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Intravenous Tomato Lectin Injection to Assess Functional Vasculature

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[Abstract] Pluripotent stem cells have recently allowed for the development of tissue models for the various organ systems within the body. These models allow scientists to study organ function, physiology, embryology, and even pathologic processes. Studies on tissue can be done *in vitro* and/or transplanted into animal models for studies *in vivo*. Recently, our lab developed a model of human small intestine derived from human pluripotent stem cells which when transplanted *in vivo*, matured into an intestinal structure similar to that of adult intestine. The maturity of the transplanted human intestinal tissue was dependent upon the development of an adequate blood supply primarily from the murine host. In order to better study the developed vascular network within our transplanted intestinal tissue, we injected Fluorescein labeled *Lycopersicon esculentum* (tomato) lectin into the mouse tail vein (Watson *et al.*, 2014). Using the property of this lectin to bind to the endothelium, we were able to visualize the vasculature within the transplant.

Materials and Reagents

1. 1 ml syringes
2. 30 gauge needles
3. Small surgical kit (scissors, ringed forceps, needle driver/holder)
4. Immune deficient NOD-SCID IL-2Rγ^{null} (NSG) mice, 8-16 weeks of age (bred in house), transplanted with a bioengineered intestine (maturation of transplant then allowed for 6-8 weeks)
5. Fluorescein labeled *Lycopersicon esculentum* lectin (Tomato-Lectin) (Vector Laboratories, catalog number: FL-1171)
6. Phosphate-buffered saline (PBS)
7. Isopropyl Alcohol
8. 4% Paraformaldehyde in PBS (PFA)
9. Water-based optical clearing solution (Ke *et al.*, 2014)

Equipment

1. Dissecting Microscope (Leica Microsystems)

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Procedure

1. Dissolve Tomato-Lectin in PBS at a final concentration of 2 mg/ml.
2. Clean the proximal portion of a transplanted NSG mouse tail with isopropyl alcohol.
3. Draw up 200 µl of the Tomato-lectin solution into a 1 ml syringe equipped with a 30 gauge injection needle.
4. Using proper restraint, hold the tail of the mouse so that the lateral tail vein is visible and then inject the vein with 200 µl of Tomato-lectin solution. Place the mouse into a clean, warm area for recovery. The method for proper tail vein injection is described elsewhere (Machholz *et al.,* 2012).
5. 15 to 30 min after injection, sacrifice the mice using CO_2 exposure and confirm the euthanasia by cervical dislocation. All animal euthanasia must be performed in accordance with the IACUC guidelines and an approved animal protocol within the institution.
6. Collect the appropriate tissues for histology at this time (transplanted tissue plus intestines for comparison) and place the tissues in ice-cold PBS. Fix collected tissue in 4% PFA at 4 °C overnight.
7. Rinse fixed tissues five times in PBS and then process in optical clearing solution according to the following protocol (Ke *et al.*, 2013).
8. Image cleared tissues under a confocal microscope to visualize the Tomato-lectin (Excitation 494 nm; Emission 521 nm) which reflects the functional vasculature (Figure 1).

Representative data

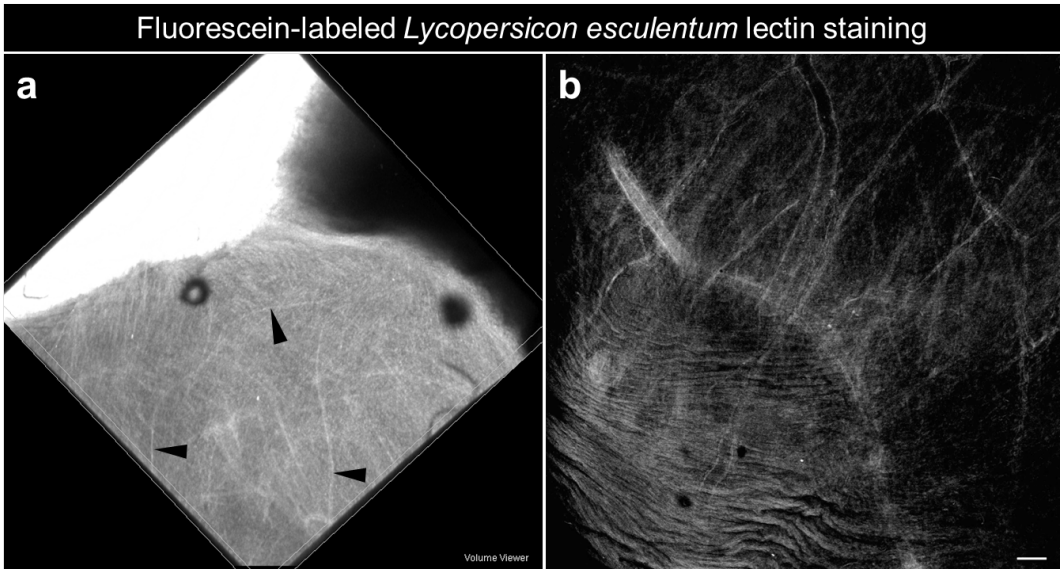

Figure 1. Functional vasculature within a human small intestine derived from human pluripotent stem cells and transplanted *in vivo* under the kidney capsule of an

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immunocompromised mouse. a. Whole mount of bioengineered intestine within the kidney following fluorescein-labeled *Lycopersicon esculentum* lectin perfusion (Black arrowheads: blood vessels). b. Confocal imaging on whole mount reveals functional fluorescent vasculature within the engraftment (scale bars 50 μm).

Notes

1. Freshly collected tissues can be directly imaged under a fluorescent stereoscope equipped with a mercury lamp and a FITC filter.
2. Fixed tissue can be kept several days in clearing agent until imaging at room-temperature.
3. This protocol allows for imaging of the functional vasculature within transplanted tissues and/or intestinal tissues.
4. This protocol was adapted from work previously performed with “human liver buds” transplanted into a murine host and using 1% tetramethylrhodamine-, FITC- and Texas-Red-conjugated dextran via tail vein injection (Takebe *et al.*, 2014). Intravital imaging is possible using a variety of fluorescent-labeled products which can be combined with immunofluorescence staining.

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