



Technical note

A novel method for murine intrahepatic islet transplantation via cecal vein



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ARTICLE INFO

Article history:

Received 17 June 2015

Received in revised form 15 September 2015

Accepted 29 September 2015

Available online 2 October 2015

Keywords:

Islet

Transplantation

Intrahepatic

Cecal vein

ABSTRACT

Islet transplantation is one of the most beneficial treatment modality to treat type 1 diabetic patients with frequent hypoglycemic unawareness. In clinical setting, human islets are infused via portal vein and are settled in the end-portal venules in the liver. However, mouse islets are transplanted into kidney subcapsule or liver through direct portal vein. These conventional transplantation methods have several drawbacks such as different physiological environments around the transplanted islets in kidney subcapsule from the liver and high mortality rate in direct portal vein approach. In this study, we introduced murine intrahepatic islet transplantation method via cecal vein to have the same surgical operation route in humans as well as guaranteeing low mortality rate after islet transplantation. With this protocol, consistent normoglycemia can be obtained in diabetic mice, while keeping operation-related mortality extremely low. This approach with easier accessibility and low mortality will make murine intrahepatic islet transplantation a useful model for studying immunological mechanisms such as strong innate and adaptive immune responses that occur in human islet transplantation.

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1. Introduction

Human islet transplantation has been successfully used to treat some patients with type 1 diabetes accompanying severe hypoglycemic unawareness (Ludwig et al., 2013). Human islets isolated from cadaveric donor pancreases are transplanted into the liver through portal vein under radiological guidance and they are engrafted in the liver. However, a relatively low rate of long-term graft survival (>3 years, <50%) hampers wide application of this therapy to most patients (Barton et al., 2012). To understand the underlying causes for these chronic graft loss, mouse model is inevitable. However, mouse islets have been generally transplanted into the kidney subcapsular space due to its easy accessibility (Hara et al., 2004). Therefore, this conventional mouse model

for islet transplantation into renal subcapsular space has limitations to study various immunological events after human islet implantation. This fact drove us to establish an experimental mouse model that can more mimic human islet transplantation. A few papers address these concerns and developed direct portal vein approach. But, as portal vein is the major vessel located in the deep peritoneal cavity, high frequency of operation-related mortality has been noted (Marzorati et al., 2014). Only one group tried ileocecal route as an islet infusion site (Yonekawa et al., 2006), however, they did not demonstrate superiority of ileocecal route to direct portal route nor described their method in detail.

The aim of this technical note is to show how we set up the islet transplantation in the liver of diabetic mice via cecal vein and achieved normoglycemia in a reproducible way. We believe that this new surgical procedure will facilitate intrahepatic islet transplantation in mice and guide many mechanistic studies in detail.

2. Material and methods

All the experiments were approved by the Seoul National University Institutional Animal Care and Use Committee (IACUC, IACUC no. SNU-130830-5).

1. C57BL/6 mice from Jackson Laboratory (Bar Harbor, ME) were used as islet donor and recipient.

Abbreviations: IBMIR, instant blood-mediated inflammatory reaction; NHP, non-human primate; IEQ, islet equivalent.

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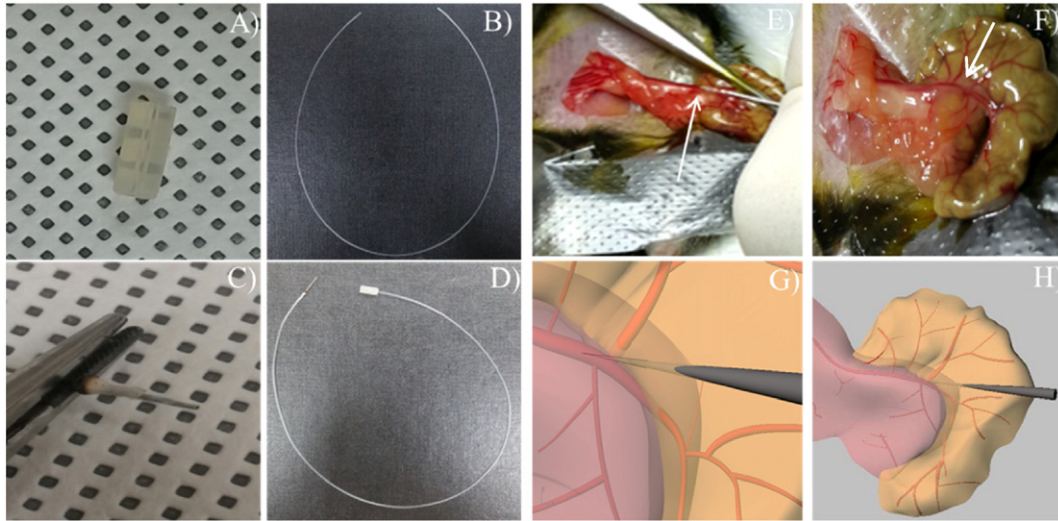


Fig. 1. Materials and procedure for islet transplantation via cecal vein. A) Silicon tube cut by 1 cm. B) Polyethylene tube cut by 40 cm. This length is fit to load 100 μ l of islet and heparin mixture. C) 26 gauge needle with the remaining plastic part that has been separated as described in the [Materials and methods](#) section. D) Assembled needle, PE tube and silicon tube. E) The appropriate site of needle insertion. F) Absorbable hemostat was placed by the puncture point and compressed immediately after the needle removal for at least 1 min. G, H) Schematic demonstration of needle insertion site.

