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Carrageenan air pouch (mice) V.1

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Guidelines

To ensure a better performance, use cold PBS 1x (4C) and room temperature 1% carrageenan

Materials

MATERIALS

⊗ Carrageenan **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C1867**

⊗ 21G Needles **Catalog #4657527**

⊗ 30G Needles **Catalog #4656300**

⊗ PBS 1X

⊗ Sterile Filter 0.22um **Corning**

⊗ Advia 120 Hemocytometer

⊗ Heparina **Catalog #654754.0**

Before start

Use the solution of 1% carrageenan fresh (prepare right before the laboratory procedure)

1- Day 0: Inflate the pouch. To anesthetize the mice, open the O2 bottle and place the O2 pressure between 0.5 and 1 and the isoflurane in 4. Place paper in the methacrylate box to anesthetize the mice, and under the nozzle in the that we placed the mouse. Open the red stopcock to get O2 and isoflurane to the box and put the mouse. After sleeping, place the mouse on the table, with the head on the nozzle, make sure the nozzle stopcock is open and lower the isoflurane pressure to 2. We can go to the next mouse in the box. Using a 10 ml syringe, a 0.22 μm filter and a 30G needle, we infuse 3 ml of sterile air into the back of the mouse. Remove the needle from the pouch by turning it so that the air does not escape. It is preferable to form the pouch something close to the head, so that the mouse does not bite it.

1

2- Day 3: Reinforcing the pouch. Refill the pouch in the manner explained above: 3ml of sterile air

2

3- Day 6: Carrageenan Injection. We dissolve 1% Carrageenan (λ -Carrageenan Sigma-Aldrich) in 1X PBS. (eg 0.03g of carrageenan and 3ml of PBS). Carrageenan takes time to dissolve completely. We heat a bath at 80 ° C, give it vortex, and so on until it dissolves completely. Fresh carrageenan should be used. With the 30G needle we puncture 0.5 ml of carrageenan in the pouch while massaging the area. Once injected, massage for 30s.

3



4- Day 7: Wash the pouch. We labeled a 2ml eppendorf per mouse and prepared a beaker with cold and heparinized 1X PBS. We keep it on ice. At 24 hours after carrageenan injection, we proceeded to wash: With 21G needle we injected the 2ml of cold PBS. Massage without removing the needle for 30s, being careful not to damage the mouse. We slowly remove the needle and syringe without exerting too much pressure.

4