Video Article

Orthotopic Small Bowel Transplantation in Rats

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Abstract

Small bowel transplantation has become an accepted clinical option for patients with short gut syndrome and failure of parenteral nutrition (irreversible intestinal failure). In specialized centers improved operative and managing strategies have led to excellent short- and intermediate term patient and graft survival while providing high quality of life [1,3]. Unlike in the more common transplantation of other solid organs (*i.e.* heart, liver) many underlying mechanisms of graft function and immunologic alterations induced by intestinal transplantation are not entirely known[6,7]. Episodes of acute rejection, sepsis and chronic graft failure are the main obstacles still contributing to less favorable long term outcome and hindering a more widespread employment of the procedure despite a growing number of patients on home parenteral nutrition who would potentially benefit from such a transplant. The small intestine contains a large number of passenger leucocytes commonly referred to as part of the gut associated lymphoid system (GALT) this being part of the reason for the high immunogenity of the intestinal graft. The presence and close proximity of many commensals and pathogens in the gut explains the severity of sepsis episodes once graft mucosal integrity is compromised (for example by rejection). To advance the field of intestinal- and multiorgan transplantation more data generated from reliable and feasible animal models is needed. The model provided herein combines both reliability and feasibility once established in a standardized manner and can provide valuable insight in the underlying complex molecular, cellular and functional mechanisms that are triggered by intestinal transplantation. We have successfully used and refined the described procedure over more than 5 years in our laboratory [8-11]. The JoVE video-based format is especially useful to demonstrate the complex procedure and avoid initial pitfalls for groups planning to establish an orthotopic rodent model investigating intestinal transplantation.

Video Link

The video component of this article can be found at https://www.jove.com/video/4102/

Protocol

1. Donor Operation

1. The donor rat should be kept fasting for 24 hr (free access to water/glucose solution).
2. To induce Isoflurane inhalation anesthesia, start with 2% on standard atomizer, and then reduce to 1% after performing laparotomy. Perform a toe pinch to check sedation.
3. Shave the abdomen and clean with skin prep 3 times (Kodan). Then perform a median incision after subcutaneous administration of the analgesic.
4. After the graft is wrapped in saline soaked gauze, separate the physiological adhesions between the pancreas and the ascending colon gently with a Q-tip (under the surgical microscope with 6x magnification).
5. Ligate and divide the ileocecal & right & middle colic vessels using 7-0 silk. After the ascending colon is spread out to the right side of the superior mesenteric vein (SMV), the ileocecal, right and middle colic vessels can be identified for ligation and division using 7-0 ties.
6. Retract the stomach upward, so that the entire SMV is straightened out and exposed. Use a mosquito clamp for retraction. The clamp is held by plasticine mass formed into shape as needed.
7. Ligate and divide the pancreatico-duodenal veins coming from the SMV. All small pancreatico-duodenal veins originating from the SMV must be carefully identified, ligated with 7-0 silk and divided before the pancreatic tissue can be removed from the graft.
8. Ligate and divide the lose connective tissue including all lymphatics between the SMV and the abdominal aorta. With the graft still on the right side of the abdomen, the lose connective tissue including all lymphatics between the SMV and the abdominal aorta is accessible. This connective tissue must be ligated using 7-0 silk and divided to avoid postoperative lymphorrhea from the intestinal graft.
9. Ligate and divide the right renal artery. After the connective tissue is divided, the right renal artery becomes accessible and is ligated and divided using 7-0 silk.

10. Systemically heparinise the rat using 200 units of heparin i.v. via the penile vein.
11. Ligate the marginal arteries, and divide the small bowel at the duodeno-jejunal junction and at the terminal ileum.
12. The aorta is ligated proximally to the origin of the SMA. The portal vein is transected at the confluence with the splenic vein. Then the graft is harvested with its vascular pedicle consisting of the SMA with an aortic segment.

2. Backtable Procedure

1. Perfuse the graft using 3 ml of University of Wisconsin solution (UW) at 4 °C via the aortic conduit and irrigate the intestinal lumen from the jejunal end with 30 ml of Uro-Nebacetin N solution at 4 °C (lumen irrigation is mandatory).

Immediately after extracting the graft, the aortic conduit is used for perfusion with 3 ml chilled UW solution. For this, a 20 G i.v. catheter on a 10 ml syringe is used. The perfusate should be observed to flow out freely from the divided portal vein. For the intestinal irrigation with Nebacetin, a 50 ml syringe is used.

