PROTOCOL FOR MICRODISSECTION OF NEPHRON SEGMENTS IN RAT KIDNEY

Materials:

- **Solutions:** all solutions are ice-cold before use.
  1) Perfusion solution: Containing (in mM) 135 NaCl, 3 KCl, 1.5 CaCl$_2$, 1 MgSO$_4$, 2 KH$_2$PO$_4$, 5.5 glucose, 5 L-alanine, and 5 HEPES (pH 7.4). Some paper used cell culture medium.
  2) Digestion solution: perfusion solution containing collagenase, 1mg/ml. (note: collagenase concentration depending on the potency of the enzyme, which differs each batch).
  3) Dissection solution: perfusion solution containing 1 mg/ml BSA (BSA stops further digestion), 0.1 mg/ml trypsin inhibitor and 20 µg/ml aprotinin.

Prepare large amount of perfusion solution and the rest solution be prepared before use.

- **Surgical instrument** for kidney perfusion: sutures, scissors, forceps (2, one curved), hemostats (2), artery clamp.

- **Other items needed:** A lid of Petri dish, a 25 ml flask, 37° C water bath, Two 10 ml-syringes filled with perfusion and digestion solutions respectively, 23g needle, Catheter: 10 cm PE 50 tubing. Apply a small piece of tape on the catheter 1 cm above the tip.

- Assembling Syringe, needle and catheter, leaving on ice for later use.
- Two fine-tip forceps or 30g needles attached to cotton tip stick for dissection

Procedure

1. **Perfuse kidneys:**
   - Open abdomen
   - Ligate both superior mesenteric and coeliac arteries together
   - Put a suture around aorta above renal arteries and make it a pre-ligation position for an easy and quick tie of aorta later.
   - Put 2 sutures around aorta below renal arteries. Tie the lower suture to ligate aorta and make the upper one into pre-ligation position.
   - Apply artery clamp above renal arteries to block blood flow
   - Cut a hole on aorta below renal arteries at a position between the two sutures.
   - Cannulate abdomen aorta through the hole with catheter assembled with syringe as indicated above.
   - Tie the upper suture to ligate aorta and catheter, and then apply the same suture to the tape on catheter to secure the catheter from pulling out of the vessel.
   - Release artery clamp and check no leakage of cannulation.
   - Ligate aorta above renal arteries.
   - Flush the kidneys with ice cold perfusion solution and then digestion solution
   - Kidneys will turn to white immediately if the above surgical preparation is good.
   - Immediately clamp renal hilus with hemostat and cut off kidneys. Put kidneys on ice

2. **digestion of renal tissue**
   - Cut kidney into 1- to 2-mm-thick transverse sections containing the entire corticomedullary axis
• Incubate tissue sections at 37°C for 30 min in digestion solution in 25 ml flask bubbled with 95% O₂-5% CO₂. Bubbling gas flow should keep the tissue sections moving.
• Watch the digestion process until tissue sections look lose and ready to fall into pieces.
• Rinse tissues twice with cold dissection solution
• Transfer tissue into petri dish filled with ice-cold dissection solution
• Mount the petri dish on the microscope stage that is maintained at 4°C during dissection.

3. Dissection of the nephron segment

• Carefully separate nephron segments under microscope using fine-tip forceps or 30g needles attached to cotton tip stick.
• Pile different types of segment together after count (glomeruli) or measure (tubules) with microscope 
  eyepiece micrometer
• Transfer piled segments into Eppendorf tube for further use using a 10 ul pipette tip.
• Transfer dissection solution for control.
• Morphological features of nephron segments are as bellow

Rat nephron segments prepared by collagenase protocol. A: tubule fragments immediately after preparation and as they appear in dissection dish before sorting. B: a proximal tubule segment extending from early proximal convoluted region to early proximal straight segment (bottom right). C: a segment containing the proximal straight tubule (left) and thin descending limb of the loop of Henle. D: thin ascending limb (left) extending through the medullary (MTAL) to the beginning of the cortical thick ascending limb (CTAL, right). E: MTAL of the loop of Henle (top) and thin ascending limb of the loop of Henle (bottom). F: CCD extending to the bottom right is formed from the confluence of two connecting tubules (CNT). Branch on left extends to a distal convoluted tubule with a glomerulus still attached at the region of the macula densa and to the CTAL. G and H: two additional examples of CCDs formed from the confluence of multiple connecting and distal tubules (DT) segments. Micrograph in A was taken with bright-field illumination and the ×2 lens; all other micrographs were taken with the ×10 lens and Hoffman contrast modulation. Nephron segments shown in A, B, and F-H came from cortical tissue slices tangential to the cortical surface, whereas those in C-E came from coronal slices of the medulla.

Reference: