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In Vivo Model of Small Intestine

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Abstract

The utilization of human pluripotent stem cells (hPSCs) offers new avenues in the generation of organs and opportunities to understand development and diseases. The hPSC-derived human intestinal organoids (HIOs) provide a new tool to gain insights in small intestinal development, physiology, and associated diseases. Herein, we provide a method for orthotopic transplantation of HIOs in immunocompromised mice. This method highlights the specific steps to successful engraftment and provides insight into the study of bioengineered human small intestine.

Keywords

Bioengineered small intestine; Human intestinal organoids; Pluripotent stem cells; Transplantation; In vivo model; Mesentery; Kidney capsule

1 Introduction

1.1 The Small Intestine

The small intestine is a complex hollow organ with several histological and functional layers that facilitate efficient digestive functions. This dynamic tube is composed of an outer layer of smooth muscle, a central layer of connective tissue, and an inner absorptive mucosa [1]. The inner lining of the small intestine is an epithelial monolayer consisting of pits called crypts that encircle and provide epithelial cells to protruding fingerlike structures called villi. The base of the crypt contains the proliferative compartment where stem cells and transit-amplifying cells divide and differentiate toward the apex of the villi. The intestinal epithelium contains four main differentiated cell lineages: absorptive, enterocytes; secretory, enteroendocrine cells; Paneth cells; and Goblet cells [2, 3]. The subjacent layers of the small intestine regroup connective tissues, smooth muscle, and an enteric nervous system. The smooth muscular layers are critical for the motor activities in the gut, including segmentation and peristalsis. They are arranged in a double layer: circular and longitudinal with intercalated enteric nerves regulating their contractions [4]. Understanding the complex interaction of the entire laminated intestine is required to appreciate the mechanisms underlying small intestinal development, physiology, and associated diseases thereof.

1.2 Model System of the Small Intestine

Limited in vivo models of human intestine exist, and the study of human intestinal biology has mostly relied on primary or established epithelial cell cultures [5]. Yet, in vitro cell culture systems do not faithfully mimic the intestinal physiology. Recent breakthroughs in

the intestinal epithelial stem cell field allowed the research community to overcome these problems. A couple groups described long-term culture conditions of three-dimensional tissue-derived epithelial organoids that uniquely reassemble intestinal epithelial dynamics and physiology [6–8]. These experimental model systems constitute useful tools for studying the regulation of gastrointestinal stem cells as well as the intestinal epithelial cell fate determination throughout the digestive tract [9]. Although the *in vivo* transplantation using this model has been reported, thus far, their *in vivo* potential to address biological questions is limited [10, 11]. Other approaches have been developed to generate tissue-engineered small intestines or TESI. Using specific bio-scaffoldings and digested tissue from patients, these organoid units transplanted in murine recipients formed functional intestinal-like tissues resembling most of the intestinal lineages and functions [12, 13]. Emerging technologies utilizing fluidic devices like the “gut on a chip” have allowed for the *in vitro* mimicry of additional features to the system such as the inclusion of microbiota, shear stress, and tension [14, 15].

1.3 Human Intestinal Organoid Model

Applying the concepts from developmental biology to human pluripotent stem cells (hPSCs) has served as the basis for generating organoid models. Using a stepwise differentiation process, the hPSCs can be driven toward a mesendodermal fate, thus resulting in the generation of three-dimensional epithelial structure surrounded by mesenchyme called human intestinal organoids (HIOs) [16, 17]. Recently, we demonstrated that HIOs produced entirely *in vitro* can be transplanted under the kidney capsule of immunocompromised mice. After 6–8 weeks post-transplantation, the HIOs engrafted *in vivo* and formed a laminated intestinal tissue with an epithelium supported by the host vasculature. While expressing all the intestinal lineages in a crypt-villus architecture, the transplanted HIO resulted in significant expansion and maturation of the epithelium and mesenchyme and displayed digestive and absorptive functions [18, 19].

The transplanted HIOs exhibit key features of the human small intestine thus offering opportunities to broaden our knowledge from intestinal developmental processes to disease mechanisms [20]. In this chapter, we provide a detailed methodology to transplant hPSC-derived HIOs in a murine model. In addition, we also describe a method to study the small intestine’s physiology using HIOs joined to the host intestinal tract.

