

# Preparation of monolayer cultures of primary rat and human islet cells on glass for super-resolution and live cell imaging

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## Method Article

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# Abstract

Pancreatic islets of Langerhans consist of interactive micro-societies of different hormone producing endocrine cells, including insulin producing beta cells, glucagon producing alpha cells, somatostatin producing delta cells and pancreatic polypeptide producing PP or gamma cells, which together control glucose homeostasis. High resolution imaging studies of the cell biology of islet cells have been limited by insufficient techniques to culture monolayers of primary pancreatic islet cells on glass coverslips. Combining the approach of using well defined matrices optimized for each species and growth factors required for culture of primary neurons, we describe a simple and reproducible method enabling robust adhesion and formation of monolayers of well connected and fully differentiated human and rodent pancreatic islet endocrine cells. This technical advance enables detailed observation of cellular processes in primary islet cells by live cell and super-resolution microscopy.

## Reagents

Laminin stock solution 1mg/ml (Life Technologies #23017015) for rat islet monolayer Prepare small aliquots (50 µl each) and store them at -20°C for up to 6 months. Collagen IV stock solution 1 mg/ml (Sigma Aldrich #C5533) for human islet monolayer Prepare small aliquots (50 µl each) and store them at -20°C for up to 6 months. Minimum Essential Medium (MEM) (Life Technologies #21090-022) Fetal Bovine Serum (FBS) Glutamax 100x (Life Technologies #35050061) Hepes Buffer 1 M (Bioconcept.ch #5-31F00-H) Na-pyruvate 100 mM (Life Technologies #11360070) B-27 Neuronal Supplement 50x (Life Technologies #17504044) 25% D-Glucose in ddH<sub>2</sub>O, sterile filtered Penicillin-Streptomycin 100x (Life Technologies #15140-122) Phosphate Buffered Saline (PBS) without Ca<sup>2+</sup>/Mg<sup>2+</sup> (PBS<sup>-/-</sup>) 1x, pH 7.4 (Life Technologies #10010-015) Hanks Balanced Salt Solution (HBSS) with Ca<sup>2+</sup>/Mg<sup>2+</sup> (Life Technologies #14025-092) Trypsin 0.05% with EDTA 0.02% (Gibco #25300054) Ethanol, absolute

## Equipment

Cell culture facility (Biosafety level 2 is required when working with human material, while islets of rodent origin can be manipulated in Biosafety level 1) Laminar flow hood 24 or 48-well cell culture plates (e.g., Cell Star, Greiner Bio-One #662160 and #677180) Round 8 or 12 mm diameter and 0.17 mm thickness borosilicate glass coverslips (Electron Microscopy Sciences #72230-01) Or Coverglass-bottom Fluorodishes 35mm (World Precision Instruments #FD35-100) Or Coverglass-bottom MatTek dishes 35mm (MatTek Corporation #P35G-0.170-14-C) Sterile forceps Glass beaker (100-150 ml) Sonicator Autoclave Research pipettes with 1000 µl, and 200 µl pipette tips with filter Standard 15-ml polypropylene lab tubes (e.g., Falcon 15-ml tubes with conical bottom and blue screw cap; Falcon, #352070) Table top centrifuge for use with 15-ml tubes Water bath at 37 °C Incubator at 37 °C and 5% CO<sub>2</sub> Disposable bottle filter (0.22 µm) (e.g., Vacuum Filtration 500 "rapid"-Fitemax, TPP, #99500) Hemocytometer for cell counting Optical microscope with 10x and 20x objectives **Reagent setup** • **Pancreatic human or rat islets:** Rat islets isolated from P5 rats (we recommend using the protocol described in Kanaani, et al., (2015) and pancreatic human islets obtained from pancreatic donors. **Important:** Viability and purity

of islets are key factors in obtaining monolayer cultures of high quality, viability and functionality. • **Laminin and collagen IV solution:** Thaw (on ice) a laminin or collagen IV aliquot (stock solution 1 mg/ml) for preparation of rat or human islet monolayer, respectively. **Caution:** Laminin/collagen IV can gel if left at room temperature. • **Culture medium:** Prepare neuronal culture medium by supplementing MEM medium with 5% FBS, 1x B-27, 1% P/S, HEPES 10 mM, Glutamax 1x, Na-pyruvate 1mM, and glucose to a final glucose concentration of 11 mM. For 50 ml of medium: MEM 44.3 ml FBS 2.5 ml Pen/Strep 0.5 ml B-27 1.0 ml Pen/Strep 0.5 ml HEPES 1M 0.5 ml Glutamax 100x 0.5 ml Na-pyruvate 100mM 0.5 ml Glucose 25% 0.2 ml • **Glass coverslips** Transfer the number of glass coverslips needed into a glass beaker and clean by sonication in 100% ethanol. Remove ethanol, cover the beaker with aluminum foil and sterilize in autoclave.

