



Pancreatic islets encapsulation with poly(2,3)GlucoseTM

I Overview

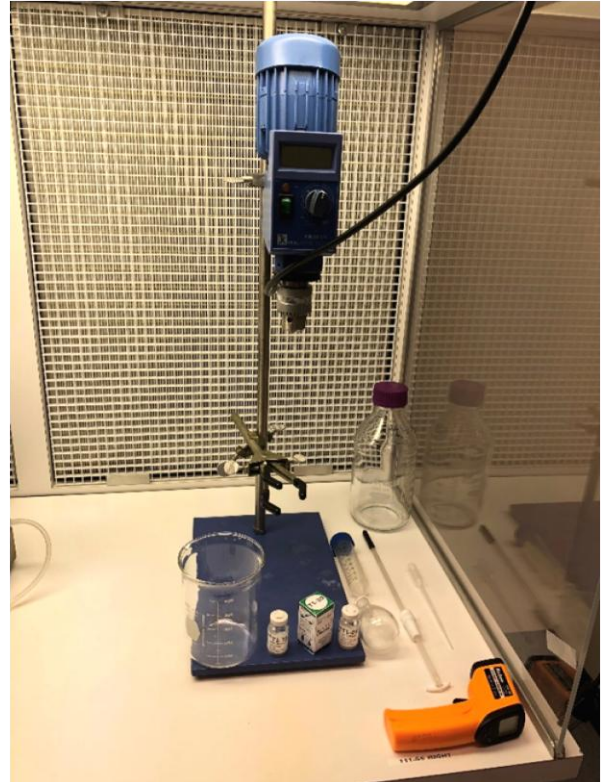
Cell encapsulation in temperature responsive poly(2,3)GlucoseTM provides immunoisolation while supporting transport of nutrients and metabolites. The cells are first suspended in an aqueous polymer solution, below the gel temperature. Spherical liquid capsules are formed by emulsifying in oil at 15° C. The capsules are solidified by heating to 37° C, which is above the gel temperature. Solid capsules are recovered, resuspended, and stored in media at 37° C.

II Materials and equipment

- Prodo Labs HIR[®] pancreatic islet suspension.
- Prodo Labs PIM(S)TM media for human islets
- TRGel poly(2,3)GlucoseTM T1-25 or T1-30.
- Sigmacote, SL2-25ML
- Cyclomethicone, High Purity USP/NF Grade
- 0.5 L sterile hot water and 0.5 L sterile cylindrical beaker
- IKA 20RW laboratory mixer
- 50 mL glass pear shaped 14/20 one neck flask
- Stirrer Shaft, 6mm OD x 305mm, ChemGlass CG-2060-11
- PTFE Blade, 11mm x 33mm CG-2060-12
- 14/20,PTFE Stopper/Stirrer bearing adapter, 7mm
- Laminar flow hood
- IR thermometer

III Encapsulation Protocol

1. Prepare 15% w/w solution of encapsulating polymer in PBS by injecting 1417 μ L of sterile PBS into the poly(2,3)GlucoseTM T1-25 (250 mg) vial. Next chill the vial at +4° C for 2-8 hours, vortex and then centrifuge to remove the foam if needed. Chill 30 mL of sterile cyclomethicone oil. Equilibrate 25 mL of islet media (to be used for recovery), and a culture flask (to store the encapsulated islets) in a 37° C incubator. Prepare ~ 450 mL of sterile water, microwave it to >60° C just prior to encapsulation. Pour it into a sterile 500 ml beaker to serve as the hot water bath.
2. Chill to 4° C, then centrifuge the islets suspension (30k IQ) in a plastic 50 mL tube, remove the top media, add 600 μ L of chilled encapsulating solution, and suspend the islets in encapsulating solution
3. Add 30 mL of cold cyclomethicone oil into the room temperature, 50 mL, pear shaped flask treated with Sigmacote. Suspend the flask by its neck, allowing the water bath to be raised up to immerse the flask.
4. Add the islet suspension to the bottom of the oil using a plastic pipette, best if slightly off-center to ensure suspension during stirring.



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5. Insert the stirrer shaft to the flask, position the blade in the center, and make sure it will not touch the walls. Connect the shaft to the mixer motor. Measure the temperature of the flask with IR gun: + 12 to + 15^o C is recommended.
6. Start spinning, gradually increasing the motor speed from 0 to 1500 RPM in 15 seconds. Maintain 1500 rpm for 60 sec, then immerse the flask in the beaker with + 60^o C water. Only heat the portion of the flask bathed with oil to avoid internal temperatures above 37^o C. Remove the water bath after 35 sec, spin for 10 sec w/o the water bath, then stop. The target final temperature of the solution is 36-37^o C
7. Let the suspension of capsules settle for 1 min
8. Decant the oil, capsules will stick to the walls
9. Add 25 mL of the recovery media equilibrated at 37^o C. Shake the islets from the walls into the suspension. Settle, then use a plastic pipette to collect the capsules from the bottom of the flask (2-3 mL). Repeat 2x-4x with increasingly vigorous shaking until ~ 100 % recovery of capsules without clusters and/or fused capsules.
10. Suspend encapsulated islets in the 37^o C media, keep at 37^o C in the incubator

IV Notes

Maintain component sterility in a laminar flow hood

Use as concentrated starting solution of encapsulating polymer as possible. 15% w/w is recommended.

