Video Article

Transplantation of Pancreatic Islets Into the Kidney Capsule of Diabetic Mice

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Abstract

Our protocol was developed to cleanly and easily deliver islets or cells under the kidney capsule of diabetic or normal mice. We found that it was easier to concentrate the islets or cells into pellets in the final delivery tubing (PE50) used to transplant the cells under the kidney capsule. This technique provides both speed and ease while reducing any undue stress to the cells or to the mouse.

Loading: Settled, hand picked, islets or pelleted cells are carefully aspirated off the bottom of a 1.5 mL microcentrifuge tube using a p200 pipetteman and a straight, thin-wall pipette tip. A length of PE50 tubing is attached to the pipette tip using a small silicone adapter tubing. Cells are allowed to settle, in the tip, and then are transferred to the PE50 tubing by slowly dialing the pipetteman. Once the cells are near the end of the PE50 tubing, a kink is made and the silicone adaptor tubing is placed over the kink. The PE50 tubing is transferred to a 15 mL conical containing a cut 5 mL pipet, and the PE50 tubing is taped over the side of the 5 mL pipet to prevent curling during centrifuging. Cells are allowed to reach 1,000 rpm and stopped.

Transplantation: Recipient mice are anesthetized, shaved, and cleaned. A small incision is made on the left flank of the mouse and the kidney is exposed. The kidney, fat, and tissue are kept moist with normal saline swab. The distal end of the PE50 is attached to a Hamilton screw drive syringe, containing a pipette tip, using the silicone adaptor tubing. A small nick is made on the right flank side of the kidney, not too large nor too deep. The beveled end of the PE50 tubing, nearest the cells, is carefully placed under the capsule, the tubing is moved around gently to make space while swabbing normal saline; a dry capsule can tear easily. A small air bubble is delivered under the capsule by slowly dialing the syringe screw drive. Islets are then slowly delivered behind the air bubble. Once the islets have been delivered kidney homeostasis is maintained and the knick is cauterized with low heat. The kidney is placed back into the cavity and the peritoneum and skin are sutured and stapled. Mice are immediately treated with Flunixin and Buprenorphine s.q. and placed in a cage on a heating pad.

Protocol

Preparation of Islets for Transplant (Tx)

1. Under an inverted microscope, hand-pick islets using a P200 pipetman and straight pipet tip from the cultured islets in a 100mm plate.
2. Count islets 100 at a time and transfer into each microcentrifuge tube (~500 islets/tube/mouse).
3. Allow islets to settle to the bottom of the microcentrifuge tubes.
4. Draw one islet pellet into a P200 pipetman (set at 130ul) using a straight thin-wall pipet tip.
5. Place a silicone tube adapter over the syringe tip. Insert a length of PE50 tubing into the silicone adapter.
6. Hang the pipetman to the side of the hood and tape the PE50 tubing to the hood wall higher than the islets in the tip. This will allow the islets to settle only in the tip of the pipette.
7. Transfer the islets into the PE50 tubing by slowly dialing the pipetman and moving the islets into the PE50 tubing, being careful not to expel the islets from the end.
8. Make a kink in the beveled end of the PE50 tubing. While maintaining the kink, disconnect the silicone adapter tubing from the pipetman and secure the kink with the silicone adapter.
9. Place the islets, in the PE50 tubing, kinked silicone adaptor tubing side down, into a 15 mL conical with a cut 5 mL pipet and tape the PE50 tubing over the side of the 5 mL pipet to prevent curling of the PE50 tubing while centrifuging.
10. Centrifuge the PE50/15mL conicals to 1000 rpm and turn off centrifuge. (Never prepare more than 10 islet preparations at one time).
11. Place tube on ice.

Preparation of Mouse for Transplant

1. Anesthetize the mice with isofluorane or with Ketamine/Xylexene (see preparation protocol).
2. After anesthetic has taken affect, shave the left flank of the mouse.
3. Swab skin of mouse, center-out, with Povidone Iodine swab and then wipe off with an ETOH swab.
4. Locate the left kidney (just right of spleen). Make a small incision in the skin, exposing the peritoneum.
5. Make a small incision in the peritoneum exposing the kidney. Keeping the incision small will help in keeping the kidney raised and exposed.
6. Apply a slight pressure to both sides of the incision, raise or pop the kidney out of the mouse.
7. Keep the kidney moist by applying normal saline with a cotton tipped swab.
8. Using a syringe 23 or 25 gauge needle, make a small scratch on the right flank of the kidney, creating a nick in the kidney capsule; not too deep or too large.

Transplantation of Islets

Note: While the mouse is being prepared for Tx, the second person should prepare the Hamilton screw-drive islet transplant syringe.

1. Slowly remove the silicone adaptor tubing from the PE50 tubing while keeping the kink in the tubing.
2. Place the opposite end of the PE50 tubing into the silicone adaptor tubing and place the silicone adaptor tubing onto the pipette tip attached to the "screw-drive" glass Hamilton syringe. Slowly release the kink in the PE50 tubing, making sure that the isles do not leak out.
3. Advance the islets slowly to the tip of the PE50 tubing using the "screw" mechanism, but keep a small air bubble in the front of the islets in the PE50 tubing.
4. Into the nick made in the kidney, carefully slide the PE50 tubing under the capsule, making a small pocket. Be very careful not to gouge the kidney or puncturing through the capsule.
5. It helps to keep the area and capsule moist with normal saline-Gentamycin soaked cotton tipped swab; a dry capsule will tear easily.
6. Gently move the tubing in all directions, creating a "pocket" for the transplanted islets to rest. Remember to reapply additional normal saline to keep the area moist.
7. Under the direction of the person who has opened the mouse and who has placed the PE50 tubing under the kidney capsule, the second person, who prepared the mouse and Hamilton transplant syringe, will slowly advance islets under the capsule, inside the "pocket", behind a small air bubble delivered by the PE50 tubing, until all the islets are transplanted.
8. Slowly remove the PE50 tubing, dry the area with a dry swab and carefully cauterize the nick with low heat.
9. Using a dry cotton tipped swab, make sure all bleeding has stopped. Once bleeding has stopped, re-moisten the kidney with sterile saline, and gently replace the kidney into the peritoneum prior to closing the mouse with suture and skin staples.

Closing/Revival of Mouse

1. Close the peritoneum with a running stitch using 5-0 silk sutures w/ a C-6 19mm needle.
2. Using forceps draw both side of the skin incision together.
3. Staple the skin together with 2 or 3 staples.
4. Clean the skin of the mouse of any blood, using a cotton tipped swab and saline.
5. Immediately treat the mouse with a subcutaneous injection of Flunixin and Buprenorphine.
6. Place the mouse in a cage, that is placed on a heating pad or below a heating lamp, until the mouse is fully active.
7. Remove the skin staples in 2 weeks.

Discussion

This protocol provides a practical and efficient option for cleanly and easily delivering islets or cells under the kidney capsule of diabetic or normal mice. The technique of concentrating and pelleting the islets or cells into the final delivery tubing (PE50) used to transplant the cells under the kidney capsule offers an easy and effective method of transplanting cells while reducing any undue stress to the cells or to the mouse.

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