## Procedure

**Coat coverslips. Timing:** 10-15 min (depending on the number of coverslips). 1. Thaw aliquots of laminin or collagen IV (1 mg/ml) prepared as described above and dilute to 50 µg/ml in HBSS with  $\text{Ca}^{2+}/\text{Mg}^{2+}$  2. Transfer the coverslips to a 24- or 48-well plate using sterile forceps 3. Coat each glass coverslip or Fluorodish with laminin or collagen solution, and incubate at 37 °C for 1-2 hours or overnight in an incubator. **Note:** Use the minimal amount of solution required for covering each coverslip or Fluorodish (typically 40 µl and 80 µl on coverslips in 24- and 48-well plate respectively, and 500 µl on Fluorodish). **Culture medium preparation. Timing:** 10 minutes 4. Prepare the neuronal culture medium as described above and filter-sterilize it with a disposable bottle filter (0.22 µm). 5. Warm up the neuronal medium and the trypsin (0.05%) at 37 °C. **Islet dissociation and seeding. Timing:** 30 minutes 6. Collect rat or human islets from Petri dishes in a 15 ml tube. **Note:** About 1 million single islet endocrine cells are usually obtained from 1,500 islets (three 10 cm Petri dishes with 500 islets each). 7. Centrifuge for 2 min at 110 x g (800 rpm) in a table top centrifuge 8. Aspirate the supernatant and wash 3x with PBS<sup>-/-</sup> 9. Remove PBS and add 300-400 µl of pre-warmed 0.05% Trypsin/EDTA solution. **Note:** The volume of trypsin for dissociating islets depends on the number of collected islets. We recommend using 300 µl and 400 µl for dissociation of about 1,500 and 2,500 islets respectively. 10. Using a micropipette with a 1000 µl tip, pipette up and down **very slowly** and without forming bubbles for about 3 minutes or until all islets are dissociated and no longer visible in solution. **Note:** 3 minutes are generally required to dissociate 1,500 rat islets. Longer incubation times are usually necessary for a higher number of rat islets and for human islets. **Caution:** This is the most critical step of the protocol: the pipetting has to be performed very slowly, avoiding formation of bubbles and continue only until no visible islets are present in solution. When islets are fully dissociated into single cells, the solution becomes opaque: at this stage, proceed immediately to steps 11 and 12 to avoid over-digestion. 11. Fill the tube up to 15 ml with the pre-warmed neuronal medium. 12. Centrifuge at 350 x g (1400 rpm) for 6 minutes. Proceed immediately to step 13. **Wash of the coverslips. Timing:** 5 min. During the centrifugation step described in 12, remove laminin/collagen solution from the coverslips/Fluorodishes and wash three times with HBSS (containing  $\text{Ca}^{2+}/\text{Mg}^{2+}$ ). Let the coverslips air dry for approximately 5 minutes. **Seeding of islet single cells. Timing:** 30 min + 3-4 days for last step 13. Gently remove

supernatant and re-suspend single islet cells in 3 ml of neuronal medium 14. Take a few microliters from the cell suspension and count the cells 15. Seed cells at 35,000 cells/cm<sup>2</sup> – or approx. 30,000 cells on each laminin or collagen-coated coverslip (48-well plate), 60,000 cells on laminin or collagen-coated coverslip (24-well plate), or 100,000 cells on laminin or collagen-coated Fluorodish. **Note:** When seeding cells on coverslips, use the minimal required volume to fully cover each coverslip (about 40 µl, 80 µl and 500 µl on coverslips for 48-well, 96-well and Fluorodishes respectively) avoiding leakage from the edges (important for maintaining the cells on top of the coverslip or Fluorodish and avoiding cells escaping underneath coverslips and outside the glass ring on Fluorodishes). Incubate for about 5-6 hours. 16. Slowly add neuronal medium on the side of the wells to reach the final volume of 500 µl, 1 ml and 2.5 ml for 48-well, 24-well and Fluorodish respectively. 17. Wait 3-4 days to reach full adhesion and spreading of islets cells before proceeding with experiments. **Note:** During the first 24 h upon seeding, cells display a rounded morphology. A more extended shape and the formation of two dimensional micro-societies of endocrine islet cells is reached 1-2 days after the seeding procedure

