Video Article

A Method for Islet Transplantation to the Omentum in Mouse

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Abstract

Islet transplantation has been proposed to be a potential treatment for type 1 diabetes. Recent compelling evidence indicates that intravascular islet infusion is far from ideal and therefore, the omentum is re-emerging as a potentially valuable site for islet transplantation. This experiment requires the isolation of high quality islets and the implantation of the islets to the diabetic recipients. Transplantation to the omentum requires surgical steps that can be better demonstrated visually. Here, the detailed steps for this procedure are presented. Two methods of mixing the isolated islets with hydrogel before placing the mixture into the omental pouch of diabetic mice are described here. Different hydrogels are used for the different conditions. Blood glucose levels of diabetic mouse recipients of syngeneic islets in the omentum were monitored for up to 35 days. Some animals were sacrificed after 14 days to perform immuno-histochemical analysis. This pre-clinical transplantation approach can be used as preliminary data leading up to translation to clinical transplantation.

Video Link

The video component of this article can be found at https://www.jove.com/video/57160/

Introduction

According to the International Diabetes Federation (IDF), diabetes mellitus currently affects 382 million people, with a projected increase to 592 million people by 2035[1]. In both allogeneic and xenogeneic islet transplantation, systemic immunosuppressive therapy is necessary. Without immunosuppression, immune rejection is a major cause of graft loss[2]. There is also a significant problem of transplanted islet loss due to the instant blood mediated inflammatory reaction (IBMIR)[3,4]. However, even in the absence of an immune response such as in syngeneic or auto-transplantation models, islet cells transplanted into the liver via the portal vein are lost due to inflammation and/or to unfavorable environmental conditions, such as poor blood supply with reduced oxygenation and/or nutrients[5,6]. As a result, in order to ensure long-term metabolic function, higher islet numbers are necessary to compensate for the initial cell loss that reduces engraftment[7].

In an attempt to optimize islet engraftment, several alternative anatomical sites have been investigated experimentally as well as clinically, with promising, yet not definitive results[8]. Whereas some of the alternative sites offer easy and safe access (*e.g.*, skin, kidney capsule, gastric submucosa and anterior chamber of the eye) or a wider surface for larger islet masses (*e.g.*, peritoneal cavity), survival and physiologic metabolic performance of the transplanted islets are still limited and remain a concern[9]. The search for a more suitable site for islet engraftment is ongoing.

The omentum was among the many anatomical sites that were investigated in the early development of islet transplantation, and proved a successful environment for islets[10,11,12,13,14]. However, intraportal islet infusion became the clinical choice due in part to the relative simplicity of the procedure and early success in animal models[6]. Also, in part, the negatives associated with this site, particularly massive early islet loss, were less understood and less constraining in the early days of experimental islet transplantation as the field matured. With more recent compelling evidence indicating that intravascular islet infusion is far from ideal, the omentum is re-emerging as a potentially valuable site for cell transplantation.

The omentum (in the form of an omental pouch) offers relative advantages over the liver[15,16]. It is well-vascularized and easily accessible. It allows retrieval of the graft (if necessary) and/or biopsy. The ischemic period experienced by the islets is reduced compared to the liver, and the omentum can accept relatively large islet masses which is not possible intraportally, where a rise in portal pressure can cause complications.

A syngeneic mouse model of transplantation was used in the protocol tested in the study, employing C57BL/6 male mice between 6–8 weeks old with a body weight of 20–25 g. Islet recipients were rendered diabetic with a single injection of streptozotocin with a dose of 250 mg/kg ip. The induction of diabetes can be considered successful if the blood glucose level of the mouse is greater than 24 mmol/L 48 h after injection and remains above that level for a minimum of 5 days.

Syngeneic islets were isolated from the pancreas of age-matched donors following previously published methods with some modifications. In brief, the collagenase was injected into the gall bladder instead of the bile duct. This was done as an improvement to facilitate the ease of injection. Collagenase infusion was followed by incubation, tissue disruption, density gradient separation and hand-picking to obtain pure islets. Islets were cultured overnight in CMRL-1066 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) in T175 flasks at 37 °C, under 95% air-5% CO_2 before transplantation.