2 Materials

2.1 Human Intestinal Organoid (HIO) Generation and Maintenance

All solutions should be prepared fresh using sterile cell culture grade reagents.

1. Human intestinal spheroids derived from human pluripotent stem cell lines (*see* Chapter 13).
2. Matrigel® Growth Factor Reduced; phenol red-free.
3. Intestinal growth medium: Advanced DMEM/F12 medium supplemented with 2 mM glutamine, 10 mM HEPES, 100 U/mL penicillin, 100 µg/mL streptomycin,

1× N2 supplement, 1× B27 supplement and filter sterilized with 0.22 µm filter (*see*^{Note 1}).

4. Human recombinant epidermal growth factor (EGF) (10,000× stock; 500 mg/mL in sterile PBS/0.1% bovine serum albumin).
5. Collagen solution: Rat tail type I collagen is made fresh prior to embedding as per the manufacturer's instructions. 30 µL type I collagen gel is typically used to embed one HIO.

2.2 Murine Recipients, Surgical Equipment, and Reagents

Aseptic technique is essential for any survival surgery and requires that all surgical instruments and supplies be sterile. All surgical instruments should be washed and sterilized in an autoclave prior to use. All surgeries are performed under a HEPA-filtered laminar flow bio Bubble to prevent microbial contamination of the surgical site.

1. Mice: Female or male immunocompromised NSG mice, NOD-scid IL2R gamma^{null}, are housed in microisolator systems in a barrier facility. The mice are used between 6 and 14 weeks of age (*see*^{Note 2}).
2. Antibiotic diet: A modified chow diet (PicoLab Rodent Diet 20; LabDiet, St. Louis, MO, USA) is supplemented with 275 ppm sulfamethoxazole and 1365 ppm trimethoprim (LabDiet) (*see*^{Note 3}).
3. Liquid diet is used for the side-to-side anastomosis surgery (Jevity® 1 Cal; Abbott Nutrition, Tipp City, OH, USA).
4. Antibiotics in water: 0.3 mg/mL of 275 ppm sulfamethoxazole and 1365 ppm trimethoprim are diluted in sterile water and are given ad libitum after the side-to-side anastomosis surgery (Bactrim®; Hi-Tech Pharmacal, Amityville, NY, USA).
5. Antibacterial drugs: 100 mg/kg of piperacillin and tazobactam are diluted in sterile saline solution and used for any surgeries (ZOSYN®; Pfizer, New York, NY, USA).
6. Buprenorphine.
7. Surgical suite (Fig. 1; *see*^{Note 4}).
8. Surgical instruments (*see* Table 1).
9. Isoflurane and anesthesia system (Fig. 1; *see*^{Note 5}).

¹Divide intestinal growth medium into 10 mL aliquots in 15 mL conical tubes, and freeze at −20 °C for up to 3 months. Store thawed aliquots up to 5 days at 4 °C without loss of activity.

²Males are preferably used for the kidney subcapsular transplantation as their kidneys are bigger and easier to work with.

³The chow diet is supplemented with antibiotics and given to the mice at least 14 days prior to any surgeries. The antibiotics decrease inflammation and risk of infection.

⁴The surgical suite consists of an operating table and a surgical scope placed under a sterile vertical laminar flow. The table is heated to 30 °C, and, additionally, a 30 °C water-heated pad is used to further control animal temperature.

⁵The anesthesia system delivers an isoflurane and oxygen mixture that can be controlled and monitored to maintain the anesthesia during surgery. The extra anesthetic gas is collected and evacuated into a canister.

10. Sterile 7–0 nonabsorbable silk suture (PERMA-HAND®; Ethicon, Cincinnati, OH, USA).
11. Sterile 4–0 coated absorbable suture (VICRYL RAPIDE™; Ethicon, Cincinnati, OH, USA).
12. Sterile 9–0 nonabsorbable nylon suture with taper cut needle (ETHILON®; Ethicon, Cincinnati, OH, USA).
13. Octyl/butyl cyanoacrylate topical tissue adhesive (GLUture; WPI, Sarasota, FL, USA).
14. Sterile 18 G blunt fill needles.