2. Store the graft in UW solution at 4 °C during the preparation of the recipient.

3. Recipient Operation

1. The recipient rat should be kept fasting for 24 hr (free access to water/glucose solution).
2. To induce Isoflurane inhalation anesthesia, start with 2% on standard atomizer, and then reduce to 1% after performing laparotomy. Perform a toe pinch to check sedation.
3. Shave the abdomen and clean with skin prep 3 times (Kodan). Then perform a median incision after subcutaneous administration of Carprofen 5 mg/kg s.c. for intraoperative analgesia.
4. Wrap the recipient bowel in normal saline soaked gauze and place it on the recipient's chest.
5. Open the retroperitoneum bluntly with Q-tips, and expose the abdominal aorta and inferior vena cava just below the renal vessels down to the level of the iliac vessels, if necessary also use microscissors. Ligate the small lumbar and spinal tribuaries from the aorta and vena cava using 7-0 silk to avoid blood loss. (To do this, change the surgical microscope zoom to 16x.)
6. Cross-clamp the aorta and the IVC below the left renal vessels proximally and above the iliac bifurcation distally using microvessel clips. Only one clamp is used proximally as well as distally to clamp both vessels simultaneously. Incise both vessels anteriorly using a microknife and wash out remaining blood.
7. Create an end-to-side anastomosis between the graft aortic segment and the recipient's infrarenal aorta using a continuous 10-0 Prolene suture.. Initially, the graft is placed on the the right side of the abdomen (the head of the rat positioned at 12 o'clock) to perform the back wall stitches of the arterial anastomosis and tying of the lower stay suture. Then, the graft is turned over to the left side of the abdomen (the head of the rat positioned at 9 o'clock) to expose and suture the front wall of the anastomosis.
8. An end-to-side anastomosis between the graft portal vein and the recipient's IVC is performed likewise by running sutures using 10-0 Prolene. With the rat still lying sideways (head in 9 o'clock position), the graft is positioned on the left side of the abdomen and a lower stay suture is placed. The anastomosis is started with the back wall from inside the vessel. After the lower stay suture is tied, the front wall stitches can be performed from outside.
9. Remove the distal clamps first, followed by the upper clamps. Any anastomotic bleeding is controlled by direct pressure using Q-tips. The graft should be checked for equal and quick reperfusion.
10. Resect the entire recipient's small intestine after ligation of the mesenteric vessels. Recipients undergo subtotal enterectomy, preserving 2-3 cm of proximal jejunum and 1 cm of distal ileum.
11. Restore enteric continuity by proximal (jejuno-jejunostomy) and distal (ileo-ileostomy) end-to end intestinal anastomoses using an interrupted one-layer suture with 6-0 Monocryl. Approximately 16 sutures are needed to complete the anastomoses.
12. Irrigate the peritoneal cavity with normal saline until clean. Administer 2 ml of normal saline intraperitoneally for fluid replacement. Then close the abdomen using a continuous suture with 3-0 Vicryl for the muscle layer plus a continuous skin suture.
13. In the postoperative period the rats should be kept fasting (with access to water and glucose solution) for another 24 hr then restarted on standard rat chow and water ad libitum. Analgesia with carprofen should be administered for 3 days (see dosage below).

Representative Results

Normal postoperative course

The transplanted animals should recover quickly from the procedure under a heat lamp for approximately 1 hr. Hypothermia is a major cause of postoperative mortality and should be carefully avoided intra- and postoperatively. Intraoperative fluid losses must be replaced by s.c. injection of 2.5 ml normal saline plus 2.5 ml Glucose 5% every 8 hr for the first 24 hr. The rats should also have free access to glucose solution (or gel diet) and water p.o. for the first 24 hr postoperatively. After this period they should regain normal feeding behavior with free access to standard rat chow and water ad libitum. Pain is controlled by administration of carprofen 5 mg/kg s.c. daily for three days, with the first shot to be administered at anesthesia induction. Perioperative antibiotic prophylaxis is only needed in the allogenic setting, and can be administered over 5-7 days (ampicillin 15 mg/kg s.c., q. 12 hr). General appearance, fur condition as well as mucosal appearance should be normal. After POD 1 the activity level should return to preoperative status, apathic or abnormal behavior suggests early surgical complications. After initial loss of up to 20% of body weight the rats will start to gain weight again around postoperative day 6-8 and will have reached around 90 % of their preoperative weight normally around postoperative day 14 (in the isogenic setting without rejection).

Postoperative complications

As stated above, a distended abdomen, apathic behavior, discontinuation of feeding and changes in general appearance (ruffled fur, secretion from the eyes) should be considered as symptoms of possible complications. The animals should be seen by the surgeon and a veterinarian.

Conditions like dehydration, inflammatory state due to peritonitis, ileus due to stenosis of the bowel anastomosis, insufficient pain medication and others must be ruled out and treated. If the animal does not recover despite treatment, discontinuation of the experiment must be evaluated on a case by case basis according to applying animal experiment regulations.

