

Video Article

Intestinal Stem Cell Isolation and Culture in a Porcine Model of Segmental Small Intestinal Ischemia

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Abstract

Intestinal ischemia remains a major cause of morbidity and mortality in human and veterinary patients. Many disease processes result in intestinal ischemia, when the blood supply and therefore oxygen is decreased to the intestine. This leads to intestinal barrier loss and damage to the underlying tissue. Intestinal stem cells reside at the base of the crypts of Lieberkühn and are responsible for intestinal renewal during homeostasis and following injury. *Ex vivo* cell culture techniques have allowed for the successful study of epithelial stem cell interactions by establishing culture conditions that support the growth of three-dimensional epithelial organ-like systems (termed "enteroids" and "colonoids" from the small and large intestine, respectively). These enteroids are composed of crypt and villus-like domains and mature to contain all of the cell types found within the epithelium. Historically, murine models have been utilized to study intestinal injury. However, a porcine model offers several advantages including similarity of size as well as gastrointestinal anatomy and physiology to that of humans. By utilizing a porcine model, we establish a protocol in which segmental loops of intestinal ischemia can be created within a single animal, enabling the study of differing time points of ischemic injury and repair *in vivo*. Additionally, we describe a method to isolate and culture the intestinal stem cells from the ischemic loops of intestine, allowing for the continued study of epithelial repair, modulated by stem cells, *ex vivo*.

Video Link

The video component of this article can be found at <https://www.jove.com/video/57647/>

Introduction

Intestinal ischemic injury, the result of decreased oxygen availability due to a reduction or complete occlusion of blood flow to the intestine, remains a significant cause of morbidity and mortality in human and animal patients^{1,2}. Ischemic damage, along with subsequent inflammation and cellular infiltration, leads to mucosal barrier compromise. The mucosal barrier is critical to the prevention of bacterial translocation and associated toxins into the systemic circulation^{3,4}. The subsequent reperfusion of ischemic tissues can result in formation of damaging reactive oxygen species that can exacerbate injury⁵. Since intestinal ischemia is rarely preventable, most current research has focused on advancing techniques for early detection of ischemia and the development of novel therapeutic approaches that reduce reperfusion injury or target mucosal repair.

Animal models have been extensively used to expand our basic science knowledge of ischemia-reperfusion injury and remain imperative for translational research. Rodent models have been the most widely used due to their ability to be genetically manipulated⁶. More recently however, the use of large animal models, specifically the pig, has been advocated for future translational studies due to a number of advantages including the pigs anatomic and physiologic similarities to humans^{7,8}. A variety of injury models have been developed to study ischemia-reperfusion injury and include complete vascular occlusion, low-flow ischemia and segmental mesenteric vascular occlusion. A full review of these models is outside the realm of this article however the authors direct readers to a recent review³.

In addition to *in vivo* models, the use of *ex vivo* cellular culture systems offers a promising tool to study intestinal homeostasis and repair following injury. Intestinal stem cells are responsible for cellular proliferation and turnover of the intestinal epithelial lining. When isolated from normal or injured intestine, intestinal stem cells can be maintained in culture, and serve as a tool or model to study stem cell and epithelial cell biology. Methods to isolate and establish these three-dimensional culture systems (termed enteroids and colonoids when derived from the small and large intestine, respectively) have been described for a variety of species and organ systems^{9,10,11,12,13}. Specifically, within the gastrointestinal tract, these culture systems have been used to model gastrointestinal disease including cancer, pathogen infection and inflammatory bowel disease¹⁴. At this time, there are no reports describing the isolation and maintenance of intestinal stem cells from ischemically injured small intestine in any species. Therefore, here we describe the process of intestinal ischemia in a novel, large animal porcine model which results in reproducible injury and the ability to isolate intestinal stem cells from normal and ischemically injured intestine for the additional study of recovery *ex vivo*.

Protocol

For these experiments, all animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) of North Carolina State University.

1. Preparation for Culture

NOTE: All reagents are listed in the **Table of Materials**. Specific growth factor concentrations are listed in **Table 1**.

