Tissue Engineering of the Intestine in a Murine Model

Erik R. Barthel (/author/Erik+R._Barthel), Allison L. Speer (/author/Allison+L._Speer), Daniel E. Levin (/author/Daniel+E._Levin), Frédéric G. Sala (/author/Fr%E9d%E9ric+G._Sala), Xiaogang Hou (/author/Xiaogang_Hou), Yasuhiro Torashima (/author/Yasuhiro_Torashima), Clarence M. Wigfall (/author/Clarence+M._Wigfall), Tracy C. Grikscheit (/author/Tracy+C._Grikscheit)

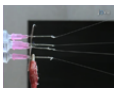
Children's Hospital Los Angeles, Division of Pediatric Surgery, Saban Research Institute, **Keck School of Medicine of the University of Southern California** (/institutions/NA-north-america/US-united-states/CA-california/16311-university-of-southern-california)

Video Article Chapters

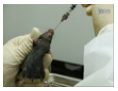
1

Author-Produced Video

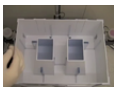
Related Videos



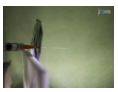
Postproduction Processing of Electrospun Fibres...
Published 8/09/2012



Chronic *Salmonella* Infected Mouse Model
Published 5/31/2010



T-maze Forced Alternation and Left-right...
Published 2/26/2012



Electrospinning Fundamentals: Optimizing Solution ...
Published 1/21/2011

Cite this Article

Barthel, E. R., Speer, A. L., Levin, D. E., Sala, F. G., Hou, X., Torashima, Y., *et al.* Tissue Engineering of the Intestine in a Murine Model. *J. Vis. Exp.* (70), e4279, doi:10.3791/4279 (2012).

Abstract

Tissue-engineered small intestine (TESI) has successfully been used to rescue Lewis rats after massive small bowel resection, resulting in return to preoperative weights within 40 days.[1] In humans, massive small bowel resection can result in short bowel syndrome, a functional malabsorptive state that confers significant morbidity, mortality, and healthcare costs including parenteral nutrition dependence, liver failure and cirrhosis, and the need for multivisceral organ transplantation.[2] In this paper, we describe and document our protocol for creating tissue-engineered intestine in a mouse model with a multicellular organoid units-on-scaffold approach. Organoid units are multicellular aggregates derived from the intestine that contain both mucosal and mesenchymal elements,[3] the relationship between which preserves the intestinal stem cell niche.[4] In ongoing and future research, the transition of our technique into the mouse will allow for investigation of the

Download

EndNote, RefWorks, Reference Manager, ProCite (/legacy/GetRIS.php?id=4279)

JoVE PDF Article (/pdf/4279)

Materials List (/pdf-materials/4279)

XML File (/pdf/default.aspx?ID=4279)

Information

Date Published	**12/01/2012**
JoVE Section	**Bioengineering (/bioengineering)**
JoVE	**70 (/archive/70)**

processes involved during TESI formation by utilizing the transgenic tools available in this species.[5]The availability of immunocompromised mouse strains will also permit us to apply the technique to human intestinal tissue and optimize the formation of human TESI as a mouse xenograft before its transition into humans. Our method employs good manufacturing practice (GMP) reagents and materials that have already been approved for use in human patients, and therefore offers a significant advantage over approaches that rely upon decellularized animal tissues. The ultimate goal of this method is its translation to humans as a regenerative medicine therapeutic strategy for short bowel syndrome.

