

Rat Aorta Ring Assay

Materials

Animals

1-3 month-old Fischer-344 male rats (Harlan Sprague Dawley, www.harlan.com)

Equipment

- ✚ Dissecting microscope
- ✚ Invert light microscope
- ✚ CO2 incubator

Reagent

- ✚ EBM-2
- ✚ Matrigel
- ✚ Petridish
- ✚ Surgical Forceps and scissors

Protocols

Excision of the Rat aorta

- ✚ A 1-3 month-old fisher 344 male rat was sacrificed by CO2 asphyxiation
- ✚ The thoracic cage were opened and both lungs inside were dissected out.
- ✚ The thoracic aorta was cut along the vertebral column from the diaphragm to aortic arch and put the new dish with EBM.

Preparation of aortic ring

- ✚ Wash and flush away residual blood inside the aorta with EBM-2
- ✚ Carefully tear periaortic fibroadipose tissue away from the aorta wall
- ✚ Cut the aorta into 1-2mm small pieces with Noyes scissors and curved microdissection forceps.
- ✚ Transfer the aorta to a new petridish and wash them with fresh EBM in

Embedding aortic rings with matrigel

- ✚ Pre-coat 48 well with 100ul matrigel and gelatinized it for 30min
- ✚ Put uniform sized aortic ring on the top of the gel
- ✚ Cover the ring with another 100ul matrigel and incubated at CO₂ incubator for 30min for polymerization.
- ✚ Pour 400ul/ wells EBM-2 with growth factor into plate and grow the aortic rings for 7 days
- ✚ Change media every 2 days

Documentation of aorta ring

Photograph aorta ring under invert light microscope with 10×power.

Neovessel area and length were calculated with Image J software.