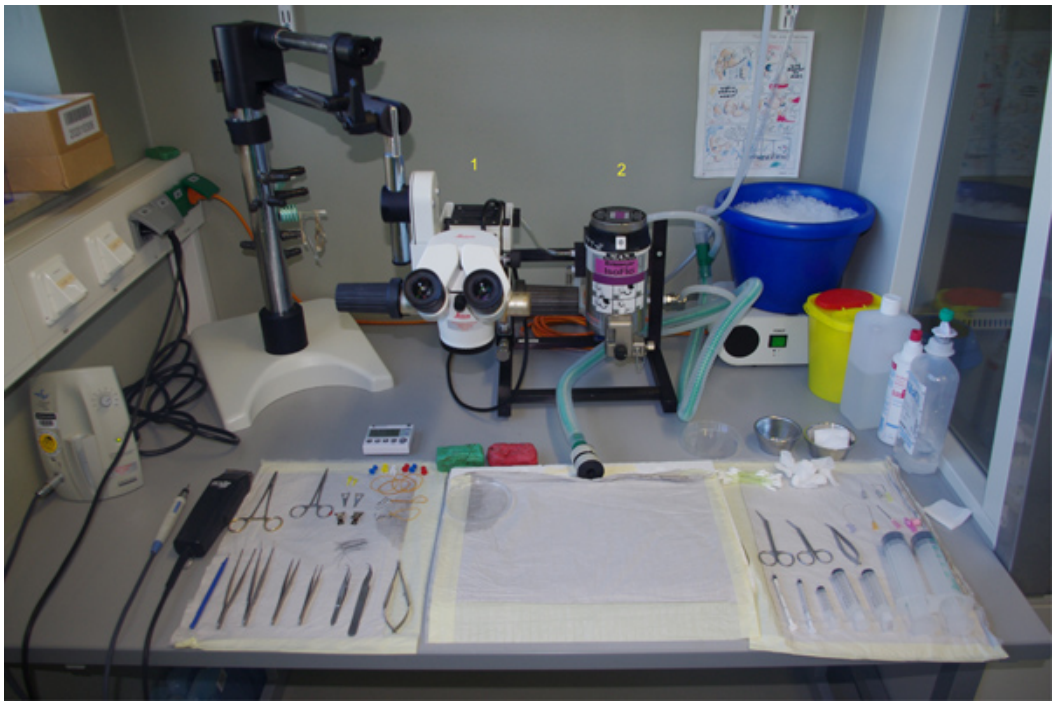

Figure 1a. Instruments.
1. Microscope (LEICA)
2. Anesthesia apparatus (EICKEMEYER)

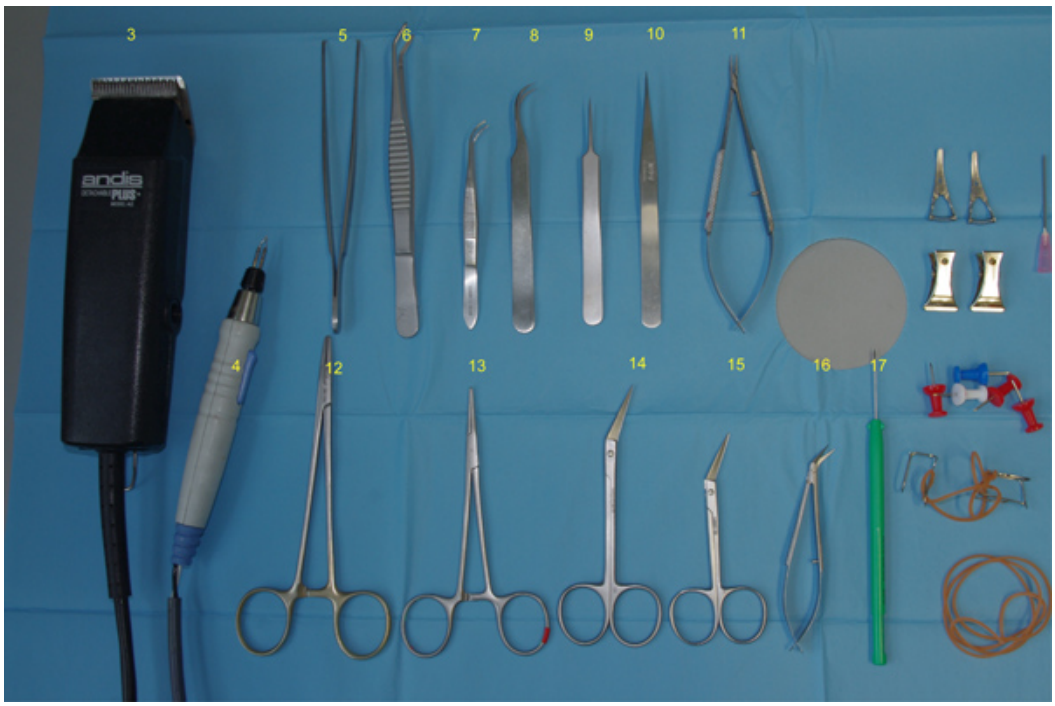

Figure 1b. Instruments.
3. Shaver
4. Electronic scalpel (GEIGER)
5. Surgical forceps
6. DeBakey forceps curved
7. Curved forceps small
8. Micro forceps curved

9. Micro forceps straight 1
10. Micro forceps straight 2
11. Micro needle holder
12. Needle holder
13. Mosquito clamp
14. Scissors 1
15. Scissors 2
16. Microscissors
17. Micro Scalpel

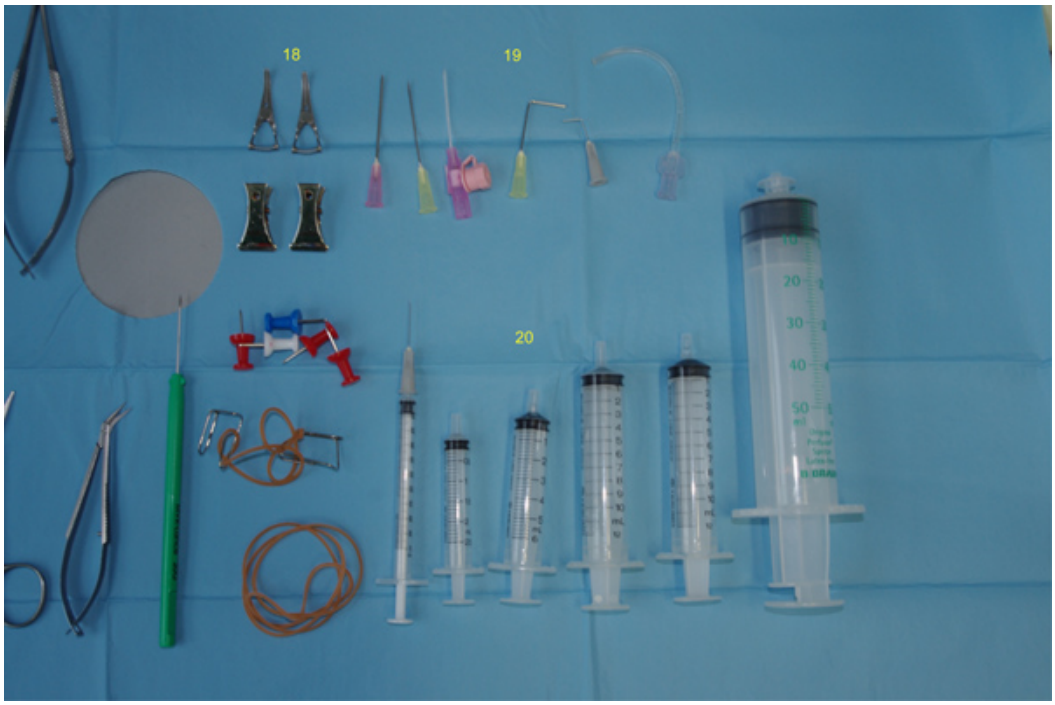

Figure 1c. Instruments.
18. Microclamps
19. Canulas and Q-Tips (not depicted)
20. Syringes (50 ml, 10 ml, 2.5 ml, 1 ml)

Figure 1c. Instruments.
21. UW solution for graft storage
22. Antibiotics (Uro-nebacetin N) for graft lumen irrigation
23. UW for graft perfusion
24. Normal saline
25. Dish with normal saline (for backtable)

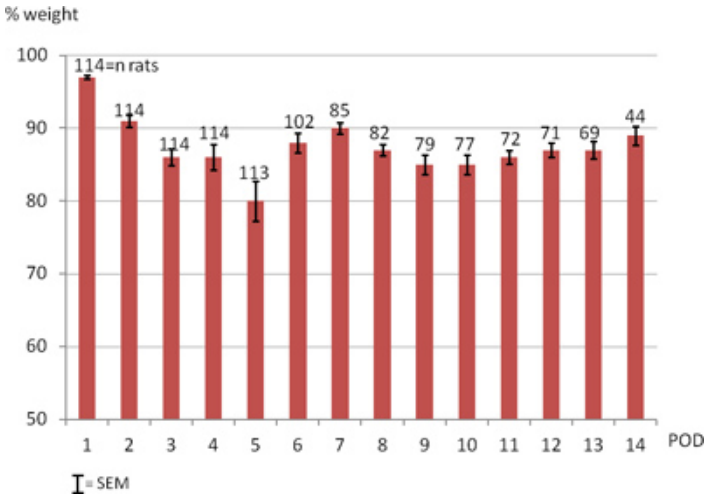

Figure 2. Average postoperative weight (cumulative data). Figure 2 shows the average postoperative weight after orthotopic small bowel transplantation. After initial loss of up to 20% of body weight the rats start to gain weight again around postoperative day 6-8 and will have reached 90% of their preoperative weight normally around postoperative day 14 (in the isogenic setting without rejection / immunosuppression). **Figure 2** represents weight loss data, not survival, the decreasing number of available animals to measure weight loss is mainly due to sacrifing of animals for experiments.