Protocol

1. Organoid Units Preparation

1. Instruments appropriate for mouse dissection (scissors and forceps) should be sterilized by autoclave.
2. Humanely euthanize the donor mouse according to local IACUC protocols. Ensure that the animal is dead before proceeding.
3. Make a midline incision to gain access to the peritoneal cavity. Skin flaps can be reflected as needed to improve exposure.
4. Eviscerate the small bowel and divide it just distal to the ligament of Treitz. Separate the small bowel from its mesentery using sharp and gentle blunt dissection. Identify the ileocecal junction and divide the small bowel 5 mm proximal to this.
5. Using scissors, open the intestine lengthwise along the antimesenteric border in a Petri dish with 10 ml 4 °C, sterile Hanks' buffered saline (HBSS, Invitrogen, Carlsbad CA) / 1X antibiotic-antifungal (Anti-Anti, Invitrogen) solution. Clear fecal matter from the opened intestine with gentle agitation then transfer opened intestine to a 15ml centrifuge tube with 10 ml of 4 °C, sterile HBSS / 1X anti-anti.
6. Wash the opened intestine three times in 10 ml of 4 ° C, sterile HBSS / 1X anti-anti in a test tube. Each wash can be performed with mild shaking of the 15 ml tube for 30 sec. After shaking, the intestinal tissue sinks to the bottom of the tube. Discard floating material, which is mesenchymal debris. Remove the washing solution carefully with a pipet.
7. Mince the washed intestine in a Petri dish with 10 ml of 4 °C, sterile HBSS / 1X anti-anti to less than 1 mm square pieces using a scissors. Gather the minced material up with an automatic pipet and place it into a test tube.
8. Centrifuge the tube at 500 rpm for 8 min. Discard the supernatant, which contains fat and mesenchyme.
9. Digest the minced, washed material with 10 ml of sterile HBSS / 1X anti-anti plus 0.125 mg/ml dispase (Invitrogen) and 800 units/ml collagenase type 1 (Worthington, Lakewood NJ). To prepare 40 ml of the digestion solution, weigh out 5 mg of dispase, 142 mg of collagenase, and add sterile HBSS up to a volume of 40 ml. Prepare this solution freshly each time organoid units are prepared, and keep at 4° C until ready to use. Add the digestion solution directly to the pellet from step 1.8.
10. Incubate the test tube containing the minced, washed material with the digestion solution at 37 °C for 20 min.
11. Retrieve the test tube and further disrupt the digested tissue by trituration with a 10 ml pipet. Repeat between 20 to 50 times until a uniform appearance is obtained.
12. Centrifuge the test tube for 5 min at 800 rpm. Discard the supernatant, which contains single cells.
13. Stop the digestion reaction with 10 ml of 4 °C, sterile Dulbecco's Modified Eagle Medium (DMEM, Invitrogen) plus 10% v/v heat-inactivated fetal bovine serum (HI-FBS, Invitrogen). Resuspend the pellet and shake the tube.

Issue Comments	**0**
View Count	**1,338** (**Details...**)
DOI	**doi:10.3791/4279 (http://dx.doi.org/10.379**

Keywords

Bioengineering (/keyword/bioengineering), Issue 70 (/archive/70), Tissue Engineering (/keyword/tissue+engineering), Biomedical Engineering (/keyword/biomedical+engineering), Medicine (/keyword/medicine), Anatomy (/keyword/anatomy), Physiology (/keyword/physiology), small intestine (/keyword/small+intestine), pediatric surgery (/keyword/pediatric+surgery), short bowel syndrome (/keyword/short+bowel+syndrome), animal model (/keyword/animal+model), mouse (/keyword/mouse)

Navigate this Article

14. Centrifuge the test tube for 5 min at 800 rpm. Remove the supernatant carefully with an automatic pipet until the last few drops. Use a disposable plastic pipet for the last few drops to avoid resuspending the pellet.

2. Loading of Polyglycolic Acid Scaffold

1. Form 2-mm long, 5-mm outer diameter cylindrical scaffolds from nonwoven polyglycolic acid (2-mm sheet thickness, 60 mg cm-3 bulk density; porosity > 95%, Concordia Fibers, Coventry RI) as described in Ref. 4.
2. Trim the distal 2 mm of a disposable 1,000 microliter pipet tip with scissors prepared with 70% ethanol in distilled water.
3. Place the scaffold into a 4-well culture plate. Load the organoid units onto the scaffold with the 1,000 microliter pipet, first into the lumen and then onto the outer surface. Use a forceps to ensure coating of the lumen. Do not disrupt or break open the cylindrical shape of the polymer.