2. Preparation of surgical materials

- 2.1. Prepare materials and tools for the experiment.
- 2.2. Prepare and assemble islet loader ([Fig. 1](#)).
 - 2.2.1. Cut silicon tube by 1 cm whose inner diameter is 0.8 mm.
 - 2.2.2. Cut polyethylene (PE) tube by 40 cm whose inner diameter is 0.58 mm and outer diameter is 0.965 mm. Shorter PE tube may cause islet clump to plug the needle outlet or needle–PE tube interface.
 - 2.2.3. Separate 26 gauge needle from 1 cm³ syringe by heating the connecting part with an alcohol lamp. *Do not* separate the needle too neatly, because the remaining plastic part on the needle shaft is essential for fitting the needle into the PE tube.
 - 2.2.4. Assemble the needle, 40 cm-long PE tube and silicon tube.

3. Preparation of antibiotics and painkiller cocktail.

- 3.1. Cefazolin sodium (90 mg/kg)
- 3.2. Meloxicam (1 mg/kg)
- 3.3. Tramadol HCl (30 mg/kg)
- 3.4. Mix these three drugs in 300 μ l PBS for each mouse.

Table 1

Materials and equipment used in this study.

Name of the materials/equipment	Company
Operation microscope	Leica. Co., Ltd.
Safer mat	YNK health care
Povidine	Sung Kwang Pharm. Co., Ltd.
KOVAX-SYRINGE 1 ml	Korea Vaccine Co., Ltd.
BD intramedic™ polyethylene tubing (PE 50)	BD
Masterflex	Cole-Parmer instrument company, Inc.
Heparin 5000 IU/ml	JW Pharm. Co., Ltd.
Hamilton 1001 TPLT 1 mL syringe	Hamilton company
Surgicel	Ethicon
Pipette Research plus 1000 μ l	Eppendorf
Pipette Research plus 200 μ l	Eppendorf
Pipette Research plus 10 μ l	Eppendorf
HyClone phosphate buffered saline	Thermo Scientific
Gentamycin reagent	Gibco
Silkam	B. Braun Medical Industries Sdn Bhd
Vicryl	Ethicon

4. Islet Preparation ([Choi et al., 2004](#)).

- 4.1. Prepare 0.5 μ l (2.5 IU) of heparin in 10 μ l PBS containing gentamycin (50 μ g/ml) per mouse to minimize instant blood-mediated inflammatory reaction (IBMIR) ([Luan et al., 2011](#)).
- 4.2. Remove the supernatant of the hand-picked 400 islet equivalent (IEQ) islets in 1.5 ml tube to a final volume of 100 μ l.
- 4.3. Add prepared heparin into islet media.
- 4.4. Load the islets and heparin mixture into surgical tube using a 200 μ l pipette. *Bubble formation must be avoided* in the process to prevent air embolism.
- 4.5. Connect surgical tube to Hamilton syringe. Hamilton syringe helps to infuse islet preparation precisely and slowly. Check whether the needle is properly plugged or not.

5. Intrahepatic islet transplantation

- 5.1. Cover the operating table with a mat and adjust an operation microscope to suit for the operator.
- 5.2. Anesthetize the mouse with oxygen and isoflurane. During induction of anesthesia, prepare hemostatics, autoclaved gauze, and aseptic drape.
- 5.3. Clip hair on the abdominal region of mouse.
- 5.4. Disinfect skin with povidone-iodine, 70% ethanol, and PBS containing gentamycin in order mentioned above.
- 5.5. Incise skin and peritoneum.
- 5.6. Spread aseptic drape on the abdomen of mouse and take cecum out.
- 5.7. Using an operation microscope, find the suitable cecal vein and infuse islet preparation (Note: Straighten the cecal vein to

Table 2

Pain killers and antibiotics.

Commercial name of the materials	Concentration	Company	Usage dose
CKD Cefazolin injection	0.2 g/ml	ChongKunDang Pharm.	90 mg/kg
Metacam	5 mg/ml	Boehringer Ingelheim	1 mg/kg
Maritrol injection	50 mg/ml	Choongwae Parm.	30 mg/kg

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