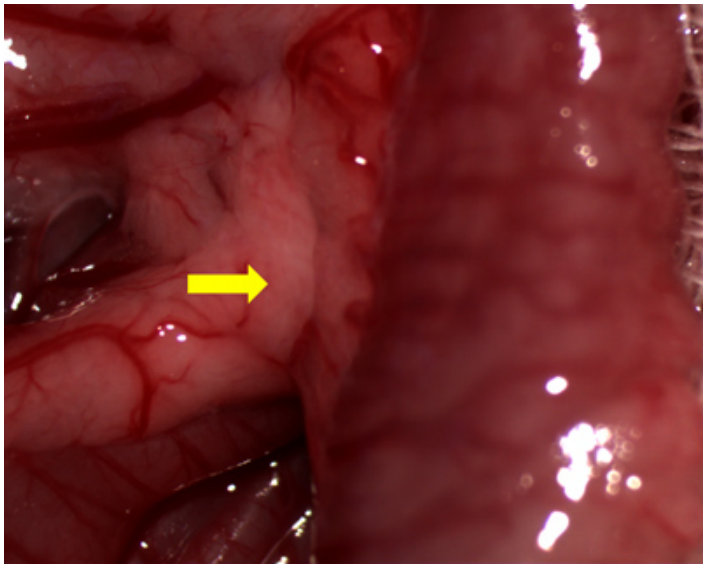

Figure 3. The pancreatic tissue (arrow) has to be removed from the colon.

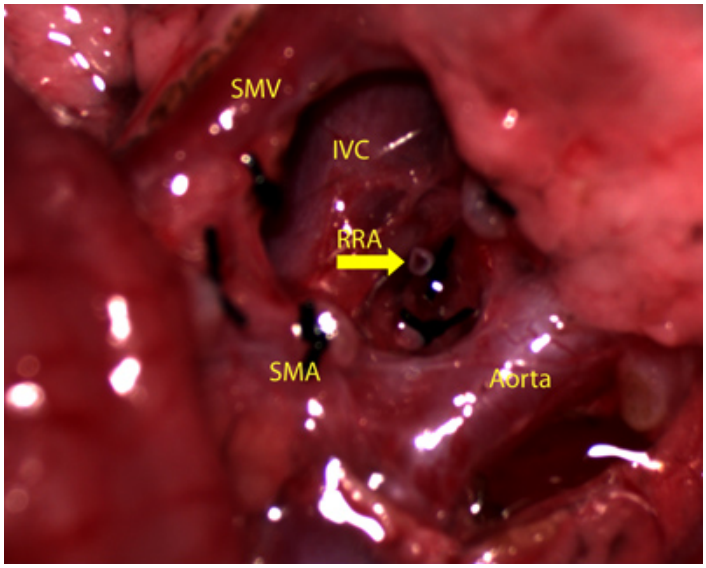

Figure 4. After ligating and dividing the lose connective tissue including all lymphatics between SMV and the abdominal aorta, the right renal artery (arrow) is divided between silk ligatures.

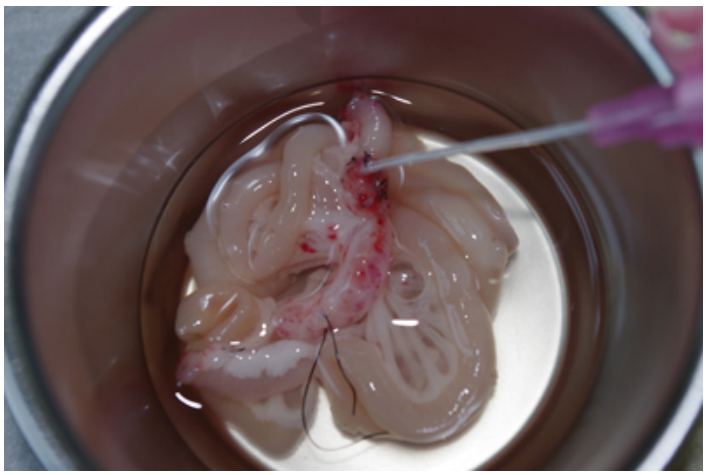

Figure 5. Perfusion of the graft with UW solution via the aortic conduit.

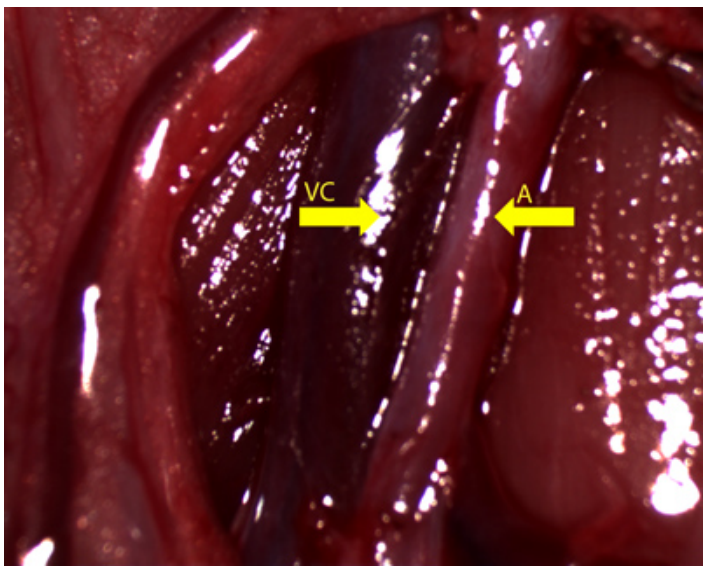

Figure 6. After preparation of recipient vena cava and aorta the vessels are exposed, ready for clamping.