3. Implantation into Host Mouse

1. Use a syngeneic host mouse on the same background as the donor if available. Otherwise, employ an immunocompromised nonobese diabetic / severe combined immunodeficient or NOD / SCID animal (Jackson Laboratories, Sacramento CA).
2. Induce general anesthesia with isoflurane. Shave, prep and drape the mouse's abdomen.
3. Make a 5 mm midline incision to gain entrance to the peritoneal cavity. Identify and carefully eviscerate the greater omentum. Place the loaded polymer onto the omentum and wrap it with the tissue. Do not tear the omentum.
4. Secure the polymer to the omentum with a 5-0 monocryl suture. Gently replace the omentum with the wrapped polymer into its anatomic position.
5. Close the abdominal incision in layers using 4-0 vicryl sutures. Run the muscle closure and take care not to injure the abdominal viscera below the incision. Use interrupted sutures for the skin.
6. Administer postoperative analgesia with 2 mg/kg ketoprofen (Ketofen, Fort Dodge Animal Health) in sterile water as a subcutaneous wheal adjacent to the incision. Animals should be evaluated daily and if the animal is demonstrating signs of pain or distress, an additional dose of ketoprofen may be administered on post operative day 2. By the third postoperative day, the animal should be fully recovered without evidence of pain or distress. If pain or distress continues on post operative day 3, this is considered abnormal and should be addressed in accordance with the IACUC and animal care facility protocols.
7. Allow the mouse to recuperate and the tissue-engineered intestine to grow for four weeks. Give the animal *ad libitum* access to rodent chow (Lab Diet 5001, PMI Nutrition, St. Louis MO) and water with Septra 200 mg / 40 mg per 5 ml (Hi-Tech Pharmacal, Amityville NY) at 1:100 dilution.

4. Harvest

1. Humanely euthanize the host animal four weeks after implantation.
2. Reopen the original incision and reflect the skin cephalad to facilitate access to the peritoneal cavity.
3. Open the muscle layer and identify the tissue-engineered construct as a globe of tissue.
4. Take down adhesions to the construct from intraabdominal viscera using sharp dissection.
5. Fix the construct in formalin for later paraffin mounting, or use the tissue fresh for biochemical assays

such as real-time PCR or protein isolation.

Representative Results

Figure 1 shows an overall schema for the protocol documented here. The end result of this protocol is a globe or spherical structure of tissue-engineered murine intestine with a lumen, mucosa, submucosa, and surrounding muscularis. **Figure 2A** shows a typical globe in comparison to a starting polymer scaffold. **Figure 2B** displays the same construct sharply bivalved to reveal its lumen. **Figure 3** demonstrates a hematoxylin/eosin-stained paraffin-mounted cross section of a typical successful construct after 4 weeks of incubation. In Ref. 4, we were able to produce tissue-engineered intestine successfully 89% of the time (39 out of 44 implants).

An unsuccessful construct is one in which no mucosa forms. It is impossible to judge based upon gross appearance at harvest whether the globe will have mucosa on final histologic analysis. Therefore if biochemical assays are performed on the fresh construct tissue, half of each globe should be fixed and paraffin mounted for histologic analysis to confirm that mucosa is present in each sample. An unsuccessful construct will demonstrate only stroma and fibrosis on hematoxylin/eosin staining.