1. Prepare a 0.5 M ethylenediaminetetraacetic acid (EDTA) solution in distilled deionized water (ddH₂O). Mix adequately and adjust the pH to 7.4 using NaOH and/or HCl solutions.
NOTE: Make up EDTA stock solution fresh before each experiment.
2. Prepare dissociation reagent #1 (DR #1) as follows and place on ice: Combine phosphate buffered saline without Ca²⁺ and Mg²⁺ (PBS), 0.5 M EDTA, 1M 1,4-Dithiothreitol (DTT), 10 μM Y27632 and 1X Antibiotic-Antimycotic solution (Anti-Anti) containing penicillin, streptomycin and amphotericin B.
NOTE: Add Y27632 immediately prior to tissue collection.
3. Prepare dissociation reagent #2 (DR #2) as follows and place in a 37 °C water bath: Combine PBS without Ca²⁺ and Mg²⁺, 0.5 M EDTA, 10 μM Y27632 and 1X Anti-Anti. NOTE: Add Y27632 immediately prior to tissue collection.
4. Prepare intestinal epithelial stem cell (IESC) media as follows: Mix 25 mL advanced DMEM/F12 medium with 1X N2 supplement, 1X B-27 supplement, 10mM HEPES, 2mM Glutamax and 1X Anti-Anti. Store at 4 °C until use.
5. Prepare a master mix of reduced growth factor basement membrane matrix (matrix) and supplemental growth factors as follows: Thaw the matrix on ice. In a microcentrifuge tube, add 100 ng/mL recombinant human Noggin, 500 ng/mL recombinant human R-Spondin 1, 50 ng/mL recombinant human epidermal growth factor (EGF), 100 ng/mL recombinant human Wnt3a, 10 mM Nicotinamide, 10 nM gastrin, 500 nM A-83-01, 10 μM Y-27632, 10 mM SB202190, 500 nM LY2157299 and 2.5 μM glycogen synthase kinase 3 inhibitor (GSK3i, CHIR99021). When the matrix is thawed, supplement with the growth factor mix (making master mix) and store on ice.
NOTE: Be careful to avoid introducing air bubbles as this will create artifact within the matrix patty during plating.
6. Pre-warm a 24-well tissue culture dish in a 37°C incubator prior to starting cell isolation procedures.

2. Surgical Model of Ischemia and Tissue Collection

1. Use eight to ten-week-old Yorkshire crossbred pigs of either sex (recommended). Remove all feed 16-18 h prior to surgery. Allow pigs access to water at all times.
2. Schedule the pigs to arrive at least 48 h before the experiments to allow for environmental acclimation.
NOTE: Trained veterinary and/or laboratory technicians supervised by a licensed veterinarian provide routine animal care and assistance with anesthetic procedures.
3. Premedicate the pig with xylazine (1.5 mg/kg intramuscularly (IM)) and ketamine (11-20 mg/kg (IM))¹⁵. Once sedated, intubate the pig orotracheally.
4. Maintain pigs under general anesthesia with isoflurane (2-5%) vaporized in 100% O₂ until the time of euthanasia.
5. Place an intravenous (IV) catheter (ear vein recommended) and administer a replacement fluid such as lactated ringers solution at a maintenance rate of 15 mL/kg/h.
6. Perform routine anesthetic monitoring including pulse oximetry, electrocardiography and indirect blood pressure measurements¹⁵. Monitor the pig's respiratory rate and effort and ventilate manually or mechanically as needed if end tidal CO₂ rises above 55-60 mmHg.
NOTE: Appropriate anesthesia is confirmed by continual monitoring of trends in heart rate (range 80-130 beats/min), non-invasive blood pressure (mean arterial pressure 75-100 mmHg), and respiratory rate (10-25 breaths/min)¹⁶, and periodic checking for absence of jaw and anal tone. Depth of anesthesia can also be judged by degree of muscle relaxation and muscle fasciculation following surgical stimulus. Ocular reflexes are usually of no value¹⁶. Analgesia can be provided as directed by the institutions IACUC policy. In this case, an opioid, such as buprenorphine, can be administered during surgery.
7. Place the pig on a heating pad and restrain in dorsal recumbency for the surgical procedure.
8. Shave the ventral abdomen and prep using surgical scrub (chlorhexidine solution) and isopropyl alcohol.
9. Make an 8-10-cm ventral midline incision using a scalpel blade centered at the umbilicus to access the abdomen.
10. Identify the small intestine (jejunum) approximately 40 cm oral to the ileocecal junction. Delineate ten-cm long loops of jejunum by circumferentially ligating the bowel twice, one cm between each ligature, prior to creating subsequent loops 10 cm located orally (**Figure 1**).
11. Create two loops per time point of ischemia adjacent to each other, one for ischemia and one for ischemia with an additional 1h of reperfusion if desired. To create complete ischemia (no flow of either arteries or veins), clamp or ligate the mesenteric vasculature using bulldog vascular clamps, curved Halstead mosquito hemostats, or 2-0 non-absorbable silk for 1, 2, 3, and 4h and then remove clamps for 1h of reperfusion, if desired.
NOTE: The authors created all loops of ischemia in order starting with the 4h ischemic loop and progressed moving proximally (to minimize total surgery time). To address the possibility that neighboring ischemic intestinal segments may damage adjacent loops, the order of the ischemic loops can be varied. This may alter total surgical time.
NOTE: The time frame of reperfusion can be varied based on the research question of interest or if therapies wish to be tested during the reperfusion period. However, as tissue damage becomes more severe from increasing durations of ischemia alone, reperfusion injury likely does not contribute to further epithelial injury. Reperfusion does likely play a role following mild periods of ischemic injury.
12. In between ischemia time points, keep the abdomen covered or closed using a sterile towel and/or a towel clamp.
NOTE: Within a single animal for this experiment, eight ischemic segments can be created. Identify an additional jejunal segment, at least 5-10 cm proximal to the last ischemic loop, that will serve as an internal normal control.
13. Obstruct approximately three mesenteric vessels per bulldog vascular clamp or curved Halstead mosquito hemostat. Care must be taken during hemostat application because the vessels are easily traumatized. Try to stack the vessels within the jaws of the clamps.