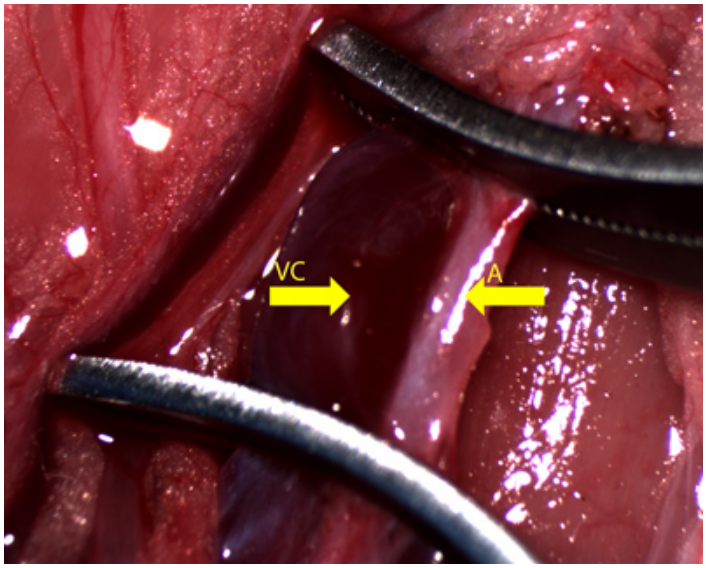

Figure 7. Cross clamp is performed on vena cava and aorta simultaneously using microclamps.

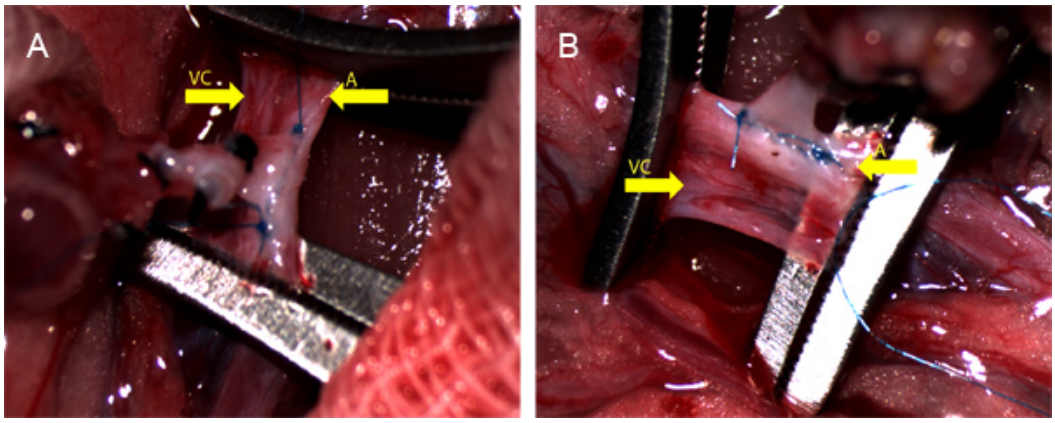

Figure 8. (a) The aorto-aortic anastomosis is performed using stay sutures. (b)After completion of the aorto-aortic anastomosis, the portocaval anastomosis will be performed next. The lower stay suture is already in place.

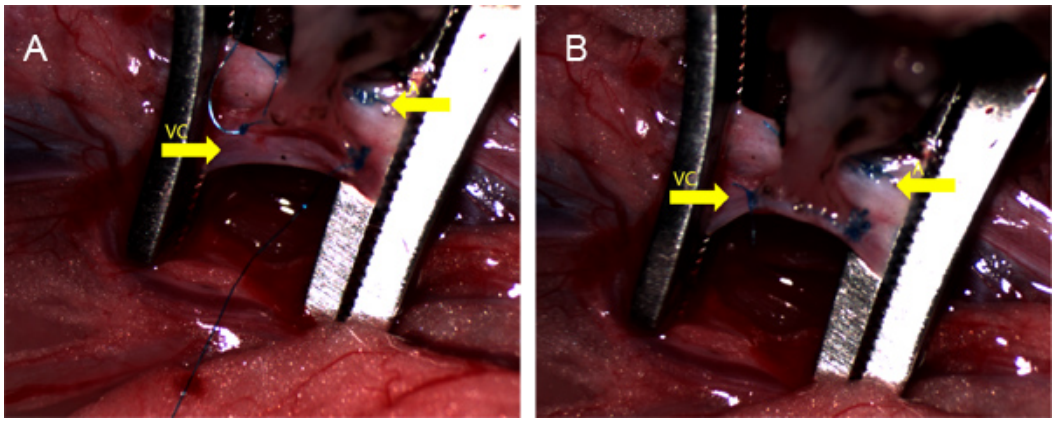

Figure 9. (a) The portocaval anastomosis is started after the second stay suture is in place. (b) After completion of the portocaval anastomosis.

