Supplemental Material

Detailed Surgical Technique

Surgical technique should be validated using the guidelines provided in Table 1 Column 4 prior to running experiments. As microsurgery can be challenging even for practicing surgeons, we recommend that operators performing surgery meet the guidelines for the selected model in 5 consecutive mice to validate that operator's surgical technique. This may require a couple weeks of practice for operators without prior surgical experience, or 2-3 days for operators with prior surgical experience. Typically, 5 to 60 mice will be required for practice. Temperature regulation is of crucial important for all rodent surgeries. An appropriate heat source (heating pad or lamp) should be used throughout surgery and recovery. Investigators need to closely monitor temperature and anesthesia during all surgical procedures.

I. IVC Models: Surgical Approach

Mice are anesthetized in a small anesthetic induction chamber using isoflurane at 5% and an oxygen flow rate of 500 mL/mn. Once the animal is at a respiration rate of 1 breath every 1-2 seconds, it is removed from the chamber, weighed, and the animal's abdomen hair is shaved or removed using a hair removal lotion. Then the animal is transferred to a nose cone, in a dorsal recumbent position, and the oxygen flow is reduced to 200 mL/mn at 2% The tail may be secured to the pad with tape to ensure the spine is elongated. The animal's shaved abdomen is wiped (cranially to caudally) with appropriate cleaning solutions (such as chlorhexidine (Nolvasan®) or betadine in combination with alcohol wipes). A midline laparotomy is performed using iris scissors—not a blade—to ensure that only the skin is incised. The muscle layer is opened along the linea-alba to reduce pain/bleeding, and a Fine Science Tools (FST) retractor system or small internal abdominal retractor/eyelid retractor is placed to keep the abdominal cavity opened. To allow for easy visualization of the IVC, the intestines are exteriorized and placed on a sterile saline moistened 2x2 inch gauze pad to the animal's left, with an additional moistened gauze pad placed over the bowel. The surgery should then proceed depending on the model being used, with each described below.

a. Surgical Approach for Ligature Based Models

The fascia tissue on either side of the IVC is dissected using straight jeweler's forceps. Dissection is performed carefully, as grabbing the IVC alone may result in puncture. Small, controlled movements should be utilized when dissecting. Model-specific surgical technique should proceed as follows:

i. Ligation Model

Once visualized, all lateral branches are ligated with nonreactive 7-0 Prolene suture, slightly away from the IVC (approximately 1-2 mm), taking caution to avoid damaging the nerves that run parallel to the IVC on either side. Using a cotton swab to roll the IVC if accessing from animal's right side (usually more accessible), or straight forceps if accessing from animal's left side, posterior venous branches are cauterized using a Bovie low temperature fine tip cautery pen (MFI Medical Equipment, Inc; San Diego, CA). Slight compression with the cotton swab should be applied to collapse the posterior branches prior to cauterization to prevent bleeding. Gentle maneuvers when handling the IVC are strongly recommended.

Dissection between the IVC and aorta is then performed immediately below the left renal vein, usually within a window of 1-3 mm below where the left renal vein enters the IVC. The gap between the IVC and aorta is used to create a path underneath the IVC, and a 7-0 Prolene suture is passed through and tied down onto the IVC as close as possible to the left renal vein. Silk or nonreactive nylon/polyprolene sutures sized 4.0-7.0 may also be used. Dilation of the IVC is immediately observed. After the retractors are removed, the intestines are moistened and placed back into abdominal cavity. The abdomen is then closed with a continuous stitch using 3-0 Vicryl for the muscle layer, and the skin is closed with tissue glue.

ii. St. Thomas Stenosis Model

If lateral branches draining into the IVC between the left renal vein and the iliac bifurcation are present, they should be ligated as described in the ligation model above. The IVC is mobilized beneath the level of the left renal vein and the posterior tributaries identified. A plane is developed behind the vena cava and between any posterior vessels, which are left alone. The abdominal aorta is separated from the IVC using blunt dissection with two pairs of fine forceps. A 2-0 silk tie (Ethicon, UK) is passed behind the IVC just inferior to the left renal vein. A 5-0 Prolene suture (Ethicon, UK) is placed longitudinally over the IVC to act as a spacer, and the silk suture is tied over the top. The Prolene is then removed. This procedure creates a stenosis that markedly reduces blood flow.