14. At the end of the experiment, following euthanasia with pentobarbital 85-100 mg/kg IV, collect all loops of tissue using metzenbaum scissors, after death has been confirmed with no heartbeat auscultated and loss of the corneal reflex. Start by collecting a control piece of normal jejunum at least 5- 10 cm proximal to the last ischemic loop.
15. Separate and store the loops from each injury time point in small containers of ice cold PBS until ready for crypt isolation.
NOTE: If desired, make ischemic loops long enough to divide into separate samples for histology and intestinal stem cell culture; however, the authors have found that loops greater than approximately 10 cm long are most likely to become hemorrhagic.

3. Crypt (Stem Cell) Isolation from Ischemic and Control Loops

1. Invert each loop of small intestine using a 20-gauge wire and tissue forceps to expose the mucosal surface. Tie the top and bottom of the loop securely to the wire using suture (2-0 non-absorbable silk or equivalent).
2. Following inversion, rinse each loop in ice cold PBS to remove luminal debris.
3. Place samples immediately into 50 mL conical tubes containing DR #1 on ice for 30 min.
4. Collect loops beginning with control and follow with loops of incrementally increasing durations of ischemia. Loops from less damaged tissue (i.e. control and 1 h ischemic tissue) can be shaken forcefully, snapping the wrist for best results. Tissues from severely damaged tissue (3 and 4 h of ischemia) should be gently rocked or inverted only as too much shaking will cause crypt disruption and additional tissue destruction. Tubes should be shaken or inverted every 5 min while on ice.
5. Transfer samples into DR #2 for 10 min in a 37°C water bath. Shake or invert tubes every 5 min.
6. After transferring the tissues, centrifuge the conical tubes containing DR #1 from the 2-4 h damaged loops to remove the EDTA supernatant (200 x g for 5 min). As these tissues are highly damaged it is possible the crypts have already become dissociated. Remove supernatant and resuspend pellet with a small volume of PBS (5 mL) and proceed to step 3.9.
7. Remove samples from DR #2 and place tissue samples directly into 25 mL of cold PBS on ice (label as Wash #1). Place the samples on an orbital shaker on ice at 60 rpm. Continue to shake or invert each tube for 30 s every 2 to 5 min.
8. After transferring the tissues, spin the conical tubes containing DR #2 from the 2-4 h damaged loops to remove the EDTA supernatant (200 x g for 5 min). As these tissues are highly damaged it is possible that the crypts have become dissociated. Remove supernatant and resuspend pellet with a small volume of PBS (5 mL) and proceed to step 3.9.
9. Remove a 50 μ L aliquot from Wash #1 (or from the DR) to check for the degree of crypt dissociation and amount of debris. Place tissue into a new 50 mL conical tube (Wash #2, #3, etc.) filled with 25 mL of cold PBS and shake until intact crypts are isolated with minimal debris and villi.
NOTE: The goal is to be able to isolate a clean fraction with intact crypts and minimal debris. Each additional wash will clean the cells however too much shaking will begin to result in secondary tissue/cell damage. Furthermore, the best results with least contamination will be achieved if crypts are plated from a wash step and not from a dissociation reagent step.
10. Remove the remaining tissue and centrifuge the 50 mL conical tubes at 200 x g for 5 minutes to remove supernatant, then resuspend remaining the crypt pellet in smaller volume of PBS (5 mL).
11. Using an inverted microscope, examine 50 μ L aliquots of each sample to determine number of crypts/fraction. The goal is 50-100 crypts/50 μ L, as this will be the size of the final matrix patty.