Protocol

All mice used in this study were obtained from the Medical Animal Center of Guangdong Province. The use of animals was approved by the Ethics Review Committee of Shenzhen Second People's Hospital, in accordance with the principles of animal welfare.

1. Islet Transplantation to the Omentum

NOTE: This protocol requires 2 persons to accomplish.

1. Assemble surgical materials which are listed in **Table 1**. Prepare aseptic field in surgical area with sterile materials such as drapes and disposables and maintain aseptic conditions throughout surgery. Sterilize all surgical instruments. Wear sterile gowns.
2. Pick the islets using a 200 µL pipette tip under the stereomicroscope. Each mouse will receive 450–500 Islet equivalent (IEQ) per tansplant. For each animal, place enough islets for a single transplant into a sterile 1.5 mL snap-top tube with 100 µL of CMRL-1066 medium.
   NOTE: Islets can be isolated on the day of transplantation, but it is better to isolate them the day before to allow recovery for the isolation process.
3. Keep the snap-top tubes with islets on ice until ready to transplant.
4. Thaw the basement membrane matrix hydrogel and keep it on ice after removing it from the -20 °C freezer. The hydrogel is liquid at 4 °C to 10 °C and solidifies at higher temperatures.
5. Weigh and tag all diabetic recipient mice. Inject 60 mg/kg pentobarbital sodium intraperitoneally. Test the the depth of anesthesia by administering a toe pinch. Give an additional 10 mg/kg pentobarbital sodium if the animal reacts to the pinch. If there is no withdraw reflex, the level of anesthesia is correct for surgery.
6. Swab the surgical site on the abdomen using 70% ethanol. Shave the hair from the site with a razer blade and disinfect the area with Iodophor. Administer vet ointment to the eyes to prevent dryness while under anesthesia.
7. Use the ophthalmic scissors to open the abdomen along the abdomen midline with an incision of 4 to 5 cm. Move the intestines to the left side and cover with saline soaked gauze to prevent excessive dehydration during the surgery procedure.
8. Use cotton swabs to fully expose the visual field of the stomach and locate the omentum (located below the stomach). Use two pair of fine forceps to distend the omentum. Take care to prevent damage from tearing.
9. The hydrogel is completely thawed at this point. Spin the tube with the islets for 30 s at 200 x g and remove the supernatant. Aspirate 50 µL of hydrogel, add it to the tube containing the islets, and resuspend the mix gently avoiding the formation of bubbles. Keep the tubes on ice during the procedure.
10. Use two pair of forceps to pick up the edges of the omentum and gently raise it to form a groove between the gastric wall and intestines that can accommodate a small volume of liquid with the islet graft.
11. Let the second person assist in the procedure and aspirate the resuspended islet-hydrogel mixture (the entire contents of the tube) with a 200 µL pipette tip, Deliver the content into the groove.
12. Ensure that the mixture is well positioned into the groove by gently raising or lowering the edges of the omentum. Complete positioning of the mixture within 3 min before the hydrogel solidifies as an effect of the body temperature. After the hydrogel sets, fold the omentum to cover the graft.
    NOTE:The omentum will adhere to the surrounding gastric wall as the hydrogel solidifies.
13. After the hydrogel is completely solidified, use cotton swabs to reposition the intestines back into the abdominal cavity, taking care not to touch the site of transplantation.
14. Add 20 µL of cephalosporin (5~10 mg) into the abdominal cavity to prevent infection, then use 4-0 suture to close the abdomen.
15. Return the mouse to its cage and repeat all steps for each mouse recipient. Keep the mice warm and monitor visually until they fully regain sufficient consciousness to maintain sternal recumbency. Keep the mice separate from other animals until they have fully recovered.
16. Inject 50 µL of cefazolin sodium (0.05 mg/mL) each day for a week as post-surgery prophylaxis. Administer Bupivicaine + Buprenorphine, i.e. Apply 1-3 drops of 0.25% Bupivicaine at the incision site topically prior to placement of wound clips. Administer Buprenorphine#0.03 mg/ml with sterile 0.9% saline, 0.05-0.10 mg/kg#via the intraperitoneal (IP) route.
17. Measure non-fasting blood glucose level from a tail-vein blood sample using a blood glucose meter once a day after the transplant. When transplanting to the omentum, the islet graft may have a delayed function and not reach a completely normal blood glucose level for 2–3 weeks.
18. For histology, remove the omental graft from the mouse at the end of the experiment following the transplantation. Fix the tissue according to the histological protocols. The graft can be analyzed for immunostaining or immunofluorescence studies.

2. Alternative Method for Transplantation to the Omentum

NOTE: An alternative fibrin-thrombin hydrogel that is used at room temperature may be substituted for the basement membrane matrix hydrogel. It consists of 2 components, a fibrin sealer protein solution (50 U/mL thrombin and 10 mg/mL fibrinogen). When the components are mixed, they form a clot that holds the islets in place.

1. Use the fibrin-thombin hydrogel compound at room temperature. As in step 1.2, each mouse will receive 450–500 islet equivalents (IEQ) per transplant. Place the islets in a sterile 1.5 mL snap-top tube with 100 µL of CMRL-1066 medium. Immediately before transplantation, aspirate the islets into a sterile PE50 tubing for a length of 10 cm and centrifuge gently (30 s at 200 x g) to form a loose pellet.
2. Prepare the animal as stated above (steps 1.5-1.8) with the omentum spread out. Mix the hydrogel components (10 µL/each) and place on the omentum. (**Figure 3D**)
3. Immediately expel the islets from the tubing onto the hydrogel. The hydrogel forms a clot around the islets. (**Figure 3E**)
4. Fold the omentum over the hydrogel and islets to form a pouch. (**Figure 3F**) Continue with steps 1.13 to 1.18.

Representative Results

Post-digestive state of the pancreas is shown in **Figure 1A**. Purified islets are shown in **Figure 1B**. Dithizone staining and viability testing of the islets are shown in **Figure 2**. The main steps of the islet transplantation to the omentum are shown in **Figure 3**. The blood glucose levels of the recipients after omental transplantation are shown in **Figure 4**. Histological analysis of grafts is shown in **Figure 5**.

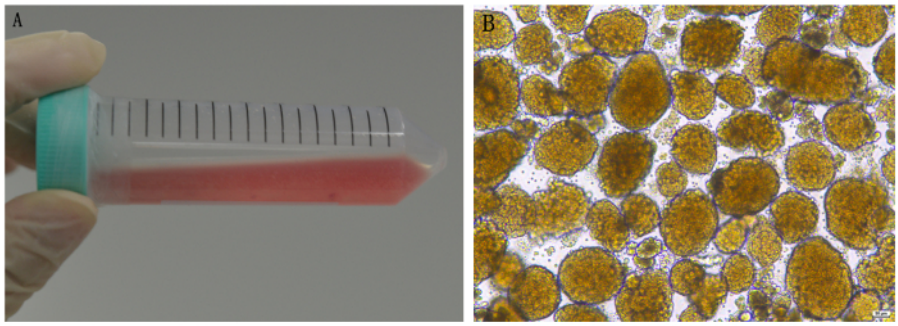

Figure 1: Pancreas digestion and islet isolation. (**A**) The pancreas following the digestion. Note the sand-sized particles in the suspension. (**B**) Light microscopy image of the islets after isolation. Please click here to view a larger version of this figure.

