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VisualSonics Application Protocol
Gene Delivery Applications into Subcutaneous Tumors

1 Objective

The objective of the **Delivery of Genetic Material for Gene Therapy Applications into Subcutaneous Tumors** protocol is to describe the activities performed during a study, including:

- Bolus injection of MicroMarker Contrast Enhancement Agent in suspension with agent to be delivered (i.e. genetic material, protein, etc)
- Acquisition of a cine loop during the injection of the suspension
- Acquisition of a cine loop during delivery of a low frequency ultrasound pulses with the Vevo 770® SoniGene™, resulting in sonoporation

This protocol is intended for mouse imaging applications.

2 Tools Used During the Study

- Vevo 770® SoniGene™ apparatus
- Vevo 770® High-resolution imaging system with software version 2.3.0 and beyond installed
- Vevo Contrast Mode software
- PN11683 *VisualSonics Preparation Protocol - Preparation for Bolus Injection using the MicroMarker™ Contrast Agent Kits*, and all tools listed within
- Medical air (oxygen content less than 31%)

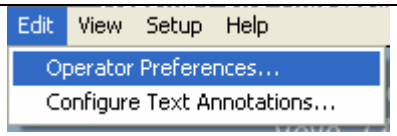
Additional materials not provided in kit that may be required:

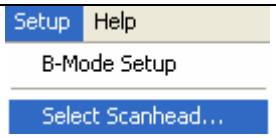
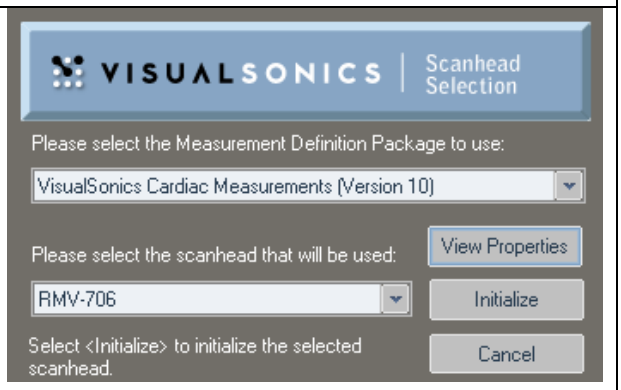
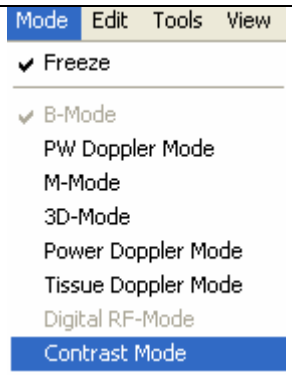
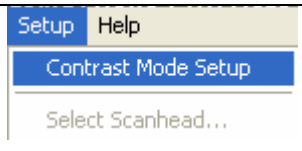
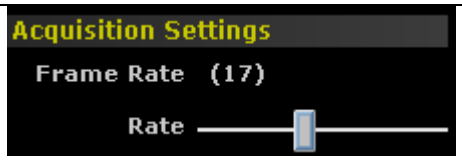
- Agent to be delivered, i.e. genetic material, protein, or intracellular dye of your choice
- Method to confirm delivery of agent, several methods should be used, for example
  - an *in vivo* fluorescence imaging modality
  - immunohistochemistry
  - biochemical assays
  - ELISA
  - western blot
  - PCR
  - Etc.

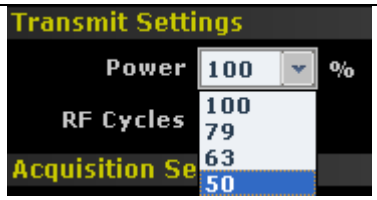
3 Preparation

3.1 Prepare the Vevo 770 for Sonoporation

All RMV's can be used for sonoporation, select the RMV based on the size of your tumor.

1. From the Edit menu, select **Operator Preferences**.	
2. In the Operator Preferences dialog, specify 800 for the Default Cine Loop Size for Contrast Mode. This defines the size of the Contrast Mode cine loop.	
3. In the same window ensure that the Auto-save Pre-Triggered cine loop option is checked.	
4. In the same window under Contrast Mode Settings select "External Destruction", and ensure that the Sequence Destroy Position is set to 50%. This will allow the Vevo 770 to recognize and trigger the SoniGene™ to deliver the low frequency pulse at 50% of a Pre-Trig cine loop.	
5. Connect the SoniGene to the Vevo 770 and adjust the settings: • Frequency - 1MHz) • Duty cycle - 50% • Intensity - 2W/cm^2 • Time - 4 x 30 sec bursts These settings are only a suggested starting point, it is suggested that researchers optimize there gene delivery experiments by testing out a range of frequency, duty cycle, intensity and time settings.	

6. Connect the RMV (RMV-706 in this example) to the Vevo 770. From the Setup menu, select **Select Scanhead**, or press the Select Scanhead key.	
7. Select RMV-706 from the Scanhead Selection dialog, and click **Initialize**.	
8. From the Mode menu, select **Contrast Mode** or press the contrast key.	
9. While scanning, adjust the Field of View setting to allow the entire tumor to be visualized, in this example the FOV was set to 9x9mm, by pressing the FOV toggle switch. If the field of view dimensions are not shown on the screen, press the mode set-up key or select contrast mode set-up from the set-up menu.	
10. From the Setup menu, select **Contrast Mode Setup**, or press the Mode Setup key.	
11. In the Acquisition Settings section, select a frame rate between 10 and 20 Hz, by sliding the Frame Rate slider.	