3 Methodology

3.1 Human Intestinal Organoid Generation and Maintenance

The generation of human intestinal organoids (HIOs) has been described in detail in Chapter 13 by *Munera and colleagues* based on original protocols [16, 17]. In this section, we highlight important steps from the spheroid generation prior to the transplantation of the HIOs.

From subsequent intestinal spheroid generation in Chapter 13:

1. Collect the floating spheroids under a stereoscope, and transfer them in a 2 mL microtube filled with 1 mL intestinal growth medium (Fig. 2a,b; *see*^{Notes 6 and 7}).
2. Let the spheroids settle out in the microtube and discard the media.
3. Mix the pelleted spheroids with ice-cold Matrigel® to a concentration of 15–20 spheroids per 40 µL of Matrigel®.
4. Apply 50 µL of spheroid suspension in Matrigel® per well on a pre-warmed 24-well plate. Slowly eject the Matrigel®-embedded spheroids in the center of the well (Fig. 2c; *see*^{Note 8}).
5. Place the 24-well plate in a 37 °C, 5% CO₂ incubator for 30 min to allow a complete polymerization of the Matrigel®.
6. Overlay the embedded spheroids with 500 µL of intestinal growth medium supplemented with 50 ng/mL of human recombinant EGF (1:10,000 dilution of 500 µg/mL stock).
7. Change intestinal growth media supplemented with 50 ng/mL of human recombinant EGF every 4 days (*see*^{Note 9}).

⁶The stereoscope is placed under a vertical laminar flow hood to prevent any contamination.

⁷Collect the spheroids using a 200 µL sterile tip, which is cut in its extremity. Make sure to collect rounded spheroids with an average size of 20–50 µm. Avoid smaller or broken spheroids that would result in a low transplantation yield.

⁸Eject the Matrigel®-embedded spheroids in the center of a pre-warmed 24-well plate where the center of each well has been pre-coated with a 10 µL Matrigel® bed. This will maintain the Matrigel® in the center of the well during polymerization.

⁹Intestinal growth medium aliquots are thawed and can be kept up to 5 days at 4 °C without loss of activity. Add the human recombinant EGF prior to media change (1:10,000 stock dilution).

8. At 14 days after spheroid plating (Fig. 2d), under a stereoscope, manually excise the HIOs out of the Matrigel®.
9. Collect the excised HIOs into a 24-well plate filled with intestinal growth media.
10. Place one HIO per center of each well of a new 24-well plate.
11. Overlay one HIO with 40 µL of fresh ice-cold Matrigel® per well.
12. Place the 24-well plate in a 37 °C, 5% CO₂ incubator for 30 min to allow a complete polymerization of the Matrigel®. Overlay the HIOs with 500 µL of intestinal growth medium supplemented with 50 ng/mL of human recombinant EGF (1:10,000 dilution of 500 µg/mL stock).
13. Change intestinal growth media supplemented with 50 ng/mL of human recombinant EGF every 4 days until transplantation.
14. At the day of transplantation, day 28, remove the HIO from Matrigel® using sterile tips and overlay with 1 mL of ice-cold DPBS (Fig. 2e–f; *see*^{Notes 10 and 11}).
15. Pipet back and forth using a 1000 µL micropipette to remove the Matrigel® from the HIO. Repeat the procedure using ice-cold DPBS twice.
16. Embed one HIO per 30 µL drop of rat tail collagen type I (*see*^{Note 12}).
17. Place the 24-well plate in a 37 °C, 5% CO₂ incubator for 30 min to allow a complete polymerization of the collagen plug. Overlay the HIOs with 500 µL of intestinal growth medium supplemented with 50 ng/mL of human recombinant EGF.
18. Incubate HIOs at 37 °C, 5% CO₂ until use (*see*^{Note 13}).

3.2 Orthotropic Transplantation of Human Intestinal Organoids

Proper surgical technique must be practiced, that is, asepsis, gentle tissue handling, minimal dissection of tissue, appropriate use of instruments, effective hemostasis, and correct use of suture materials and patterns. The person performing the procedures must be appropriately trained and work under a mentor or veterinarian to perform the procedure. During a surgical procedure, the person performing the procedures must wear clean scrubs, sterile surgical gown, mask, cap, and sterile gloves. Sterile surgical gown and gloves must be donned and maintained in an aseptic manner. All NSG mice are maintained on regular chow supplemented with antibiotics prior to transplantation.

