that most of the islets we selected were devoid of attached acinar cells (Figure 3B). The islets were also functional in terms of Ca^{2+} signaling. Some cells on the mantle of islets responded to kainate, an agonist for AMPA-type glutamate-receptor, suggesting that they are probably α- and δ-cells (Supplementary Figure S3A and B, and Supplementary Movie S1) (4). As we expected, these cells did not respond to high glucose. The majority of islet cells responded to the high glucose concentration (16 mM); therefore they are probably insulin-secreting β-cells (Supplementary Figure S3C and Supplementary Movie S2). Islets secreted insulin upon glucose stimulation (Figure 3C). The basal insulin secretion was negligible in a normal saline solution containing 2 mM glucose, but insulin secretion increased in a 16 mM glucose solution. Both basal and glucose-stimulated insulin secretion increased when 10% FBS was included in the saline solution, probably due to activation of protein kinases (5).

In addition to collection of islets, our method may be used to detect physical changes in cells, such as textured or loose surfaces due to excessive treatment with collagenase, mechanical trituration, or prolonged incubation in culture. Equation S2 in the Supplementary Methods predicts that the height of an islet in the micropipette tip will depend on islet density (ρ_I), which is reduced in loose islets due to the presence of buffer solution between cells. Textured and rough surfaces will also increase the frictional drag force of solution flow, causing the damaged islets to move to a higher position in the tip. In fact, after loosening cell junctions with trypsin, we observed a slow upward movement over 10–20 min, while islets perfused with a trypsin-free saline solution stayed at the same height (Supplementary Figure S4). This suggests that our method can be used to test the quality of islet preparation. However, for clinical or research applications requiring islets (e.g., transplantation) further validation such as a careful calibration of flow rate and tip geometry will be required.

In conclusion, islets can be collected continuously into a micropipette tip

based on simple physical characteristics. This method reduces collection time and lessens hand strain compared with conventional protocols. Thus far, our approach has been used for several projects (4, 6–10). We expect that, with minor modifications, this method may be used to collect cells from a variety of tissue types.

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Competing interests

The authors declare no competing interests.

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