JMN-MSMP Volumetric Muscle Loss Surgery V.2

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[1]JMN-MSMP

Cellular Senescence Network (SenNet) Method Development Community

SenNet JMN-MSM

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VERSION 2

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OPEN ACCESS

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We use this protocol and it's working

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ABSTRACT

VML Surgery

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1 Disinfect all surfaces and anesthetic equipment (induction chamber, nose cone) with Vimoba (Quip Laboratories VIMTAB).

2 Prepare sterile surgical area and a separate surgical prep area (shaving, Rimadyl injection). Anesthesia should be available in both areas.

3 Anesthetize the mouse. The following settings should be used for anesthesia during induction: 3-4% isoflurane, 100% oxygen, 1.5-2 liter per minute (LPM) flow rate. The following settings should be used for maintenance: 2-3.5% isoflurane, 100% oxygen, 1.5-2 LPM. Mouse should be continually monitored for regular breading and adequate anesthetic depth via toe pinch reflex. Anesthesia levels should be adjusted if breathing becomes to fast or slow, or if the mouse is responsive to toe pinch.

4 Once the mouse is fully anesthetized, place the mouse under maintenance anesthesia at the surgical prep site.

5 Administer 5 mg/kg Rimadyl via subcutaneous injection.

6 Shave the skin around the quadriceps region of both hind limbs.

7 Spray the shaved regions with 70% ethanol and use sterile gauze to wipe off excess hair.

8 Transfer mouse to sterile surgical area under maintenance anesthesia.

9 Prior to making an incision, confirm anesthetic depth using toe pinch. If the animal is responsive, adjust the anesthetic dose and repeat under proper anesthetic depth is maintained.

10 Sterilize the incision area about the mouse quadriceps muscle by spraying 70% ethanol. Wipe off excess ethanol with

a sterilie gauze and wait for skin to completely dry.

11 Move the tail underneath the leg to be operated on, and tape down the foot of the leg prior to making the incision.

12 Make a 1 cm longitudinal skin incision just above the quadriceps muscle.

13 Remove the fascia overlaying the quadriceps muscle using forceps and scissors.

14 At the muscle portion near the kneecap, grip the quadriceps muscle with tweezers and make an initial ~3 mm cut through the cross-section of the muscle. Then, cut ~3 mm of the muscle along the longitudinal plane of the muscles (i.e. parallel to the underlying tibia), and then pull the muscle up and perform a final cut to remove the muscle. The muscle defect should be approximately 3 mm x 3 mm x 4 mm in size.

15 Using the Abadie cuvette (Moria 1121B or similar), place on scoop of PCL particles into the muscle defect.

16 Suture the muscle to close the wound.

17 Repeat process on the other leg, starting from Step 10.

18 Place mouse in clean cage on a heat pad to enable recovery. Remove the cage from the heat pad once all mice in the cage are alert and ambulatory, and do not appear to be in distress. Note: If mice are out for >1 hour, place a water gel pack or water bottle in cage to ensure they remain hydrated.

19 Sterilize tools using the bead sterilizer for 30 sec at 225°C.

20 Monitor animals daily following surgery for the first week to ensure mice are not in pain or distress. If any mice are in pain or distress, follow necessary procedures to treat the pain or euthanize the mice according to the approved institutional animal protocol.

21 Sutures should be removed if still present after 2 weeks. Mice should be monitored regularly until the terminal harvest timepoint.