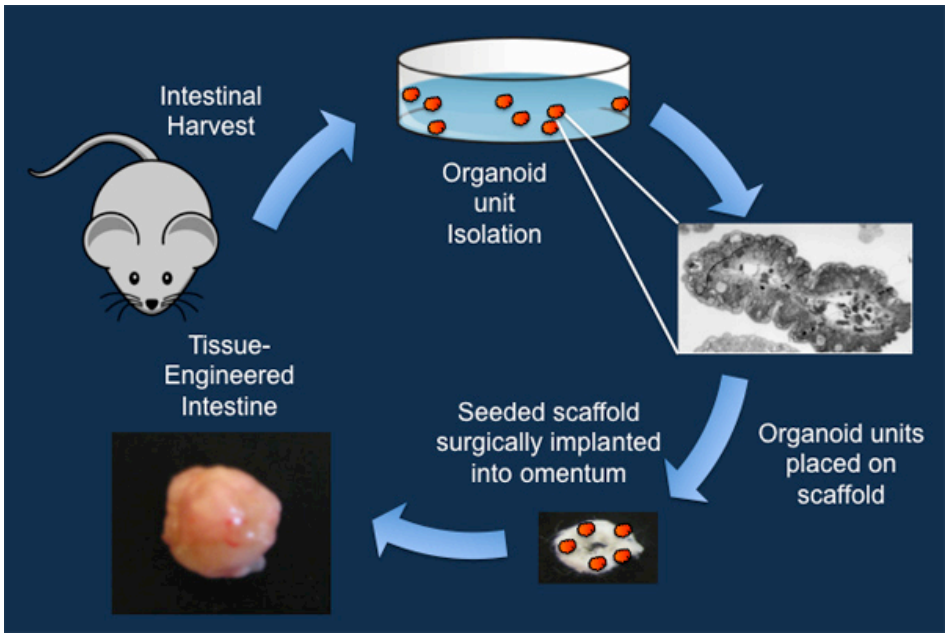


Figure 1. Schema for the production of tissue-engineered intestine in the mouse. In brief, donor tissue is harvested and processed into organoid units. The organoid units are loaded onto a porous polyglycolic acid scaffold, which is then implanted into a host and allowed to incubate for 4 weeks. The engineered construct is then retrieved and can be characterized via histology or biochemical assays.

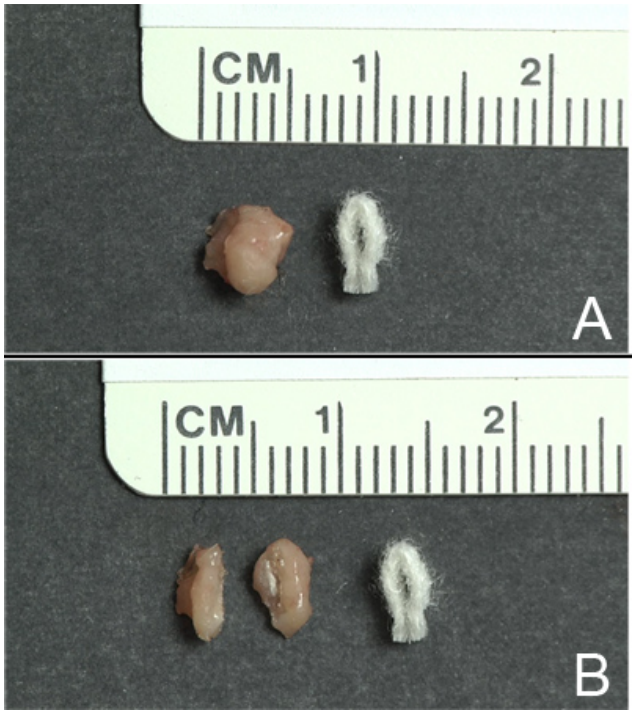


Figure 2. Example of tissue-engineered intestine construct harvested at 4 weeks. A: Construct in comparison to starting polymer scaffold. B: The same construct, bivalved to reveal its lumen.

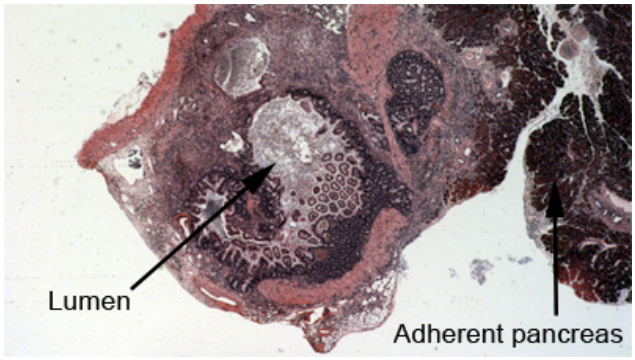


Figure 3. Low-power hematoxylin/eosin micrograph of a typical successful tissue-engineered intestine construct. The labels and arrows indicate the lumen with intestinal mucosa, and adherent host pancreas.

Discussion

We present a protocol for producing tissue-engineered intestine in the mouse using an organoid units-on-scaffold approach. The most critical steps are those of the organoid units preparation. Care must be taken to adequately clean and mechanically process the tissue, but equal care must be taken not to overdigest or overtriturate the organoid units after the digestion is performed (step 1.11). If this is done, the organoid units can be reduced to single cells, which can be lost in the supernatant of step 1.12, and are unlikely to survive to produce tissue-engineered intestine, as the intestinal stem cell niche would not be preserved in this case.

