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 We use this protocol and it's working

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Procedure for Seeding Cells on the Disque Platform

Peter Anthony Jones*¹, Kisuk Yang*¹, Jeffrey M Karp¹

¹Department of Anesthesiology Perioperative and Pain Medicine Brigham and Women's Hospital Boston MA 02115, Harvard Medical School Boston MA 02115, Harvard Stem Cell Institute Cambridge MA 02138, Harvard-MIT Division of Health Sciences and Technology Cambridge MA 02139, Broad Institute of MIT and Harvard Cambridge MA 02142

Peter Anthony Jones*: Current address: Takeda Pharmaceuticals U.S.A., Inc., 40 Landsdowne Street, Cambridge, MA 02139

Kisuk Yang*: co-first author

Human Islet Research Network

Lili Liang

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.

MATERIALS

Equipment	
Laser machining tool with 75-Watt 10-µm CO2 laser	NAME
PLS6MW	TYPE
Universal Laser Systems	BRAND
PLS6MW	SKU
https://www.ulsinc.com/products/platforms/pls6mw	LINK

Equipment	
1.5 mm thick cast PMMA sheet for Disque and support pedestal	NAME
1.5 mm thick	TYPE
McMaster-Carr	BRAND
8560k171	SKU
https://www.mcmaster.com/catalog/127/3896	LINK

Equipment	
PETE polyester track etched membrane filter	NAME
1.0-µm pore size	TYPE
Sterlitech	BRAND
PET1047100	SKU
https://www.sterlitech.com/polyester-membrane-filter-pet1047100.html	LINK

Equipment	
Acrylic solvent cement to attach membrane filter	NAME
IPS CORP. SCIGRIP	BRAND
10300	SKU
https://www.ellsworth.com/products/by-manufacturer/ips-corp/adhesives/solvent-based/ips-corp.-scigrip-3-acrylic-plastic-cement-solvent-based-adhesive-clear-1-pt-can/	LINK

Equipment	
96-well plate	NAME
Corning	BRAND
N/A	SKU
https://ecatalog.corning.com/life-sciences/b2c/US/en/Microplates/Assay-Microplates/96-Well-Microplates/Corning%C2%AE-96-well-Polypropylene-Microplates/p/corning96WellPolypropyleneMicroplates	LINK

Equipment	
Transwell inserts	NAME
Corning	BRAND
N/A	SKU
https://www.fishersci.com/shop/products/costar-transwell-permeable-supports-9/p-176666	LINK

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 **Merck MilliporeSigma (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 ($\pm$ 3.0 mm inner diameter) were engraved by laser cutter from $\pm$ 1.5 mm thick acrylic sheets.
2. A 1.0 μ m 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

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.

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 3µm 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 μ L) while they are waiting inside the incubator, so they don't dry out.