Use 20 uL of encapsulation solution per 1000 IQ of islets as a starting point. View capsules under a microscope and then adjust this ratio to optimize volume utilization and islets position. Islets should be positioned at the center of the capsules with recommended 1 min of emulsification stage.

The size of the capsules is determined by the rotation speed. 1500 rpm will typically result in 235 um average capsule size. Faster the rotation, the smaller size of the capsules obtained. It is recommended to perform blank runs w/o loading islets to adjust encapsulation parameters.

Set the room temperature to 20-21^o C 1-2 hrs prior to encapsulation runs with T1-25. T1-25 solution in PBS (15%) will gel at 25^o C:

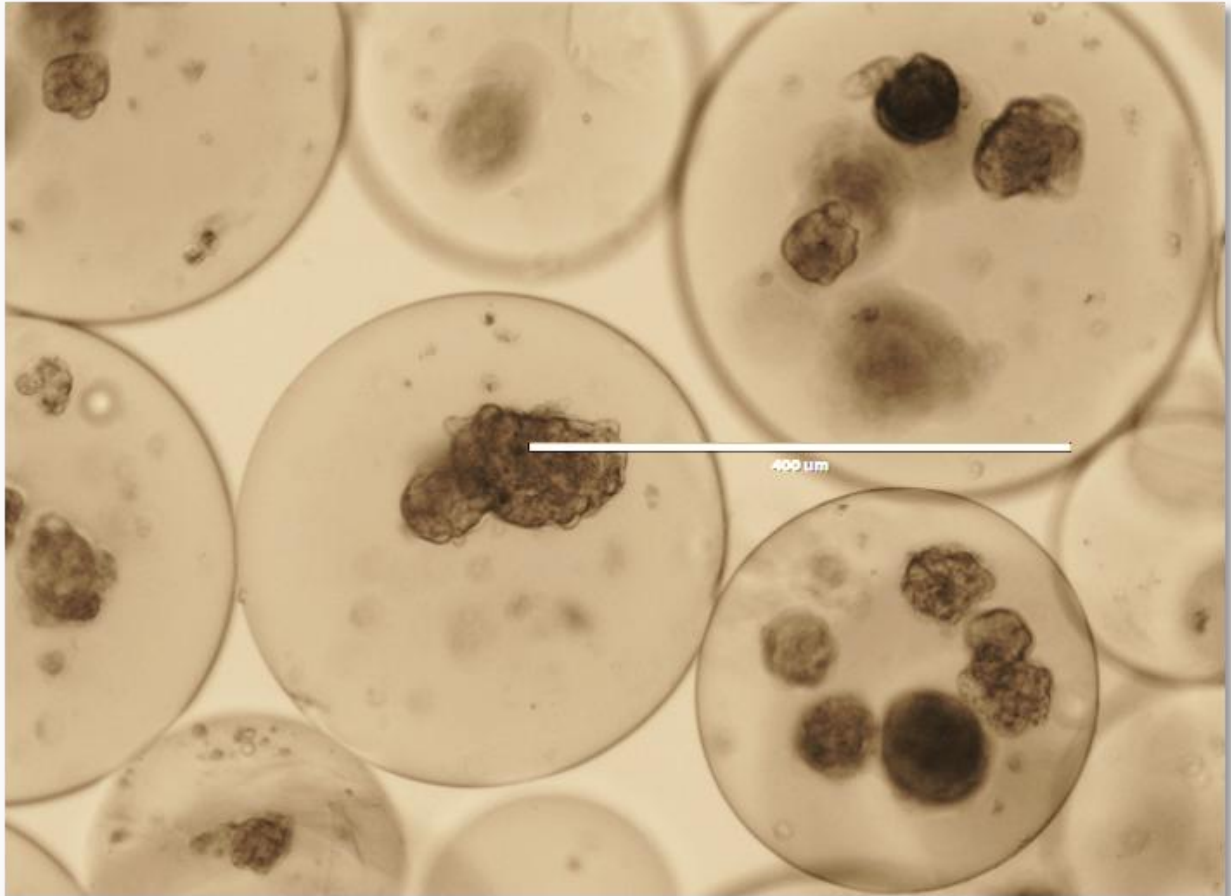
Table 1. Gel Temperature of poly(2,3)Glucose™ polymer solutions in PBS

Conc, w/w	T1-35	T1-30	T1-25
%	Tgel, °C	Tgel, °C	Tgel, °C
15	33.5	29.5	25
10	34.5	31.0	27.0
5	35	31.5	27.5
2	35.5	32	28
1	37	33.5	29.5

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Hydrophobic coating of the glassware (flask and shaft): Clean, wash, treat with 37 % HCl at RT, wash, dry, apply Sigmacote for 5 min, collect solution for reuse, dry, wash with DI water, dry.

Use 30 mL of chilled cyclomethicone oil per run (50 mL flask). Use the same oil volume irrespective of the encapsulating solution volume.



Human islets encapsulated in poly(2,3)Glucose™