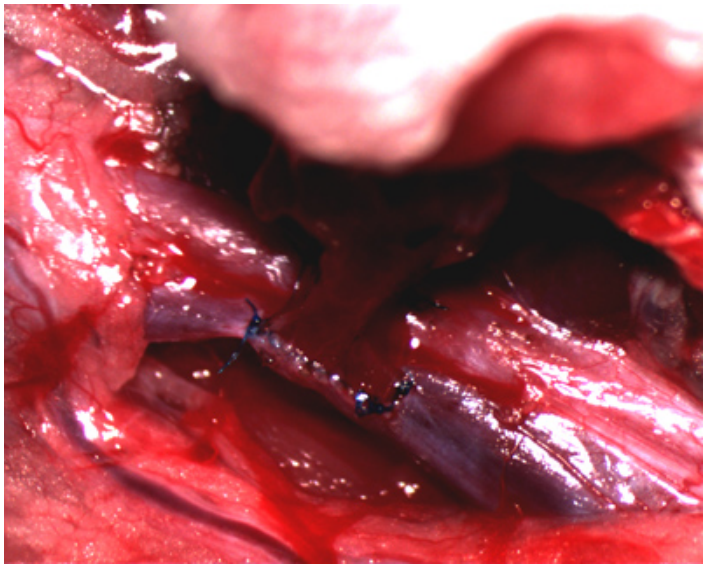

Figure 10. After removal of the clamps the graft reperfuses well.

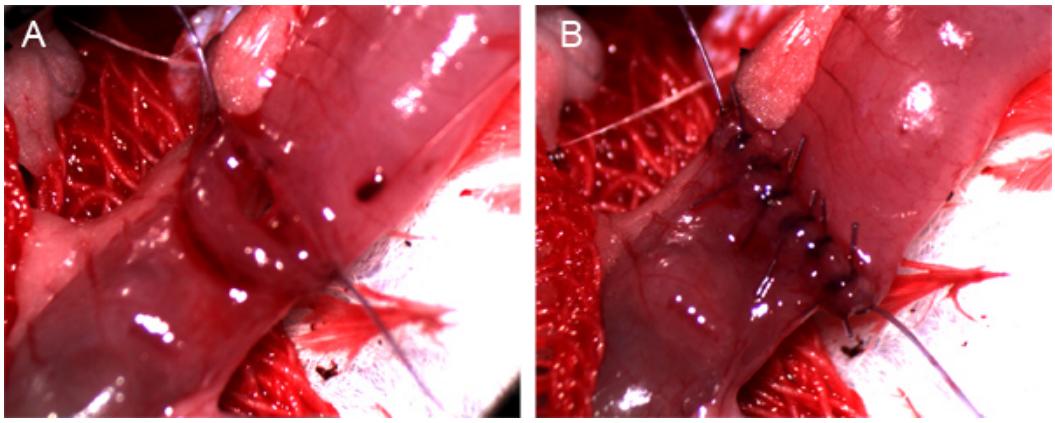

Figure 11. (a) Placement of stay sutures for the bowel anastomosis. (b) Completed bowel anastomosis.

Discussion

While intestinal transplant models in rats have been described as early as in the 1970ies [5] most of the recently employed models involve heterotopic intestinal transplantation using different techniques [13]. While the heterotopic procedures in general have the advantage of easier microsurgical techniques and easier accessibility of the graft for assessment, heterotopic intestinal transplantation has the big disadvantage of not taking into account the multiple interactions of the transplanted small bowel and its functional aspects like contractile activity and mucosal barrier function that characterizes an orthotopic graft in the context of a vast host of commensals and pathogens. Our group has gained a large experience with the herein described orthotopic model and our findings suggest that many of the specific alterations caused by inflammatory and adaptive immune responses have to be assessed in context with the functional properties of the transplanted small bowel like contractility and mucosal integrity. Of note, the anastomotic techniques employed here do not include the usage of arterial or venous cuffs, some of which have been shown to facilitate the procedure and reduce the critical warm ischemia time, especially in models of multivisceral transplantation [12,14]. Although it may be necessary to employ cuff techniques in rodent models of multivisceral transplantation, the complicative potential of the cuffs has led us to avoid similar techniques in this model of single intestinal transplantation. We have not attempted to use portal drainage techniques in this model. Apart from being technically challenging (the small portal vein diameter could lead to venous outflow problems), which would make the whole model more difficult to establish - it is a fact that in clinical intestinal transplantation systemic drainage is used in the majority of cases. Thus, the described technique reflects clinical practice combined with technical feasibility. Portal drainage of the grafts has not been shown to be associated with superior outcomes in several clinical and experimental studies [2,4]. Sufficiently short ischemia times of around 35 minutes necessary for stable animal survival can be achieved after completion of the learning curve for this model.