¹⁰Prepare HIOs 1 to 2 h prior to surgery.

¹¹HIOs can be transplanted from day 26 to day 42 of culture. Before or beyond these time points, success of engraftment is not guaranteed. Control the quality of the HIOs using specific gene and protein expression to assess correct intestinal differentiation, i.e., CDX2, CDH1, and SOX2.

¹²Collagen embedding can be facultative, but improve the handling of the HIOs during transplantation.

¹³For early morning surgeries, HIOs can be embedded in the evening preceding the transplantation.

3.2.1 Renal Subcapsular Transplantation of HIOs

1. Bring the mice to the operating suite where weighting and assessment of health status are performed.
2. Anesthetize the mouse in an anesthetic gas vaporizer delivering an isoflurane/O₂ mixture (*see*^{Note 14}).
3. Shave the left flank of the abdomen between the last rib and the iliac crest and the spine and the lower third of the abdominal wall. Remove loose fur (Fig. 3a; *see*^{Note 15}).
4. Prepare the surgical site using povidone-iodine with a cotton swab. Repeat the procedure with new cotton swabs three times.
5. Repeat the procedure using 70% isopropyl alcohol with cotton swabs. Repeat the procedures with new cotton swabs three times.
6. Place ophthalmic ointment on the eyes to prevent drying of the cornea and administer buprenorphine (0.05 mg/kg) subcutaneously (*see*^{Note 16}).
7. Restrain the mouse on lateral recumbency, the left kidney facing upward, and secure the mouse to a nose cone vaporizing isoflurane/O₂ mixture (*see*^{Note 17}).
8. Monitor respiratory rate and effort, along with the surgical plane of anesthesia. Confirm the loss of pedal reflex by pinching the toe with forceps.
9. Use straight forceps and fine scissors to make an 8–10 mm left posterior subcostal skin incision just below the last rib.
10. Use fine scissors to make a subsequent 8–10 mm retroperitoneal muscle incision (Fig. 3b).
11. Identify the kidney using ring forceps and mobilize it into the wound.
12. Stabilize the kidney in the wound by placing a 7–0 silk suture loop with an untied square knot around the incision. Secure one ear of the suture with a needle holder to hold the knot and leave the other ear free (Fig. 3c).
13. Lift the kidney caudal pole through the abdominal incision and tie the silk suture by gently pulling the free ear until the kidney remains still (Fig. 3d; *see*^{Note 18}).
14. Use a cotton swab to dry out the renal capsule.
15. Grasp the capsule under a surgical stereoscope with fine forceps and make a 2–3 mm incision with Vannas spring scissors in the capsule over the lateral aspect of the kidney (Fig. 3e).

¹⁴Final anesthetic gas concentration is achieved by delivering 2% isoflurane with 2.5–3 L/min O₂.

¹⁵The left kidney is used for ease of access.

¹⁶Analgesia provisions are most effective at reducing the intensity of painful stimulation when given prior to the surgery. Any animal showing evidence of pain should be provided with analgesia. Other opioids like buprenorphine can be used, i.e., butorphanol (0.2–2 mg/kg subcutaneous or intraperitoneal) or oxymorphone (0.2–0.5 mg/kg subcutaneous).

¹⁷Keep the animal warm using a 37 °C heating pad. Adjust anesthetic gas concentration to 1.5–1.75% isoflurane with 2–3 L/min O₂.

¹⁸This technique allows you to hold the kidney outside the abdominal cavity. Do not completely tie the knot to avoid renal vascular ligation and permanent kidney damage. Alternatively, curved forceps can be used to lift the kidney.

16. Create a subcapsular pocket by gently sliding back and forth straight suture-tying forceps under the kidney capsule (*see*^{Note 19}). Allowing the forceps to gently open when inside the capsule helps create enough space for the HIO (Fig. 3f).
17. Grab one collagen-embedded HIO using straight suture-tying forceps and insert it in the subcapsular pocket (Fig. 3g–h; *see*^{Note 20}).
18. Cut the 7–0 silk suture and return the kidney within the abdominal cavity.
19. Flush the abdominal cavity with 2–3 mL of piperacillin/tazo-bactam solution to help prevent bacterial infection.
20. Close the incision in double layers with continuous over- and-over sutures using 4–0 VICRYL RAPIDE[®] suture (Fig. 3i; *see*^{Note 21}).
21. Allow mice to recover in a warm and dry incubator (30 °C) and monitor at least every 15 min until they resume activity and are able to maintain a sternal or sitting position.
22. After recovery, place mice back into cages with regular bedding and provided ad lib Bactrim diet and water.
23. Evaluate animals 12 h later and then daily throughout the remainder of the experiment. Appetite, attitude, and hydration should be noted as an indication of recovery from the surgery. Supplemental fluids and/or analgesics should be administered postoperatively as needed.
24. Sacrifice the mice at a desired time point (Fig. 3j–l; *see*^{Note 22}).

3.3 Mesenteric Transplantation of the HIO

1. Bring the mice to the operating suite where weighting and assessment of health status are performed.
2. Anesthetize the mouse in an anesthetic gas vaporizer delivering an isoflurane/O₂ mixture.
3. Shave the abdomen between the last rib and the iliac crest. Remove loose fur (Fig. 4a).
4. Prepare the surgical site using povidone-iodine with a cotton swab. Repeat the procedure with new cotton swabs three times.
5. Repeat the procedure using 70% isopropyl alcohol with cotton swabs. Repeat the procedure with new cotton swabs three times.

¹⁹Slide the closed straight suture-tying forceps under the capsule and open the forceps while pulling it back. Repeat the motion until an appropriate size of the subcapsular pocket is achieved.

²⁰Inserted HIOs will not dislodge from under the subcapsular pocket.

²¹VICRYL RAPIDE[®] sutures are synthetic coated absorbable sutures, and the animals will not chew them. Alternatively, skin staplers can be used.

²²In our experience, 6–8 weeks post-transplanted HIOs provide us with a fully laminated small intestinal tissue that can be further used for downstream applications ranging from physiological to molecular assays. HIOs transplanted beyond a year do not exhibit common intestinal epithelial features probably due to the accumulation of mucus and debris within the lumen.

6. Place ophthalmic ointment on the eyes to prevent drying of the cornea and administer buprenorphine (0.05 mg/kg) subcutaneously.
7. Restrain the mouse on dorsal recumbency and secure the mouse to a nose cone vaporizing isoflurane/O₂ mixture.
8. Monitor respiratory rate and effort, along with the surgical plane of anesthesia. Confirm the loss of pedal reflex by pinching the toe with forceps.
9. Use straight forceps and fine scissors to make an 8–10 mm midline abdominal skin incision parallel to the spine and in between the iliac crest and the last rib (Fig. 4b).
10. Use fine scissors to make a subsequent 8–10 mm retroperitoneal incision in the *linea alba* (muscle sparing) to gain access to the peritoneum.
11. Identify the cecum in the right upper quadrant, and use ring forceps and cotton swabs to expose delicately the small intestine and the mesentery (Fig. 4c; *see*^{Note 23}).
12. Use a cotton swab to dry out the mesentery.
13. Place a collagen-embedded HIO on the mesentery using straight suture-tying forceps.
14. Apply an octyl/butyl cyanoacrylate glue in between the mesentery and the HIO (Fig. 4d–f; *see*^{Note 24}).
15. Wait for 5 min to allow the complete curing of the glue.
16. Return the small intestine within the abdominal cavity. Carefully replace the organ avoiding any torsion of the gut or its blood supply.
17. Flush the abdominal cavity with 2–3 mL of piperacillin/tazobactam solution to help prevent bacterial infection.
18. Close the incision in double layers with continuous over-and-over sutures using 4-0 VICRYL[®] suture (Fig. 4i).
19. Allow mice to recover in a warm and dry incubator (30 °C) and monitor at least every 15 min until they resume activity and are able to maintain a sternal or sitting position.
20. After recovery, place mice back into cages with regular bedding and provided ad lib Bactrim diet and water.
21. Evaluate animals 12 h later and then daily throughout the remainder of the experiment. Appetite, attitude, and hydration should be noted as an indication of

²³Multiple mesenteric sites can be utilized for transplantation as long as they provide blood supplies and are not in area that would create volvulus and therefore intestinal obstruction.