NOTE: If the sample is too concentrated, continue to add cold PBS until desired concentration is reached. If sample is too dilute, determine number of aliquots needed to reach desired crypt yield and adjust.
12. Aliquot appropriate volume(s) of crypts (determined by aliquot observations) into a microcentrifuge tube. For example, if sample contains 50 crypts/50 μ L then aliquot 50 μ L per patty X 3 replicates = 150 μ L/ microcentrifuge tube. If the sample only contains 25 crypts/50 μ L then 2 aliquots needed/patty X 3 replicates= 300 μ L/tube.
13. Pellet crypts at 200 x g for 5 min at 4°C.
14. Gently resuspend pelleted crypts with appropriate volume of master mix (50 μ L per well). Mix thoroughly by rapidly pipetting 15 times without creating air bubbles.
NOTE: Pre-cool the pipette tip with cold sterile PBS prior to aspirating the master mix. This will help keep the master mix from spreading out. Tips can also be kept in the refrigerator and placed in the hood immediately prior to use.
15. Dispense a 50 μ L master mix plus crypt droplet into center of each well of a pre-warmed 24 well plate.
16. Incubate the culture plate for 30 min at 37 °C.
17. Overlay each matrix patty with 500 μ L/ well of IESC media (no additional growth factors needed on day 0 as they are contained within the master mix).
18. Add 500 μ L sterile PBS to any unused wells left on the plate to maintain humidity.
19. Count number of plated crypts on Day 0.
NOTE: It is helpful to pre-grid each cell culture plate into quadrants to help ensure more accurate counting.
20. Count number of enterospheres every 24 h and monitor for enteroid development daily.
21. Add growth factors to each well every 48 h. Remove the IESC media every 96 h and replace with 500 μ L fresh media (growth factors must then be added to media).

Representative Results

Complete intestinal ischemia was created in small intestinal loops by utilizing vascular occlusion with suture or clamps as shown in **Figure 1**. By releasing the clamps, a controlled period of reperfusion can be performed, allowing for additional study of subsequent reperfusion injury if desired. All animals survived during the procedure with minimal complication until euthanasia. The most common surgical complication was hypotension, which resolved with supplementation of a positive inotrope such as dobutamine.

If performed correctly, ischemic injury will begin at the tip of the intestinal villus and migrate down within the crypt as the duration of ischemia increases (**Figure 2**). One common mistake with the surgical technique can occur when the blood vessels are not ligated or clamped evenly. The result is a hemorrhagic ischemia (**Figure 3**), in which the thin-walled vein collapses before the artery, allowing for additional blood to infiltrate the tissues. This is seen grossly as a dark purple serosal surface (**Figure 3**, left) compared to a paler surface during complete ischemia (**Figure 3**, right).

Following removal of the ischemic intestinal loops, intestinal crypts were successfully isolated following the dissociation protocol (**Figure 4**). As expected, crypts from more severely damaged timepoints were often broken (f; fragment) and crypt fractions contained more background cellular debris when compared to those that underwent no or mild damage. During the protocol, the severely injured intestine must be shaken gently to avoid additional damage to the underlying tissue. Shaking too roughly can result in further crypt damage and the majority of the crypts ending up in the DR solutions containing EDTA, resulting in additional disruption.

