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VisualSonics Preparation Protocol

Preparation for Bolus Injection using MicroMarker™ Contrast Agent Kits for Gene Therapy

Introduction

The MicroMarker Ultrasound Contrast agents are made by Bracco Research SpA for improved vascular enhancement imaging:

- MicroMarker agents are lyophilized microbubbles with a lipid based shell containing polyethylene glycol, phospholipids and fatty acids, and a perfluorobutane gaseous core.
- They are stored in a glass vial containing a gas head-space consisting of nitrogen and perfluorobutane.
- Prior to use the microbubbles must be re-suspended in saline, gently agitated, and allowed to rest at room temperature for 10 minutes
- MicroMarker Kits can be stored at room temperature and have a shelf life of 1 year
- Each MicroMarker vial which has been reconstituted is stable within the vials for 4-6 hrs

1 Objective

The objective of the **Preparation for Bolus Injection using the MicroMarker™ Contrast Agent Kits** for Gene Therapy protocol is to prepare the microbubbles and agent to be delivered by sonoporation. This suspension is prepared so that upon injection a low frequency pulse can be delivered by the SoniGene™ probe thereby delivering the agent via sonoporation

NOTE: This is a generic protocol describing the preparation of the microbubbles for agent delivery into the subject. Refer to application-specific protocols for details about visualizing the images using the Vevo 770®.

This protocol is intended for mouse imaging applications.

2 Required Tools

- 1x1mL syringe – pre-filled with 0.7mL sterile saline
- 1x1mL syringe – pre-filled with sterile saline for flushing the cannula

- 1x1mL syringe
- 2x21G 5/8" needle
- 2x27G 1/2" needle
- MicroMarker ultrasound contrast agent – 1 vial

Additional materials not in provided in kit that may be required:
- Ice
- Saline
- Eppendorf tubes
- Pipettes and tips
- Agent to be delivered, i.e. genetic material, protein, dye, etc.

3 Preparation

3.1 Prepare MicroMarker Contrast Agent for Gene Delivery in Mouse Imaging

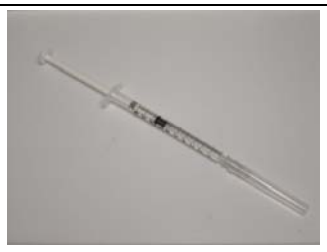
1. With each 1mL syringe – pre-filled with sterile saline (green cap), attach the 21G 5/8" needle (green) and reconstitute the MicroMarker vial by injecting in 500µl of saline directly into the vial. Remove the syringe and leave the needle in to vent, then remove the needle. Gently agitate for 10 seconds, let sit at room temperature for 5 minutes.

2. Dilute 50µg-100 µg of agent to be delivered to a volume of 200µL with saline. For example, add 50µl from a 1mg/ml protein suspension to 150µL of saline.

For the example above, using pipettes, add 150ul of saline to an eppendorf tube, then add 50ul of the protein suspension to the saline and gently mix.

Using a 21G needle draw up the entire contents of the tube into a syringe and inject into the vial of prepared MicroMarker contrast agent. This will give you a total of 50µg of agent to be delivered per vial of MicroMarker contrast agent. Gently agitate the vial for 10 seconds and let sit on ice for 10 minutes to allow the agent to be delivered to become well suspended with the microbubbles.

2. Attach the 27G 1/2" needle (grey) to the 1mL syringe pre-filled with sterile saline (white cap). This is the "flush syringe".



3.2 Prepare for Injection

1. Using the empty 1mL syringe, and the second 21G 5/8" needle (green), gently agitate the vial of microbubbles before drawing up approximately 170µL of prepared MicroMarker from the vial to compensate for the dead space in the needle hub

Note: Ensure that the suspension is well mixed prior to removing the volume for bolus delivery.

2. Replace the 21G 5/8" needle (green) with the second 27G 1/2" needle (grey) to prepare for immediate bolus injection. Remove the air that was drawn up, and adjust the plunger to the volume mark of 100µL. This is the bolus amount to be injected into the cannulation set-up (the "contrast syringe") and will contain 2 x 10^8 microbubbles.

Note: The amounts and concentrations outlined in this protocol are simply a suggested starting point; this protocol should be optimized for use in specific models, both by varying the concentration of the agent within the microbubble suspension, as well as the volume being delivered.

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
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- 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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