²⁴Topical adhesive glue can be used to glue the HIOs to the mesentery. Alternatively, a purse-string suture can be created using 9–0 nonabsorbable nylon sutures with taper cut needles to form a mesenteric pocket for the HIO (Fig. 4g–h).

recovery from the surgery. Supplemental fluids and/or analgesics should be administered postoperatively as needed.

22. Utilize the mouse for side-to-side anastomosis or sacrifice the mice at a desired time point (Fig. 4j, k; *see*^{Note 25}).

3.4 Side-To-Side HIO Mouse Small Intestine Anastomosis

All mice undergoing intestinal side-to-side anastomosis are provided with ad libitum liquid diet for 24–48 h prior to surgery and housed in cages with nonedible bedding (*see*^{Note 26}).

1. Bring the mice to the operating suite where weighting and assessment of health status are performed.
2. Anesthetize the mouse in an anesthetic gas vaporizer delivering an isoflurane/O₂ mixture.
3. Shave the abdomen between the last rib and the iliac crest. Remove loose fur.
4. Prepare the surgical site using povidone-iodine with a cotton swab. Repeat the procedure with new cotton swabs three times.
5. Repeat the procedure using 70% isopropyl alcohol with cotton swabs. Repeat the procedures with new cotton swabs three times.
6. Place ophthalmic ointment on the eyes to prevent drying of the cornea and administer buprenorphine (0.05 mg/kg) subcutaneously.
7. Restrain the mouse on dorsal recumbency and secure the mouse to a nose cone vaporizing isoflurane/O₂ mixture.
8. Monitor respiratory rate and effort, along with the surgical plane of anesthesia. Confirm the loss of pedal reflex by pinching the toe with forceps.
9. Use straight forceps and fine scissors to make an 8–10 mm midline abdominal skin incision in the midline.
10. Use fine scissors to make a subsequent 8–10 mm incision in the *linea alba* to gain access to the peritoneum.
11. Identify the transplanted HIO, and use ring forceps and cotton swabs to expose it outside the abdominal cavity (Fig. 5a).
12. Orient the HIO and the murine small intestine to perform a side-to-side anastomosis. The HIOs are very mobile and should be oriented in a manner that will not obstruct the intestine after the anastomosis is performed.
13. Perform an incision of 5–6 mm using Vannas spring scissors on the HIO, and irrigate out the luminal debris with saline (Fig. 5b).

²⁵Similar to the subcapsular transplantation, 6–8 weeks post-transplanted HIOs provide us with a developed small intestinal tissue that can be further used for downstream applications or subsequent surgeries.

²⁶Liquid diet is well tolerated and isocaloric when compared to regular chow. The use of this diet prior to surgery prevents obstruction at the anastomosis site increasing the survival of the animals.

14. Perform a 5–6 mm incision on the antimesenteric side of the small intestine to provide a bypass of around 5–6 mm.
15. Place the initial sutures on the posterior sides of the HIO and small intestine with interrupted 9–0 nylon over-and-over suture under a stereoscope microscope. The first stitch should be placed at 6 o'clock (middle of the posterior wall) and left long to help exposure for future posterior sutures. The two corner sutures are placed next. Retracting on the middle suture, the posterior wall is exposed, and additional sutures are placed from a nice mucosa to mucosa anastomosis (Fig. 5c–d).
16. Restore the continuity of the intestine by applying interrupted stitches on the anterior sides (Fig. 5e–f).
17. Check for any leakage using a cotton swab.
18. Return the small intestine within the abdominal cavity. Carefully replace the organ avoiding any torsion of the gut or its blood supply.
19. Inject 2–3 mL of piperacillin/tazobactam solution into the abdominal cavity to help prevent bacterial infection.
20. Close the incision in double layers with continuous over-and-over sutures using 4–0 VICRYL® suture.
21. Allow mice to recover in a warm and dry incubator (30 °C), and monitor at least every 15 min until they resume activity and are able to maintain a sternal or sitting position.
22. After recovery, place mice back into cages with nonedible bedding and provided ad libitum antibiotic diet and water.
23. Evaluate animals 12 h later and then daily throughout the remainder of the experiment. Appetite, attitude, and hydration should be noted as an indication of recovery from the surgery. Supplemental fluids and/or analgesics should be administered postoperatively as needed. Evaluate mice and weigh and provide new liquid diet daily. In addition, change bedding as needed (usually every other day).
24. Utilize the mouse at a desired time point and analyze the transplant (Fig. 5g–i).