Once plated, crypts from all time points of ischemia survive and are able to become established in culture (**Figure 5**). When normal and mildly damaged intestinal crypts are plated in culture, enterospheres form within 24-48 h. With severe ischemic damage (3 and 4 h), the intestinal crypts survive but are damaged, resulting in the formation of much smaller spheres initially. By 72-120 h, enteroids become more complex with obvious central lumens and budding structures. Overall, there is a decreased growth efficiency of crypts as well as a decreased size of enteroids derived from the severely damaged intestinal tissue (Gonzalez, L.M., Unpublished Data, 2017).

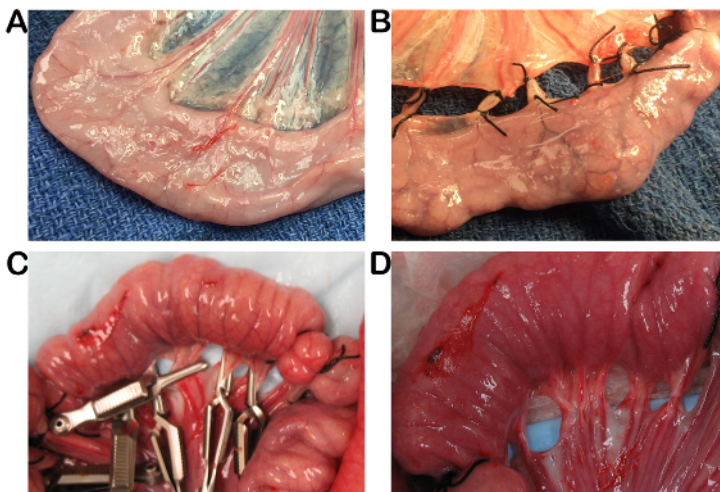


Figure 1: Surgical model of complete intestinal ischemia in a porcine model. A) Normal porcine jejunum exteriorized. B) Mesenteric vessels have been ligated with suture creating intestinal ischemia. C) Ischemia created using bulldog vascular clamps to allow for tissue reperfusion if desired. D) Intestinal vasculature immediately following removal of the vascular clamps. [Please click here to view a larger version of this figure.](#)

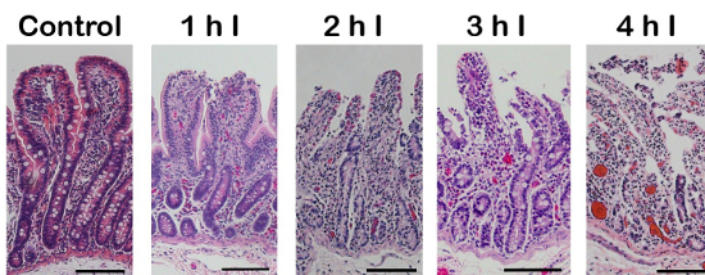


Figure 2: Histologic evidence (hematoxylin and eosin (H&E) stain) of increasing epithelial damage following longer durations of complete intestinal ischemia. Damage starts at the tip of the villus with gradual loss of the single cell epithelial layer, villus blunting and cellular damage extending down to the crypt base with severe injury (up to 4 h duration). 100 μ m scale bar. I = Ischemia. [Please click here to view a larger version of this figure.](#)

