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Procedure for Seeding Cells on the Disque Platform

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ABSTRACT

Advances in treating β cell loss include islet replacement therapies or increasing cell proliferation rate in type 1 and type 2 diabetes. We previously developed a proliferation-inducing prodrug (ZnPD6) that targets the high concentration of zinc ions in β cells, and which exhibits a 2.4-fold increase in β cell proliferation compared to the DYRK1A inhibitor harmine. These prodrugs were identified through screening on the Disque Platform (DP)—a high-fidelity culture system where stem cell–derived β cells are reaggregated into thin, 3D discs within 2D 96-well plates that mimic in vivo conditions.

The Disque Platform allows for the formation of 3D micro-tissues within an automation-friendly design, and is capable of systematically manipulating the cell niche in order to identify chemical and physical cues that enhance β cell proliferation. The Disque Platform better replicates the zinc content of native islets, enabling for the screening of zinc-activated prodrugs whose activity cannot be detected in 2D culture systems, which typically display a markedly lowered zinc content. The Disque Platform is a reliable screening platform that bridges the advantages of 2D and 3D culture systems and responds to interventions when conventional systems cannot produce a clear signal or readout. Here we describe a standard protocol for the formation of 3D micro-tissues in the Disque Platform.

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MATERIALS TEXT

Laser machining tool with 75-Watt 10-µm CO2 laser
PLS6MW

Universal Laser Systems **PLS6MW**

1.5 mm thick cast PMMA sheet for Disque and support pedestal
1.5 mm thick

McMaster-Carr **8560k171**

PETE polyester track etched membrane filter
1.0-µm pore size

Sterlitech **PET1047100**

Acrylic solvent cement to attach membrane filter

IPS CORP. SCIGRIP **10300**

96-well plate

Corning **N/A**

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Transwell inserts

Corning N/A

TrypLE Express **Thermo Fisher Scientific Catalog #N/A**

Ultrapure Laminin Mouse 1 mg **Corning Catalog #354239**

Collagen IV Mouse 1 mg **Corning Catalog #354233**

70% ethanol **Sigma Aldrich Catalog #470198**

PBS **Contributed by users**

SC β cell clusters **Melton Lab**

Culture media for SC β cells **Melton Lab**

Human primary islets **Prodo Laboratories, Inc**

Culture media for human islets: PIM(S) supplemented with PIM(ABS G and 3X) **Prodo Laboratories, Inc**

incubator 37°C, 5% CO2, 100% humidity **Contributed by users**

UV sterilizer (UV lamp in biosafety cabinet) **Contributed by users**

1 DP fabrication

1. Disques (**3.0 mm** inner diameter) were engraved by laser cutter from **1.5 mm** thick acrylic sheets.

2. A 1.0µm pore sized-hydrophilic PTFE membrane was attached to the bottom of a Disque using acrylic glue.

3. The reverse side of the membrane was attached to a supporting pedestal engraved by a laser cutter.
Laser cutter settings: Vector cut - 90% power / 5% speed / 500 ppi

4. The Disques were sterilized by incubation with 70% ethanol overnight and ultraviolet (UV) radiation for 1 hour before placement into the bottom of 96well plates.

2 **Preparation of culture plate** (at least two days in advance to seeding)

Coating the DP

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1. Thaw collagen IV and laminin on ice at 4 °C overnight.

2. Break up collagen IV fibers with 23-gauge needles (lubricate needle and syringe with media first).

3. Spin down collagen IV solution at 100g for 5 minutes to pellet remaining large fibres.

4. Collect supernatant – this is what we will use.

5. Make coating solution with concentration of 100 µg/mL of collagen IV and laminin in media.

6. Add 50 µl coating solution to each insert (for 24 well plates).

7. Tap well plate to make sure coating solution covers the bottom of each insert.

8. Incubate inserts in coating solution in a 37 °C incubator for 2-2.5 hours.

9. Wash inserts 2-3 times with PBS.

10. Store the coated Transwell inserts in 4 °C .

Placing the washers into Transwell inserts

1. Using a tweezer, place sterilized washers into coated Transwell inserts.

2. Make sure the washer is lying flat at the bottom of the insert.

3 Preparation of Media and Growth factors

1. Estimate the amount of media and factors needed.

2. In TC hood, transfer the required amount of media into a Falcon tube. Warm in 37 °C water bath for 10-15 minutes.

3. Meanwhile, thaw the growth factors on ice.

4. Add factors to new media with the correct dilutions.

5. Mix factors in media by pipetting up-and-down.

4 Dispersal

1. Warm up sterile TrypLE solution in a 37 °C water bath.

   a.*Tip: It is recommended that 1 mL TrypLE is used to disperse ~5 M cells.

2. Disperse cell clusters in TrypLE for 10 minutes, pipette to break up the clusters every 5 minutes.

3. During the 5 minute-breaks, prepare media with Rock-I and counter balance for cell suspension.

4. Make sure the cell suspension looks like a homogenous, milky solution.

5. Centrifuge at 250g for 4 minutes to pellet single cells, then remove TrypLE.

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6. Add media with Rock-i (1:1000 dilution) to wash cells.

7. Centrifuge at 250g for 4 min. to pellet single cells, remove wash media.

8. Re-suspend cells in media with Rock-i.

a. *Tip: To seed 0.8M cells/well, since each washer holds ~ **20 µl** of volume, it is convenient to re-suspend dispersed cells at ~40M cells/mL.

5 Cell Count

9. Dilute a small sample of cell suspension for cell counting.

a.*Tip: It is convenient to dilute the cell suspension at 1:100 for counting (it makes the math easy). For dilution of Trypan Blue, we use 1:10. Typically, we dilute cells for counting by mixing **1 µl** of cell suspension with **89 µl** of PBS and **10 µl** of Trypan Blue.

10. Count cells on a hemocytometer.

11. Determine the amount of cell suspension to put into each well.

6 Seeding

12. Place the appropriate amount of cell suspension into the centre of the washer.

13. Centrifuge at 200g for 4 min to form cell discs.

a.Note: used to do it at 250g, but media leaks through the 3um membrane at 250g. Pilot experiments done before to show that centrifugation speed and duration do not affect cell disc thickness, so changed speed to 200g . Now media doesn't leak.

14. Gently add the media with the full recipe of growth factors into and under the insert.

***If media is not ready upon forming cell discs in well-plate, make sure to cover cell discs with a small amount of media **with Rock-i** (~100 µL) while they are waiting inside the incubator, so they don't dry out.

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