References

1. Simons BD, Clevers H. Stem cell self-renewal in intestinal crypt. *Exp Cell Res.* 2011; 317:2719–2724. [PubMed: 21787769]
2. van der Flier LG, Clevers H. Stem cells, self-renewal, and differentiation in the intestinal epithelium. *Annu Rev Physiol.* 2009; 71:241–260. [PubMed: 18808327]
3. Noah TK, Donahue B, Shroyer NF. Intestinal development and differentiation. *Exp Cell Res.* 2011; 317:2702–2710. [PubMed: 21978911]
4. Cheng LK, O'Grady G, Du P, et al. Gastrointestinal system. *Wiley Interdiscip Rev Syst Biol Med.* 2010; 2:65–79. [PubMed: 20836011]
5. Simon-Assmann P, Turck N, Sidhoum-Jenny M, et al. In vitro models of intestinal epithelial cell differentiation. *Cell Biol Toxicol.* 2007; 23:241–256. [PubMed: 17171431]

6. Ootani A, Li X, Sangiorgi E, et al. Sustained in vitro intestinal epithelial culture within a Wnt-dependent stem cell niche. *Nat Med.* 2009; 15:701–706. [PubMed: 19398967]
7. Sato T, Stange DE, Ferrante M, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology.* 2011; 141:1762–1772. [PubMed: 21889923]
8. Sato T, Vries RG, Snippert HJ, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature.* 2009; 459:262–265. [PubMed: 19329995]
9. Sinagoga KL, Wells JM. Generating human intestinal tissues from pluripotent stem cells to study development and disease. *EMBO J.* 2015; 34:1149–1163. [PubMed: 25792515]
10. Fukuda M, Mizutani T, Mochizuki W, et al. Small intestinal stem cell identity is maintained with functional Paneth cells in heterotopically grafted epithelium onto the colon. *Genes Dev.* 2014; 28:1752–1757. [PubMed: 25128495]
11. Yui S, Nakamura T, Sato T, et al. Functional engraftment of colon epithelium expanded in vitro from a single adult Lgr5(+) stem cell. *Nat Med.* 2012; 18:618–623. [PubMed: 22406745]
12. Grant CN, Mojica SG, Sala FG, et al. Human and mouse tissue-engineered small intestine both demonstrate digestive and absorptive function. *Am J Physiol Gastrointest Liver Physiol.* 2015; 308:G664–G677. [PubMed: 25573173]
13. Spurrier RG, Grikscheit TC. Tissue engineering the small intestine. *Clin Gastroenterol Hepatol.* 2013; 11:354–358. [PubMed: 23380001]
14. Kim HJ, Li H, Collins JJ, et al. Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip. *Proc Natl Acad Sci U S A.* 2016; 113:E7–15. [PubMed: 26668389]
15. Kim HJ, Huh D, Hamilton G, et al. Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. *Lab Chip.* 2012; 12:2165–2174. [PubMed: 22434367]
16. McCracken KW, Howell JC, Wells JM, et al. Generating human intestinal tissue from pluripotent stem cells in vitro. *Nat Protoc.* 2011; 6:1920–1928. [PubMed: 22082986]
17. Spence JR, Mayhew CN, Rankin SA, et al. Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. *Nature.* 2011; 470:105–109. [PubMed: 21151107]
18. Watson CL, Mahe MM, Munera J, et al. An in vivo model of human small intestine using pluripotent stem cells. *Nat Med.* 2014; 20:1310–1314. [PubMed: 25326803]
19. Finkbeiner SR, Hill DR, Altheim CH, et al. Transcriptome-wide analysis reveals hallmarks of human intestine development and maturation in vitro and in vivo. *Stem Cell Reports.* 2015 pii:S2213-6711(15)00122-8.
20. Wells JM, Spence JR. How to make an intestine. *Development.* 2014; 141:752–760. [PubMed: 24496613]

Fig. 1.
Example of a surgical suite with an anesthetic system

Fig. 2.