The small size of the mouse makes it challenging to directly anastomose the tissue-engineered construct to the host animal's intestine to test its function *in vivo*. Alternatively, the rat represents a larger model for TESI growth that facilitates *in vivo* anastomosis and has already been performed for this reason[1]. Nevertheless, the size of the mouse is not an insuperable obstacle, as others have been able to perform small bowel resection and anastomosis,[6] or anorectal surgery,[7] in these animals. To address these issues, experiments to characterize the function of our tissue-engineered intestinal constructs in an *in vitro* fashion are ongoing.

Additional limitations to this technique include an 89% success rate in the generation of TESI[4]. That is, 89% of OU loaded scaffolds will successfully generate TESI. The application of this technique by others may help refine and improve this technique increasing the overall yield to 100%. In addition, this technique could be improved if the total tissue mass generated could be increased. Currently, flow cytometry has demonstrated that the total number of cells present in the harvested TESI construct is 3-fold greater than the number present at implantation ($3.07 \times 10^6 \pm 0.5 \times 10^6$)[4]. Improving the volume of TESI production is an important step toward translation to therapy.

We note also that the mouse intestine, being very thin-walled and of small caliber, is particularly easy to process in comparison to that of other species such as swine or the rat. In humans, we expect that some of the details of the organoid units preparation steps would have to be modified to both adequately digest the tissue and avoid overdigestion to single cells, in particular the concentrations of dispase and collagenase in the digestion solution and the time of digestion at 37 °C.

The ultimate goal of this technique is a transition to human therapy. Tissue engineering of human intestine from the patient's own tissue could potentially offer a durable, long-term cure for short bowel syndrome (SBS) with none of the drawbacks of existing therapies. Short bowel syndrome is a morbid condition caused by resection of a significant fraction of the total length of the small bowel, usually greater than 50-75 percent, such that its absorptive capacity is severely reduced and the patient cannot obtain sufficient nourishment from enteral nutrition.[2] In children, the most common causes of SBS are massive small bowel resection secondary to necrotizing enterocolitis or malrotation with midgut volvulus.[8,9] Also, though less common, SBS can occur in adults because of multiple resections in the setting of Crohn's disease, or with mesenteric ischemia secondary to vascular disease.[10,11] Because SBS patients cannot maintain sufficient nutrition with enteral intake, they may require long-term total parenteral nutrition, which itself can be complicated in children by liver failure and cirrhosis.[12] SBS patients therefore endure significant healthcare costs, recently estimated to be on the order of $1.6 million per patient over 5 years.[13] The current standard of care for intestinal failure secondary to SBS is intestinal, liver/intestinal, or other multivisceral transplantation, but this confers only a 60% 5-year survival and consigns the patient to a lifelong course of immunosuppressive therapy.[14] Further, limited donor organ availability results in an inevitable mismatch in demand and supply and long wait times.[15] Therefore, tissue engineering from the patient's autologous tissue would be an attractive alternative.

Disclosures

No conflicts of interest declared.

Acknowledgements

Tracy C. Grikscheit, Erik R. Barthel, and Frédéric G. Sala are supported by the California Institute for Regenerative Medicine (CIRM), grant numbers RN2-00946-1 (TCG) and TG2-01168 (ERB, FGS). Allison L. Speer is a Society of University Surgeons Ethicon scholar. Yashuhiro Torashima is funded by a Children's Hospital Los Angeles Saban Institute Research Career Development Fellowship.

Materials

Name	Company	Catalog Number	Comments

HBSS	Gibco	114170-112	
Antibiotic-Antimycotic 100X	Invitrogen	15240-062	
Dispase	Gibco	17105-041	
Collagenase Type 1	Worthington	LS004194	
DMEM High Glucose 1X	Gibco	11995-065	
Heat inactivated FBS	Invitrogen	16140-071	
Biofelt 100% PGA	Concordia Medical	FELT01-1005	For polymer preparation as in Ref. 4
Poly-*L*-lactic acid	Durect	B6002-1	For polymer preparation as in Ref. 4
Type I Collagen, rat tail	Sigma-Aldrich	C3867-1VL	For polymer preparation as in Ref. 4
Ketoprofen 100 mg/ml	Fort Dodge Animal Health	71-KETOI-100-50	
LabDiet 5001 rodent chow	LabDiet	5001	
Septra 200 mg / 40 mg per 5 ml, USP	Hi-Tech Pharmacal	50383-824-16	
Isoflurane, USP	Phoenix Pharmaceuticals	57319-507-06	