Orthotopic small bowel transplantation according to this protocol can be learned by a microsurgically experienced researcher after performing approximately 30-40 procedures. The visualization as achieved by the JoVE format allows for direct visual observation and accurate reproduction of the employed techniques that possibly leads to quicker establishment of the method and less animal sacrifice. Critical points are bleeding, cold and warm ischemia time and bowel anastomotic stenosis/insufficiency. The cold ischemia time in this experimental setting is not as crucial as warm ischemia time but should not exceed 45 minutes. The warm ischemia time should be around 35 minutes and also not exceed 45 minutes, as this may cause higher postoperative mortality. The ideal donor and recipient weight is around 200 g because smaller rats do not tolerate the procedure well and weight over 300 g is associated with excessive intraabdominal fat. The rats lose up to 20% of body weight in the direct postoperative period but should start to gain weight again after 6-8 days postoperatively (**Figure 2**). Daily health checks

(alertness, fur and mucosal appearance, weight, stool quality and frequency) should be performed until the animal is sacrificed. We recommend daily administration of antibiotics and analgesia for at least the first three days as described above. After initial practice, particularly of the microvascular and bowel anastomoses, this model provides reliable and stable long term survival of around 80-90% in the isogenic setting. In the allogenic setting survival is generally lower, depends mainly on immunologic phenomena like acute and chronic rejection and may vary widely according to the immunosuppressive regimen used and tested.

Technical notes: The donor operation time should be about 45 minutes. The recipient operation should not much exceed 1.5 hours. A heating pad should be used routinely to avoid hypothermia in the recipient. For easy vascular access, the lateral tail vein of the recipient can be cannulated at the beginning of the procedure using a 22 G intravenous catheter. Irrigation of the intestinal lumen, as described above may not be necessary, omitting this step has to our knowledge no negative effects on outcome and mortality.

Animal study requirements: The animals were kept according to applying laws and regulations of the Federal Republic of Germany, State North Rhine-Westfalia. The document numbers under which the experiments were approved can be requested from the corresponding author.

Disclosures

No conflicts of interest declared.

References

1. Abu-Elmagd, K.M., *et al.* Five Hundred Intestinal and Multivisceral Transplantations at a Single Center: Major Advances With New Challenges. *Ann. Surg.* **250** (4), 567 (2009).
2. Berney, T., *et al.* Portal versus systemic drainage of small bowel allografts: comparative assessment of survival, function, rejection, and bacterial translocation. *J. Am. Coll. Surg.* **195** (6), 804 (2002).
3. Fishbein, T.M. Intestinal transplantation. *N. Engl. J. Med.* **361** (10), 998 (2009).
4. Hernandez, F., *et al.* Is portal venous outflow better than systemic venous outflow in small bowel transplantation? Experimental study in syngeneic rats. *J. Pediatr. Surg.* **40** (2), 336 (2005).
5. Monchik, G.J. & Russell, P.S. Transplantation of small bowel in the rat: technical and immunological considerations. *Surgery.* **70** (5), 693 (1971).
6. Murase, N., *et al.* Graft-versus-host disease after brown Norway-to-Lewis and Lewis-to-Brown Norway rat intestinal transplantation under FK506. *Transplantation.* **55** (1), 1 (1993).
7. Murase, N., *et al.* Immunomodulation of intestinal transplant with allograft irradiation and simultaneous donor bone marrow infusion. *Transplant Proc.* **31** (1-2), 565 (1999).
8. Pech, T., *et al.* Perioperative infliximab application ameliorates acute rejection associated inflammation after intestinal transplantation. *Am. J. Transplant.* **10** (11), 2431 (2010).
9. Schaefer, N., *et al.* Resident macrophages are involved in intestinal transplantation-associated inflammation and motoric dysfunction of the graft muscularis. *Am J Transplant.* **7** (5), 1062 (2007).
10. Schaefer, N., *et al.* Mechanism and impact of organ harvesting and ischemia-reperfusion injury within the graft muscularis in rat small bowel transplantation. *Transplant. Proc.* **38** (6), 1821 (2006).
11. Schaefer, N., *et al.* Acute rejection and the muscularis propria after intestinal transplantation: the alloresponse, inflammation, and smooth muscle function. *Transplantation.* **85** (10), 1465 (2008).
12. Xue, L., *et al.* Surgical experience of refined 3-cuff technique for orthotopic small-bowel transplantation in rat: a report of 270 cases. *Am. J. Surg.* **198** (1), 110 (2009).
13. Zhang, X.Q., *et al.* Simplified techniques in rat heterotopic small bowel transplantation. *Transplant. Proc.* **38** (6), 1840 (2006).
14. Zhao, Y., *et al.* The establishment of a new en bloc combined liver-small bowel transplantation model in rats. *Transplant. Proc.* **39** (1), 278 (2007).