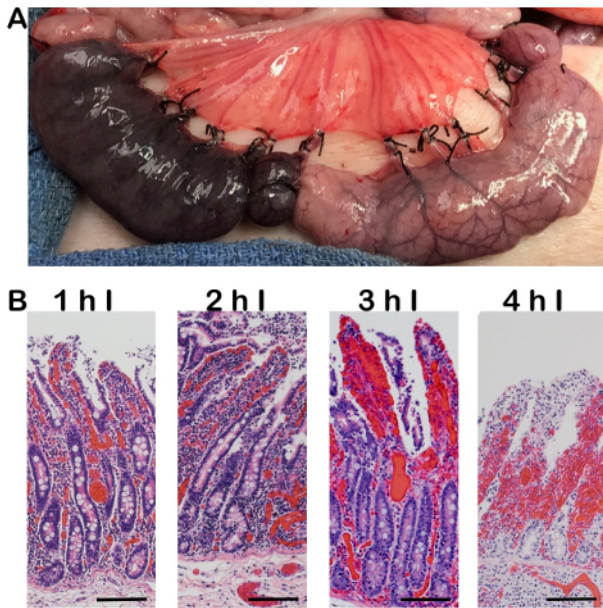


Figure 3: Gross and histologic evidence of hemorrhagic ischemia. A) Gross photograph comparing hemorrhagic ischemia (left loop) and complete ischemia (right loop). When the vasculature is not ligated or clamped evenly, blood can continue to infiltrate the tissues, resulting in additional inflammation and damage. B) H&E images of hemorrhagic ischemia of increasing duration from 1-4 h. In addition to cellular damage seen with complete ischemia, there is evidence of red blood cell infiltration within the surrounding lamina propria. 100 μ m scale bar. I = Ischemia. [Please click here to view a larger version of this figure.](#)

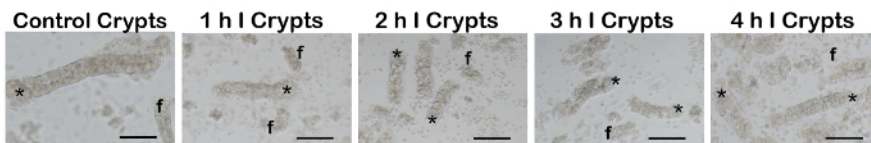


Figure 4: Aliquots of intestinal crypts isolated from loops of ischemic and normal intestine. Complete, intact intestinal crypts (asterisks) were successfully isolated from each loop of intestine. As expected, crypts from more severely damaged time points were often broken (f; fragment) and crypt fractions contained more background cellular debris when compared to those that underwent no or mild damage. 100 μ m scale bar. I = Ischemia. [Please click here to view a larger version of this figure.](#)

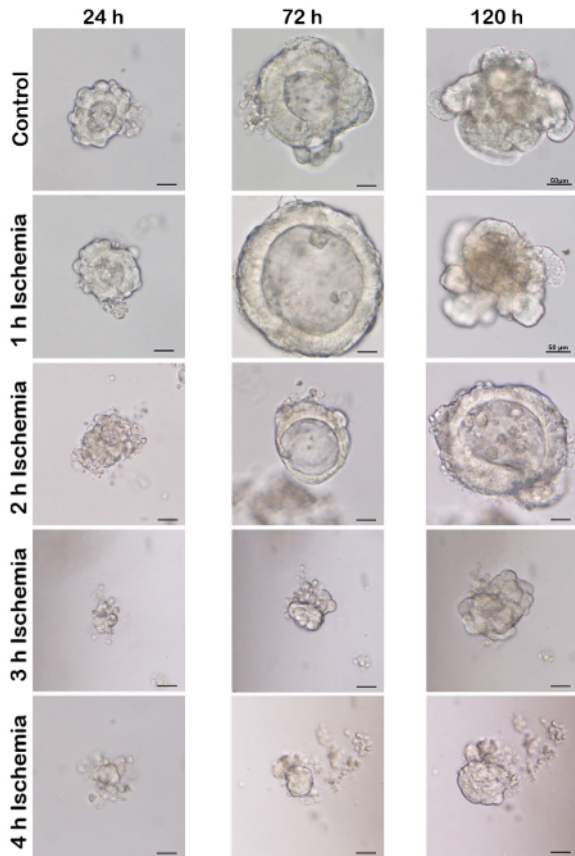


Figure 5: Time course of intestinal stem cell growth following isolation from ischemic loops of small intestine. When normal intestinal crypts were plated in culture, enterospheres formed within 24-48 h. With severe ischemic damage (3 and 4 h), crypts survive but form much smaller spheres initially. By 72-120 h, enteroids become more complex with obvious central lumens and budding structures. 20 μm scale bar unless noted. [Please click here to view a larger version of this figure.](#)

Growth Factor	Diluent	Stock Concentration	Stock Dilution	Working Dilution
R-Spondin	PBS	100X	100 $\mu\text{g/ml}$	1 $\mu\text{g/ml}$
Noggin	SW/0.1%BSA	1000X	100 $\mu\text{g/ml}$	100 ng/ml
EGF	10mM Acetic acid	10,000X	500 $\mu\text{g/ml}$	50 ng/ml
A-83-01	DMSO	1000X	500 μM	500 nM
SB202190	DMSO	3000X	30 mM	10 μM
Nicotinamide	SW	1000X	1 M	1 mM
Gastrin	PBS	10,000X	100 μM	10 nM
Y-27632	PBS	1000X	10 mM	10 μM
LY2157299	DMSO	10,000X	5 mM	0.5 μM
CHIR99021	PBS	1000X	2.5 mM	2.5 μM
Wnt3a	PBS	2000X	200 $\mu\text{g/ml}$	100 ng/ml

Table 1: Growth factor reagent table. Summary of growth factor stock solutions and working solutions used in this protocol.