Human intestinal organoid generation and maintenance prior to transplantation. **(a)** Intestinal spheroids generated after definitive endoderm differentiation and patterning from human pluripotent stem cells (data from H9 embryonic stem cell are shown). **(b)** Close-up picture on representative intestinal spheroids picked up before embedding in Matrigel®. **(c)** Embedded intestinal spheroids in Matrigel®. **(d)** Human intestinal organoids after 14 days in Matrigel®. **(e–f)** Human intestinal organoids after 28 days in Matrigel®, expressing green fluorescent protein (GFP) (*scale bar*, 250 μ m)

Fig. 3.

Human intestinal organoid transplantation under the kidney capsule. **(a–b)** A left incision is performed in the skin and the subjacent muscle layer to access the left kidney. **(c–d)** A silk suture is placed to secure the kidney in the wound. **(e–h)** A small incision is made on the lateral aspect of the kidney, and a pocket is created under the capsule to introduce the collagen-embedded human intestinal organoid. **(i)** The kidney is returned to the abdominal cavity, and both abdominal and skin layers are sutured. **(j)** Photograph of a human intestinal organoid after 10 weeks posttransplantation. Transplanted organoid size ranges from 1 to 3 cm at the most. **(k)** Hematoxylin and eosin staining showing crypt and villus structures of the epithelium along with a laminated submucosal layer. **(l)** Human nuclear staining (HuNUC, red) shows the human origin of the transplanted organoid (*scale bars, 100 μ m*)

Fig. 4.

Human intestinal organoid transplantation in the mesentery. **(a–c)** A midline incision is performed in the skin and the subjacent muscle layer to access the mesentery. **(d–f)** A collagen-embedded human intestinal organoid is placed and glued adjacent to the mesenteric vasculature (*black arrows*). **(g–h)** Alternatively, a string-purse suture can be performed to fold the organoid within the mesentery (*black arrows*). **(i)** The mesentery is returned to the abdominal cavity, and both abdominal and skin layers are sutured. **(j)** Photograph of a human intestinal organoid after 10 weeks posttransplantation. **(k)** Hematoxylin and eosin staining of the transplant showing multilobular cavities. Morphology of the transplant showing crypt and villus structures of the epithelium along with a laminated submucosal layer

Fig. 5. Side-to-side anastomosis of the human intestinal organoid with the murine small intestine. (a–b) A 6–8 weeks transplanted human intestinal organoid is identified and irrigated to remove debris. A 5–6 mm incision on the antimesenteric side of the small intestine is performed to provide a bypass of around 5–6 mm. (c) Initial sutures are placed on the posterior sides of the HIO and small intestine with interrupted 9–0 nylon over-and-over suture under a stereoscope microscope. The first stitch should be place at 6 o'clock (middle of the posterior wall) and left long to help exposure for future posterior sutures. (d) Retracting on the middle suture, the posterior wall is exposed, and additional sutures are placed to from a nice mucosa to mucosa anastomosis. (e–f) The continuity of the intestine is restored by applying interrupted stitches on the anterior sides. (g–h) Hematoxylin and eosin and PAS stainings demonstrate the continuity between the murine small intestine (*black asterisk*) and the transplant (*black dotted line*). (i) Human nuclear staining (HuNUC, red) shows the human origin of the transplant. The cadherin 1 (CDH1) staining demonstrates the continuity between the murine and human epithelia

Table 1

List of surgical instruments used for the different surgical procedures

Procedures	Instruments	Functions
All surgeries	Suture-tying forceps	Suture tying, HIO, and tissue handling
All surgeries	Ring forceps	Handling of the organs
All surgeries	Dissecting scissors	Muscle and skin incisions
All surgeries	Bishop-Harmon forceps	Muscle and skin handling
All surgeries	Halsey needle holder	Muscle and skin suturing
All surgeries	Sterilization tray	Gas sterilization for surgical instruments
Anastomosis	Microneedle holder	HIO and small intestine suturing
Anastomosis	Round handled suture-tying forceps	HIO and small intestine suturing
Subcapsular transplantation, anastomosis	Vannas spring scissors	Subcapsular pocket creation, HIO, and small intestine incisions