PROTOCOL FOR MICRODISSECTION OF NEPHRON SEGMENTS IN RAT KIDNEY

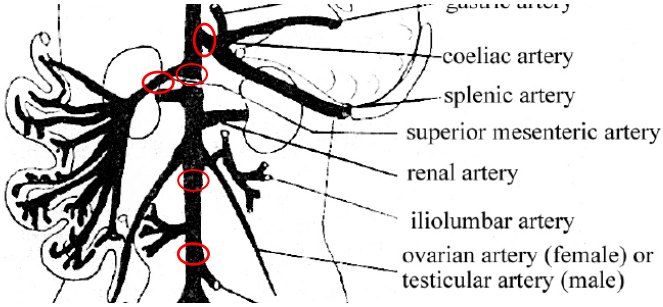
Materials:

- **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_2PO_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 flusk, 37^o 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:

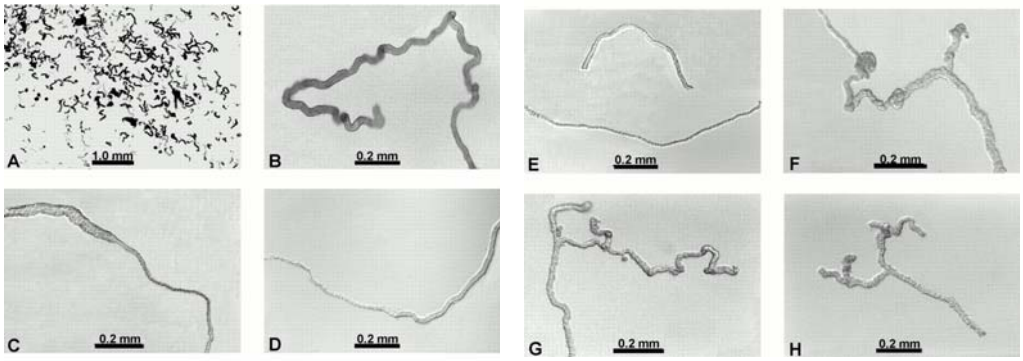
- Open abdomen
- Ligate both superior mesenteric and coeliac arteries together
- Put a suture around aorta above renal arteries and make it a pre-ligation position for an easy and quick tie of aorta later.
- Put 2 sutures around aorta below renal arteries. Tie the lower suture to ligate aorta and make 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% O_2-5% CO_2. 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:

Li N, Yi FX, Spurrier JL, Bobrowitz CA, and Zou AP. Production of superoxide through NADH oxidase in thick ascending limb of Henle's loop in rat kidney. *Am J Physiol Renal Physiol* 282: F1111–F1119, 2002

Li N, Zimpelmann J, Cheng K, Wilkins JA, Burns KD. The role of angiotensin converting enzyme 2 in the generation of angiotensin 1-7 by rat proximal tubules. Am J Physiol Renal Physiol. 2005 Feb;288(2):F353-62. Epub 2004 Oct 5.

Schafer JA, Watkins ML, Li L, Herter P, Haxelmans S, and Schlatter E. A simplified method for isolation of large numbers of defined nephron segments. *Am J Physiol Renal Physiol* 273: F650–F657, 1997