Discussion

The development of a porcine model of segmental intestinal ischemia expands upon previous murine models by allowing for the study of multiple time points of tissue injury within the same animal. There are several critical discussion points of this protocol including proper vessel ligation, tissue reperfusion and successful crypt cell culture.

Proper vessel ligation is essential to the creation of a model of complete ischemia. If the suture is tied unevenly or the clamp not tightened completely, blood from the thick-walled artery may continue to enter the tissue and cannot exit due to the collapse of the thin-walled vein.

This results in extravasation of blood into the lamina propria causing additional tissue damage. However, depending on the type of ischemic injury being studied, complete or hemorrhagic ischemia may be desired. For example, in the process of intestinal transplantation, the bowel is completely separated from the vascular supply (artery and vein) during the resection phase of the procedure, which results in complete intestinal ischemia. Alternatively, however, when the mesentery is twisted during an event such as an intestinal volvulus, the venous return is often obstructed first, leading to additional blood within the tissue prior to the arterial supply being obstructed, thus creating hemorrhagic ischemia.

Ischemic injury results in tissue damage starting at the villus tip and extending down to the base of the crypt³. During ischemia, energy in the form of adenosine triphosphate continues to be used and generates the metabolite hypoxanthine. When the tissue is reperfused with oxygen, hypoxanthine becomes metabolized by xanthine oxidase and produces superoxide free radicals leading to mucosal injury and attraction of tissue damaging neutrophils^{17,18}. Species differences in mucosal vascular architecture as well as varying expression of xanthine oxidase, result in varying degrees of reperfusion injury³. Feline and rodent models of ischemia-reperfusion are more susceptible to reperfusion injury from reactive oxygen metabolites^{19,20}. In contrast, pigs were found to have less xanthine oxidase, and therefore less reperfusion injury, making this model more comparable to that of human intestinal ischemia²¹. At this time, the use of knockout or transgenic porcine models to study intestinal injury has not been described, making this a major limitation of this model. Selection of the proper animal model depends on the disease process or specific condition the researcher wishes to study. For example, porcine models of ischemic injury up to 6 h have been described²², whereas most ischemic procedures in murine models are 45-60 min²³.

Successful isolation of intestinal crypts from normal and ischemically damaged intestine allows for the study of epithelial recovery in culture. This system allows the researcher to focus uniquely on the epithelium alone, as there is not vascular supply or immune cell component to consider. This offers the opportunity to study epithelial cell interactions and recovery following injury in addition to the response to different growth factors, or treatments administered during surgery or following crypt isolation by supplementing the culture media. This step remains the most difficult, as isolation from the severely damaged loops requires gentle shaking and quick removal of the EDTA-containing solutions in case the crypts have become prematurely dissociated. If these loops of intestine are not washed thoroughly, the crypts have the potential to become contaminated in culture. As a result, antibiotic-antimycotic solution was added to both DR solutions in addition to the IESC media. Another discussion point focuses on the intestine collected as a normal control. As the animals undergo anesthesia with possible alterations in systemic tissue perfusion, along with the possibility of circulating inflammatory mediators secondary to ischemia, even "normal" control tissue may not represent a true control. In these experiments, it is of note that the control tissue appeared grossly and histologically comparable to tissue from animals that did not undergo ischemia in other experiments (Gonzalez, L.M., Unpublished Data, 2017).

In summary, this method describes a reproducible model of porcine intestinal ischemia, that closely models what occurs in human ischemic injury. Additionally, the isolation of intestinal stem cells from ischemic loops is described, which serves to study epithelial repair and possible response to treatment in culture.

Disclosures

The authors have nothing to disclose.