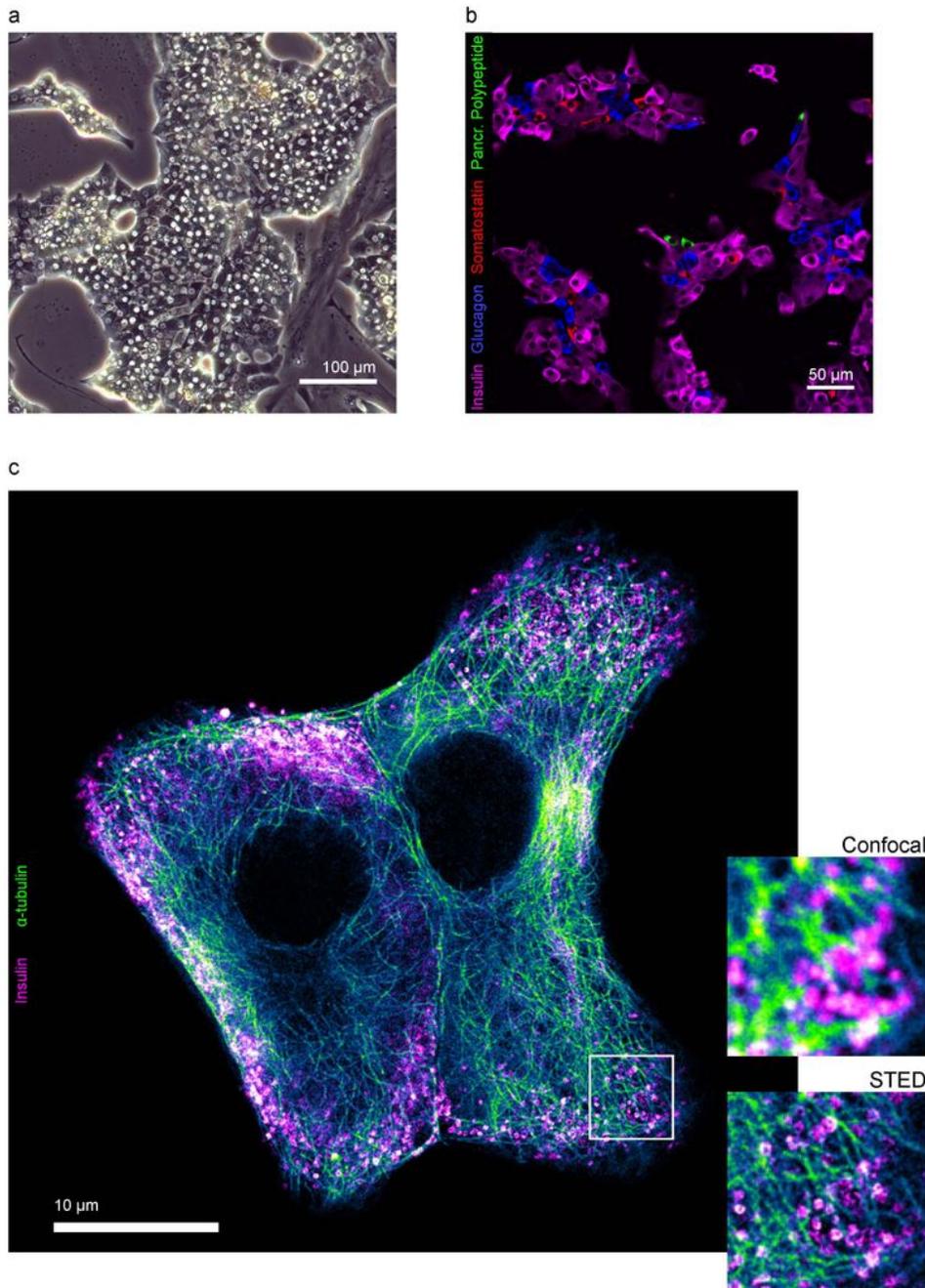
## Anticipated Results

During the first 24 h upon seeding, cells display a rounded morphology. A more extended shape and the formation of two-dimensional micro-societies of well-differentiated endocrine islet cells is reached 1-2 days after the seeding procedure. Transmitted light and immunostained images of two-dimensional human and rat islet cells on glass are shown in Figure 1. The islet beta cells in those microsocieties display wavelike propagation of calcium signals across neighboring cells consistent with establishment of well-functioning cell-cell contacts and interactions (Phelps et al., 2017. Associated Publication). The monolayer cultures facilitate sophisticated super-resolution image analyses of the cell biology of beta cells (Phelps et al., 2017. Associated Publication).

## References

Kanaani, J. et al. Compartmentalization of GABA synthesis by GAD67 differs between pancreatic beta cells and neurons. *PLoS One* 10, e0117130 (2015).

## Figures



**Figure 1**

Representative images of islet cell monolayer cultures on glass surfaces. (a) Representative phase-contrast transmitted light image of rat islet cell monolayer on glass showing well-spread and adherent islet endocrine cells. (b) Representative immunostained confocal microscopy image of a different rat islet cell monolayer on glass showing incorporation of four major islet endocrine cell types. (c) Representative high-magnification and stimulated emission depletion (STED) super-resolution image of two beta cells

from a rat islet cell monolayer on glass. Inset frames show higher magnification of the boxed region comparing standard confocal to STED super-resolution microscopy.

## Supplementary Files

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- [supplement0.docx](#)