Next, endothelial damage is induced using a neurovascular surgical clip (Braun Medical OR Mini Bulldog Serrefines, #18053-28, Fine Scientific Tools, Germany) applied to the vessel in two separate positions—one cephalic and one caudal—below the renal vein, for twenty seconds at a time. The bowel is then placed carefully back into the abdominal cavity. The laparotomy is closed using 3-0 monocryl in layers. Subcutaneous buprenorphine (0.01-0.1mg/kg) may be immediately administered for post-operative analgesia.

iii. Stenosis Model

Lateral and posterior branches are not manipulated, cauterized, or ligated in any way. The IVC and aorta are separated just distal to the left renal vein, as described above in the ligation and stenosis models. A 30-gauge blunted needle spacer is placed on top of the exposed IVC and a non-reactive permanent narrowing ligature (8-0 monofil polypropylene) is secured around both the IVC and spacer directly below the left renal vein. A 5-0 Prolene suture or 0.38-mm wire may also be used for the spacer. Once the suture is secure, the spacer is removed. The surgical site is then closed with 5.0 nylon sutures; the muscle layer is closed using a continuous stitch and the skin layer is closed by individual sutures. Following recovery, mice may be dosed with 0.1 mg/kg sterile buprenorphine, repeated every 12 hours for 48 hours or longer if needed.

b. Surgical Approach for Electrolytic IVC Model (EIM)

Prior to surgery, equipment should be prepared as follows. The EIM needle is prepared by cutting a 25-gauge needle to a length of 0.5 cm using a #11 blade and scoring the needle completely around until it is broken cleanly (no sharp edges and without decreasing the lumen of the needle). A 30-gauge silver-coated copper wire is measured to a length of approximately 20 cm, and the coating is stripped off on each end (0.3 cm off one end, approximately 2 cm of the other end). The 0.3 cm stripped wire end is inserted into the blunt end of the 0.5 cm 25-gauge needle, which is held in place using alligator forceps. A needle holder is used to clamp the needle onto the wire to secure it. The 2-cm end of the stripped wire is attached to a positive anode of a constant current machine. The needle is bent, bevel facing up, at 60-70° from the wire (less than 90° to avoid slipping out of the IVC, so that upon insertion the needle will be angled upwards along the anterior vein wall). A second piece of copper wire of the same length is prepared with the shorter stripped end bent into a small hook to be inserted into subcutaneous tissue. This second wire is connected to the negative terminal of the constant current source.

Mice are prepared and surgery is initiated as described above. If lateral branches draining into the IVC are present, the immediately surrounding fascia tissue should be carefully dissected and a nonreactive 7-0 Prolene suture used to ligate the lateral branches. Care should be taken to avoid damaging the nerves that run parallel to the IVC on either side. To better visualize the iliac bifurcation, a moistened cotton tip broken off a cotton swab (without any rough edges) may be used as a laparotomy pad for bladder and seminal vesicles, held in place with FST retraction to keep these tissues from touching the IVC during the procedure.

The copper wire is bent into an upside down "U" shape and taped such that the needle lies directly on top of the distal IVC, with care taken to avoid poking the IVC with the needle. The hook end of the second wire is inserted into subcutaneous tissue near the proximal end of the abdominal incision.

Next, the EIM needle connected to the anode is inserted into the IVC at the level of the lymph node just proximal to the iliac vein bifurcation. To accomplish this, a cotton swab is used to press on the IVC just below the left renal vein prior to needle insertion in order to dilate the IVC to prevent sticking the needle through the back of the IVC. A needle driver or forceps is used to hold the wire by the base of the needle to insert into the IVC, keeping the needle parallel to the vessel, taking care not to insert the needle all the way to where the needle meets the wire (insert approximately 90%) as this can increase the risk of bleeding. The inserted needle is elevated in order to make contact with the ventral surface of the IVC. This elevation can be accomplished by taping to a fixed structure such as the microscope, or through construction of a stable hook.

A direct current of 250 μA is applied over 15 minutes, which will produce a large thrombus from the iliac bifurcation extending to approximately the left renal vein by day 2. If modifying current or time to achieve a different thrombus size, these modifications should *decrease* one or both of these parameters, as higher current or longer application may result in paralysis.