References

1. Grikscheit, T.C., Siddique, A., Ochoa, E.R., *et al.* Tissue-engineered small intestine improves recovery after massive small bowel resection. *Ann. Surg.* **240**, 748-754 (2004).
2. Wales, P.W. & Christison-Lagay, E.R. Short bowel syndrome: epidemiology and etiology. *Sem. Ped. Surg.* **19**, 3-9 (2010).
3. Evans, G.S., Flint, N., Somers, A.S., *et al.* The development of a method for the preparation of rat intestinal epithelial cell primary cultures. *J. Cell Sci.* **101**, 219-231 (1992).
4. Sala, F.G., Matthews, J.A., Speer, A.L., *et al.* A multicellular approach forms a significant amount of tissue-engineered small intestine in the mouse. *Tiss. Eng. Part A.* **17**, 1841-1850 (2011).
5. Speer, A.L., Sala, F.G., Matthews, J.A., & Grikscheit, T.C. Murine tissue-engineered stomach demonstrates epithelial differentiation. *J. Surg. Res.* **171**, 6-14 (2011).
6. Haxhija, E.Q., Yang, H., Spencer, A.U., *et al.* Intestinal epithelial cell proliferation is dependent on the site of massive small bowel resection. *Pediatr. Surg. Int.* **23**, 379-390 (2007).
7. Zhao, L., Cheng, Z., Dhall, D., *et al.* A novel corrective pullthrough surgery in a mouse model of Hirschsprung's disease. *J. Pediatr. Surg.* **44**, 759-766 (2009).
8. Petrosyan, M., Guner, Y.S., Williams, M., *et al.* Current concepts regarding the pathogenesis of necrotizing enterocolitis. *Ped. Surg. Int.* **25**, 309-318 (2009).
9. Shew, S.B. Surgical concerns in malrotation and midgut volvulus. *Ped. Radiol.* **39**, S167-S171 (2009).
10. Sampietro, G.M., Corsi, F., Maconi, G., *et al.* Prospective study of long-term results and prognostic factors after conservative surgery for small bowel Crohn's disease. *Clin. Gastroenterol. Hepatol*. **7**, 183-

191 (2009).

11. Klempnauer, J., Grothues, F., Bektas, H., & Pichlmayr, R. Long-term results after surgery for acute mesenteric ischemia. *Surgery.* **121**, 239-243 (1997).
12. Fitzgibbons, S.C., Jones, B.A., Hull, M.A., *et al.* Relationship between biopsy-proven parenteral nutrition-associated liver fibrosis and biochemical cholestasis in children with short bowel syndrome. *J. Ped. Surg.* **45**, 95-99 (2010).
13. Spencer, A.U., Kovacevich, D., McKinney-Barnett, M., *et al.* Pediatric short bowel syndrome: the cost of comprehensive care. *Am. J. Clin. Nutr.* **88**, 1552-1559 (2008).
14. Kato, T., Tzakis, A.G., Selvaggi, G., *et al.* Intestinal and multivisceral transplantation in children. *Ann. Surg.* **243**, 756-766 (2006).
15. Reyes, J., Bueno, J., Kocoshis, S., *et al.* Current status of intestinal transplantation in children. *J. Ped. Surg.* **33**, 243-254 (1998).

Ask the Author

0 Comments

Post a Question / Comment / Request

Your comment (in English, please):

Allowed HTML tags: i, b, u, sup, sub

You are signed in as jzacka@worthington-biochem.com

Post Comment

About JoVE

The Journal of Visualized Experiments (JoVE) is a PubMed-indexed video journal. Our mission is to increase the productivity of scientific research.

Follow JoVE

blog (http://blog.jove.com) rss (/rss)

facebook (http://www.facebook.com/JoVEjournal)