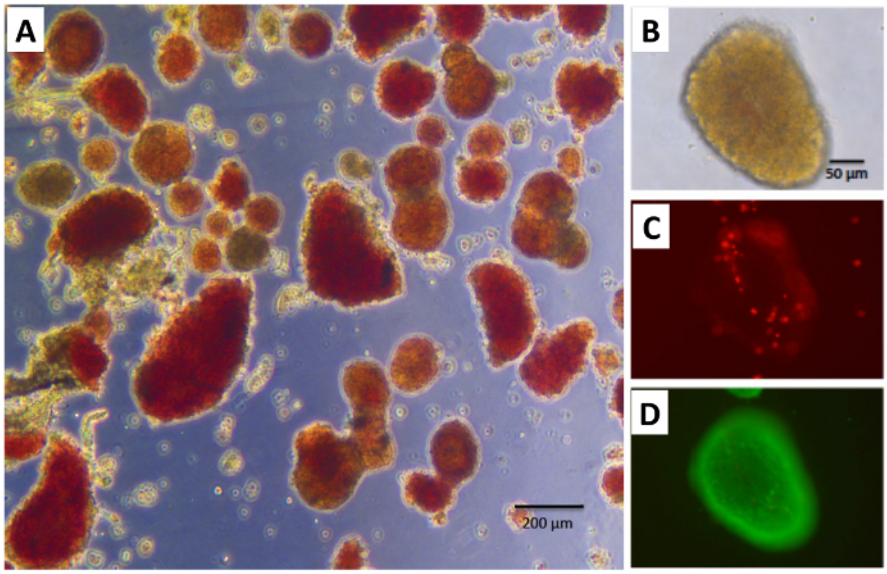

Figure 2: Islet dithizone staining and fluorescence viability dyes. (**A**) Islets are stained in red by dithizone. (**B**) Light microscopy. (**C**) Propidium Iodide fluorescence red staining (dead cells). (**D**) Calcein-AM green staining (viable cells). Please click here to view a larger version of this figure.

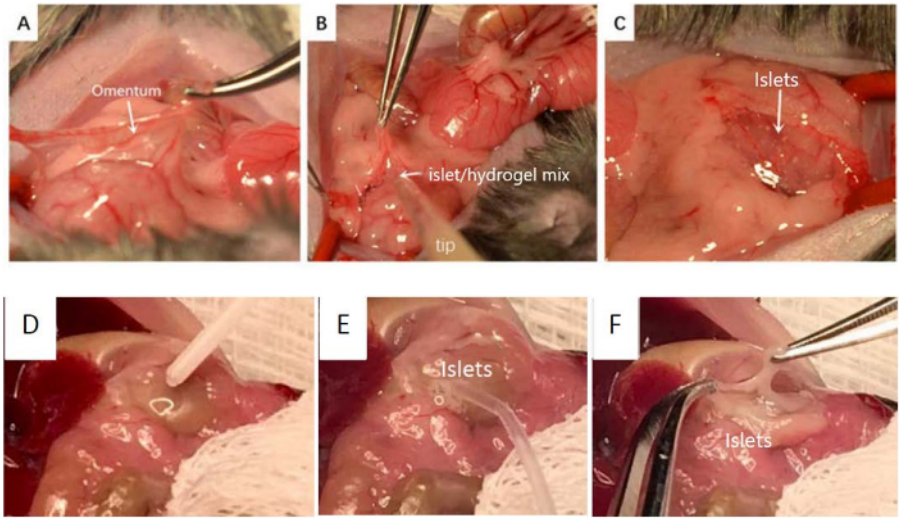

Figure 3: Transplantation in the omentum. (**A**) After the recipient laparotomy, the stomach is exposed and the omentum located. (**B**) Islets are mixed in hydrogel and slowly placed onto the omental tissue. (**C**) Islet graft at higher magnification. The hydrogel is a semi-solidified state. (**D**) Alternative method. Hydrogel is placed on the omentum without islets. (**E**) Islets are placed on top of the hydrogel. (**F**) The omentum is folded around the islets. Please click here to view a larger version of this figure.

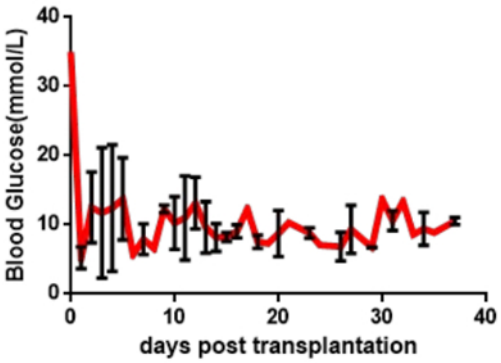

Figure 4: Blood glucose levels post-transplant. Non-fasting blood glucose levels of recipients (n = 7) up to 35 days after transplantation. The graft recipients did not all receive the same batch of islets so there are differences in quality and function resulting in large fluctuations in blood glucose level.