12. In the Transmit Settings section, select 50% for the Power setting.	

3.2 Prepare contrast agent

Prepare the contrast agent according to the instructions provided in *VisualSonics Preparation Protocol – Preparation for Bolus Injection using the* **MicroMarker™ Contrast Agent Kits for Gene Therapy**

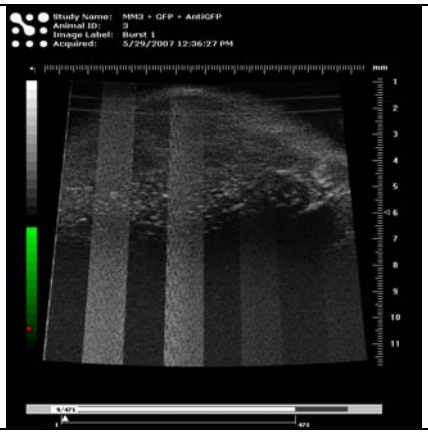
3.3 Prepare subject animal

1. Prepare the subject animal for contrast agent injection via tail vein, jugular vein or retro-orbital sinus. 2. The area of interest for imaging should be displayed on the screen. 3. Place the SoniGene™ low frequency probe in the rail system and align the probe to delivery the pulse in the area of interest, this may require tilting both the RMV scanhead as well as the animal.

Note: It is important to prevent motion of the target tissue once imaging has begun. Prepare materials prior to starting the study and avoid touching, or moving the animal in any way.

4 Delivery of Genetic Material and Image Acquisition

1. After all preparation steps have been completed, press **Scan/Freeze** to begin scanning in Contrast Mode.	
2. Select an appropriate imaging plane with the target tissue centered inside the focal zone.	
3. Press the **Pre-Trig** key to acquire an 800 frame baseline cine loop. Label the cine loop by pressing the Image Label key, typing a label, and clicking **OK**.	
4. Press the Scan/Freeze key to begin imaging again.	
5. Begin a Pre-Trig cine loop, wait approximately 5 seconds and deliver the bolus containing the	

MicroMarker Contrast Agent suspension with the agent to be delivered. The bolus should be delivered over 4-5 seconds.	
6. Save and lable the cine loop in which the agent was delivered.	
7. Slowly and gently flush the cannulation setup with 20-30uL of saline from the flush syringe.	
8. Immediately start imaging and trigger the SoniGene™ low frequency probe: this is done by pressing the Scan/Freeze button to begin imaging, then press the Imaging Sequence button in the bottom right corner of the screen to initiate a pre-triggered cine loop with the low frequency pulse being delivered by the SoniGene in the middle of the cine loop.	
9. The low frequency pulse will appear as interference on the screen as the microbubbles are destroyed in the path of the low frequency beam. The frames acquired post destruction contain only contrast agent which has recirculated into the area of interest.	
10. Save and label the cine loop where the low frequency pulse was applied to cause sonoporation.	
11. Agent delivery should be confirmed by another technique of choice at an appropriate time point.	

Suggested readings for protocol optimization:

› Inagaki et. al. 2006: Ultrasound-Microbubble-Mediated NF- B Decoy Transfection Attenuates Neointimal Formation after Arterial Injury in Mice J Vasc Res 2006;43:12–18

- Sato, M. *et.al* (2005) Enhancement of adenoviral gene transfer to adult rat cardiomyocytes in vivo by immobilization and ultrasound treatment of the heart. Gene Ther., 12(11):936-41.
- Nakaya H. *et.al.*2005. Microbubble-Enhanced Ultrasound Exposure Promotes Uptake of Methotrexate Into Synovial Cells and Enhanced Antiinflammatory Effects in the Knees of Rabbits With Antigen-Induced Arthritis. Arthritis and Rheumatism, 52, 8, 2559–2566
- Tsunoda *et.al.* (2005): Sonoporation using microbubble BR14 promotes pDNA/siRNA transduction to murine heart. Biochemical and Biophysical Research Communications 336, 118–127
- Sakakima Y. *et.al.* 2005: Gene Therapy for hepatocelluar carcinoma using sonoporation enhanced by contrast agents. Cancer Gene Therapy, 12, 884-889
- Hashiva N. *et.al.* 2004: Local delivery of E2F decoy oligonucleotides using ultrasound with microbubble agent (Optison) inhibits intimal hyperplasia after balloon injury in rat carotid artery model. Biochem Biophys Res Commun., 317(2):508-14
- Huang S. & Mac Donald. R. 2004: Acoustically active liposomes for drug encapsulation and ultrasound-triggered release. Biochimica et Biophysica Acta 1665, 134– 141
- Shimamura M. *et.al.*2004: Development of efficient plasmid DNA transfer into adult rat central nervous system using microbubble enhanced ultrasound. Gene Ther., (20):1532-9
- Ohta, S. *et.al.* (2003). Microbubble-enhanced sonoporation: efficient gene transduction technique for chick embryos. Genesis, 37(2):91-101
- Li. T. *et.al.2003:* Gene Transfer with Echo-enhanced Contrast Agents: Comparison between Albunex, Optison, and Levovist in Mice—Initial Results1. Radiology 2003; 229:423–428
- Yo-Ichi Yamashita *et.al*.(2002). In Vivo Gene Transfer into Muscle via Electro-Sonoporation. Human Gene Therapy.13:2079-2084
- Taniyama Y. *et.al.*2002. Development of safe and efficient novel non-viral gene transfer using ultrasound: enhancement of transfection efficiency of naked plasmid DNA in skeletal muscle. Gene Ther, 9, 6, 372-380
- Taniyama Y. *et.al.*2002. Local delivery of plasmid DNA into rat carotid artery using ultrasound. Circulation, 105(10):1233-9

Need help?

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