The direct current generates free radicals in the copper wire, which results in endothelial cell activation. Saline is intermittently dripped into the abdomen while the current is being applied, and the brightness of the microscope is reduced to diminish tissue desiccation. Dark spots should become visible surrounding the needle during current application, indicating successful thrombus formation. Following electrolysis, the negative wire is removed first, and sterile saline is applied to the IVC around the anode needle to ease the removal process. While gripping the wire with a needle driver, small up and down motions should be used to release the needle from the anterior IVC wall before removal. Light or minimum pressure is applied to the insertion site without compressing the IVC following removal of the EIM needle. A laminar thrombus (visible during current application) forms immediately with maintenance of a flow channel and a solid thrombus can be harvested beginning at 2 hours following induction, depending on the mouse strain. The cotton tip is removed from the bladder area, and the intestines are moistened and placed back into the abdominal cavity. The muscle layer is closed with a continuous stitch using 3-0 Vicryl, and the skin is closed with tissue glue.

c. *Surgical Approach for the Ferric Chloride Model*

A solution of 3.5% ferric chloride by weight in water is used to saturate a small piece of filter paper (2 x 4 mm). The filter paper is applied to the exposed IVC for 3 minutes and then removed. The concentration of ferric chloride solution, size of the filter paper, and duration of application may be adjusted to vary the size of the subsequently forming thrombus, which has a 100% incidence. The thrombus may be harvested 30 minutes later. If longer time points are desired, it is not recommended to recover the animal due to complications from the $FeCl_3$ solution on the surrounding tissue such as GI perforation, peritonitis, and synechia.

d. *Surgical Approach for the Recurrent VT Model*

The primary VT is created using the EIM procedure as described above, because this constant flow model will allow for a recurrent injury without the use of a ligature and without damaging endothelial cells. The animal's incision is closed in two layers using 5-0 Vicryl sutures: the abdominal muscle wall is closed in a continuous suture pattern and the skin is closed in a simple interrupted pattern. Skin glue is not used after the primary VT surgery because it is more difficult to re-open the incision for the secondary VT procedure. Also, occasionally the glue seeps through the abdominal wall and adheres to the intestines and liver causing

additional difficulties in opening the wound. The animal is removed from anesthesia and allowed to recover with supplemental heat until ambulatory.

Twenty-one days after the primary VT insult, a secondary VT is created using either the IVC Ligation model or EIM (other models can also be used), as described above. If the EIM is used as the secondary VT insult, the needle is placed in a slightly different location (e.g. with the first surgical placement slightly to the left, and the second surgery placing the needle slightly to the right) and the electrical stimulus time is decreased to 10 minutes. The animal's incision is closed in two layers: the abdominal muscle wall is closed in a continuous pattern using 5-0 Vicryl suture and the skin is closed using skin glue.

Sham groups are studied with the primary VT performed as a sham surgery. This is done in the same manner as the EIM procedure, except the electrical current is not turned on. The secondary VT procedure is done in the same manner as stated above.

II. Femoral Vein Electrolytic Model Surgical Approach

Adult mice are anesthetized with pentobarbital (40-60 mg/kg) or isoflurane (1-2%) and the groin skin overlying the femoral vessels is shaved, cleaned, and opened. Simple retraction provides wide exposure of the femoral vein; no further dissection is needed. A 70-75-micron diameter microsurgical needle (e.g., Sharpoint, Reading, PA) is used with the suture removed from the swaged end to create a blunt circular ending for the needle. A jeweler's forceps, connected to the positive end of a 1.5-volt direct-current source, is used to grasp this needle and touch the blunt end to the top surface of the exposed femoral vein. Touching the negative pole of the electric source to remote subcutaneous tissue completes the circuit. Continuous current delivery of 1 to 180 seconds can be used to induce a thrombus by electrolytic deposition of iron from the needle onto the surface; a standard time of 30 seconds gives a consistent, non-occlusive thrombus that becomes evident ~5 minutes after the electrolytic injury and peaks around 25-45 minutes later. The resulting thrombus is non-occlusive and forms downstream of the induction site.