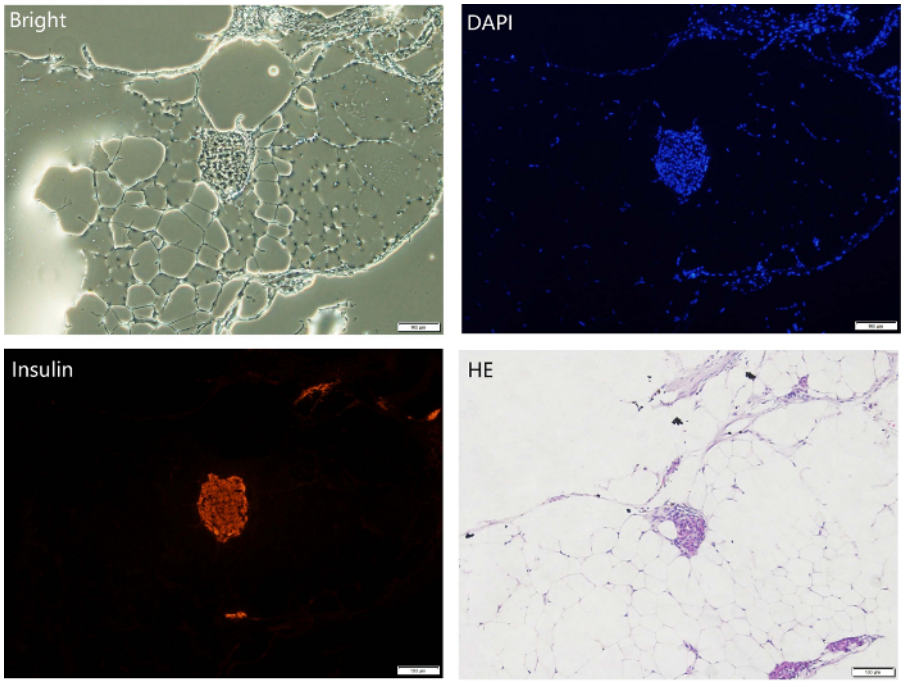

Figure 5: Histology. Graft sections (graft retrieval 14 days after transplantation) were stained with DAPI (nuclei), anti-mouse insulin antibodies, and hematoxylin. Please click here to view a larger version of this figure.

Discussion

Islet transplantation to the liver via the portal vein is the most commonly used method of islet transplantation in humans, but there are still efficiency and safety concerns such as portal vein thrombosis and liver steatosis[17]. Recent studies show that the omentum may be a suitable alternative to the liver, but more research needs to be conducted prior to clinical translation[12,14,18,19].

The mouse is a suitable model for testing the omentum as a site for islet transplantation. In the mouse, the omentum is located under the stomach wall and is generally folded. However, the size of the omentum in mice is small and challenging to access. In order to use it for transplantation, it is necessary to gently spread it out to its full size then fold it over after the islets/hydrogel mix is deposited. In a larger animal, the omental tissue is more extensive and can be sutured to construct one or more pouches into which the islets are deposited. The mouse omentum is fragile and does not easily accept sutures without breaking. For this reason, a hydrogel is used to make the pouch without sutures.

In the first method (steps 1.1–1.18), the hydrogel used is a basement membrane matrix and is composed of laminin, collagen IV and growth factors. It solidifies as it warms to body temperature. In the second method (steps 2.1–2.4), a different hydrogel is used composed of 50U/mL thrombin and 10 mg/mL fibrinogen. Two different hydrogels are used to show the versatility of this technique. It may be tailored to fit many circumstances and to establish proof of principle. For clinical applications, whether a sutured pouch or a clinical-grade hydrogel is used, the technique can be combined with a minimally invasive approach, (*e.g.*, an endoscopic method of delivery to the omentum). To this aim, additional studies using larger mammal pre-clinical models are required.

The time to normalize the blood glucose levels in the mouse recipients after transplantation in the omentum in our as well as other studies suggest that the islets in the omentum require slightly longer time to achieve sufficient insulin production. Revascularization efficiency appears to be one important factor, as outlined in the study. Whether the presence of a hydrogel affects the speed of islet revascularization and performance, that remains to be determined.

Disclosures

The authors report no conflicts of interest.

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