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References

- Eltzschig, H.K., Eckle, T. Ischemia and reperfusion--from mechanism to translation. *Nat Med.* **17** (11), 1391-1401 (2011).
- Blikslager, A.T. Treatment of gastrointestinal ischemic injury. *Vet Clin North Am Equine Pract.* **19** (3), 715-727 (2003).
- Gonzalez, L.M., Moeser, A.J., Blikslager, A.T. Animal models of ischemia-reperfusion-induced intestinal injury: progress and promise for translational research. *Am J Physiol Gastrointest Liver Physiol.* **308** (2), G63-75 (2015).
- Podolsky, D.K. Mucosal immunity and inflammation. V. Innate mechanisms of mucosal defense and repair: the best offense is a good defense. *Am J Physiol.* **277** (3), G495-499 (1999).
- Collard, C.D., Gelman, S. Pathophysiology, clinical manifestations, and prevention of ischemia-reperfusion injury. *Anesthesiology.* **94** (6), 1133-1138 (2001).
- Mallick, I.H., Yang, W., Winslet, M.C., Seifalian, A.M. Ischemia-reperfusion injury of the intestine and protective strategies against injury. *Dig Dis Sci.* **49** (9), 1359-1377 (2004).
- Gonzalez, L.M., Moeser, A.J., Blikslager, A.T. Porcine models of digestive disease: the future of large animal translational research. *Transl Res.* **166** (1), 12-27 (2015).
- Ziegler, A., Gonzalez, L.M., Blikslager, A.T. Large animal models: The key to translational discovery in digestive disease research. *Cell Mol Gastroenterol Hepatol.* **2** (6), 716-724 (2016).
- Sato, T., et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature.* **459** (7244), 262-265 (2009).
- Sato, T., et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology.* **141** (5), 1762-1772 (2011).
- Meneses, A.M.C., et al. Intestinal organoids--Current and future applications. *Veterinary Sciences.* **3**(4) (2016).
- Clevers, H. Modeling development and disease with organoids. *Cell.* **165** (7), 1586-1597 (2016).
- Mahe M.M., Sundaram N., Watson C.L., Shroyer N.F., Helmuth M.A. Establishment of human epithelial enteroids and colonoids from whole tissue and biopsy. *J Vis Exp.* (97) (2015).
- Dedhia, P.H., Bertaux-Skeirik, N., Zavros, Y., Spence, J.R. Organoid models of human gastrointestinal development and disease. *Gastroenterology.* **150** (5), 1098-1112 (2016).

15. Swindle M.M., Smith A.C. *Swine in the Laboratory: Surgery, Anesthesia, Imaging & Experimental Techniques.*, 3rd Ed. Boca Raton, FL: CRC Press (2015).
16. Riebold, T.W., Geiser, D.R., Goble, D.O. *Large Animal Anesthesia: Principles and Techniques.*, 2nd Ed. Ames, Iowa: Iowa State University Press (1995).
17. Parks, D.A., Granger, D.N. Contributions of ischemia and reperfusion to mucosal lesion formation. *Am J Physiol.* **250** (6), G749-753 (1986).
18. Schoenberg, M.H., *et al.* Involvement of neutrophils in postischaemic damage to the small intestine. *Gut.* **32** (8), 905-912 (1991).
19. Nilsson, U.A., *et al.* Free radicals and pathogenesis during ischemia and reperfusion of the cat small intestine. *Gastroenterology.* **106** (3), 629-636 (1994).
20. Osborne, D.L., Aw, T.Y., Cepinskas, G., Kvietys, P.R. Development of ischemia/reperfusion tolerance in the rat small intestine. An epithelium-independent event. *J Clin Invest.* **94** (5), 1910-1918 (1994).
21. Blikslager, A.T., Roberts, M.C., Rhoads, J.M., Argenzio, R.A. Is reperfusion injury an important cause of mucosal damage after porcine intestinal ischemia? *Surgery.* **121** (5), 526-534 (1997).
22. Shegarfi, H., *et al.* Regulation of CCN1 (Cyr61) in a porcine model of intestinal ischemia/reperfusion. *Innate Immun.* **21** (5), 453-462 (2015).
23. Gubernatorova, E.O., Perez-Chanona, E., Koroleva, E.P., Jobin, C., Tumanov, A.V. Murine model of intestinal ischemia-reperfusion injury. *J Vis Exp.*(111) (2016).