FINAL SCRIPT: APPROVED FOR FIMING

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Title: Echocardiography-Guided Injection for Targeted and Reliable Intramyocardial Stem Cell Delivery in a Rat Model of Myocardial Infarction

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **NO**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **All done**

3. **Filming location:** Will the filming need to take place in multiple locations? **NO**

Current Protocol Length
Number of Steps: 17
Number of Shots: 41

Ethics Title Card

This research has been approved by the Local Ethical Committee of UHasselt

Protocol

1. Animal Preparation for Echocardiography-Guided Intramyocardial Injections

Demonstrators: Ellen Heeren, Vincent Vandenboer

1.1. To begin, use a sterile applicator to apply ophthalmic gel onto both eyes of an anesthetized rat, to prevent dryness **[1]**.~~shave the anesthetized rat's chest **[1]** and apply depilatory cream to remove any remaining hair **[2-TXT].**~~ After complete hair removal, disinfect the injection site by alternating an appropriate surgical scrub agent with 70 percent ethanol **[2-TXT]**.
NOTE: VO has been edited as per the moved shots

~~1.1.1. WIDE: Talent shaving the rat's chest. **TXT: Anesthesia: 2.5% isoflurane supplemented with O_2 (Flow rate: 2 L/min)**~~

1.1.2. ~~Talent removing hair with depilatory cream.~~
Videographer's Note: Shots 1.1.1-1.1.2 were not filmed

1.1.3. Talent wipes the skin with 70 percent ethanol to disinfect the surgical area. **TXT: Repeat 3x**

1.1.4. Talent applying ophthalmic gel to the eyes of the rat. AUTHOR's NOTE: Please move shot 1.1.4 before shot 1.1.3

1.2. Transfer the rat from the heating pad onto the animal platform of the ultrasound imaging system **[1]**. Apply a small amount of electrode gel to attach the rat's paws to the platform electrodes, enhancing signal quality **[2]**. Then, insert the rectal temperature probe to monitor body temperature continuously **[3]**.

1.2.1. Talent moving the anesthetized rat onto the imaging platform. **TXT: Anesthesia Maintenance: 1 - 3% isoflurane in 1 - 1.5 L/min O_2**

1.2.2. Talent applying electrode gel and attaching each paw to the platform electrodes.
Videographer's Note: Use take 3

1.2.3. Talent gently inserting the rectal temperature probe into the rat.

1.3. Now, acquire the desired baseline or pre-injection images, including parasternal long axis **[1]** and short axis views in both B-mode and M-mode **[2],** as well as four-chamber images for anatomical and functional assessment **[3]**.

1.3.1. SCREEN: 68775_screenshot_1.mp4: 00:02-00:04

1.3.2. SCREEN: 68775_screenshot_2.mp4: 00:02-00:04 and 68775_screenshot_3.mp4: 00:02-00:04 *Video editor: Use split screen and show both the videos together*

1.3.3. SCREEN: 68775_screenshot_4.mp4

2. Echocardiography-Guided Intramyocardial Injection Procedure

2.1. To prepare the injection system, attach a 22-gauge guide needle to a 1-milliliter syringe **[1]**. Mount the syringe with the guide needle onto the injection clamp and secure it in place **[2]**.

2.1.1. Talent attaching a 22-gauge needle to a 1 milliliter syringe. Videographer's Note: Use take 2

2.1.2. Talent placing the syringe into the injection mount and tightening the clamp .

2.2. Next, align the ultrasound transducer with the injection mount to ensure the guide needle is visible in the imaging field **[1]**. Adjust the transducer position using the transducer mount and holding clamp **[2-TXT]**.

2.2.1. Talent aligning the ultrasound transducer with the injection mount.

2.2.2. Talent adjusting the transducer using the mount and holding clamp. **TXT: Rotate the injection mount to achieve alignment if required**

2.3. Without moving the rat, rotate the animal platform until the notch on the transducer points toward the rat's right shoulder **[1-TXT]**.

2.3.1. Talent rotating the animal platform to orient the transducer notch toward the rat's right shoulder. **TXT: Use the 3D motor to realign the transducer if misaligned**

2.4. To fine-tune the imaging, adjust the micromanipulator screws on the animal platform rail **[1]**. Keep the transducer steady to maintain alignment with the needle **[2-TXT]**.

2.4.1. Talent using the micromanipulator screws on the rail to fine-tune imaging alignment.

2.4.2. Shot of the talent with the transducer. **TXT: Rotate or translate the animal platform further to optimize image clarity**

2.5. Now, using parasternal long axis B-mode and M-mode imaging, visualize the infarcted area **[1]**. Evaluate the extent of infarction, regional wall motion, and areas of wall

thinning to determine injection feasibility **[2-TXT]**.

2.5.1. SCREEN: 68775_screenshot_5.mp4, 68775_screenshot_6.mp4 *Video editor: Please use markedscreenrecordings.pptx to annotate 68775_screenshot_5.mp4*

2.5.2. SCREEN: 68775_screenshot_7.TIFF **TXT: Use 16-segment scoring to detect akinetic/severely hypokinetic regions**

2.6. Identify the peri-infarct zone and choose a hypokinetic area next to the infarct core that has an end-diastolic wall thickness greater than 1 millimeter **[1]**.

2.6.1. Display side-by-side images of infarct core and adjacent hypokinetic zone with measured wall thickness.

2.7. Next, using the rail system, advance the injection mount towards the animal platform **[1]**. Then, adjust the injection mount's micromanipulator screws to fine-align the needle tip with the exact center of the transducer field **[2]**.

2.7.1. Talent sliding the injection mount along the rail toward the rat.

2.7.2. Talent adjusting micromanipulator screws to center the needle tip in the ultrasound field.

2.8. Now, to slowly advance the guide needle and puncture the skin, turn the inject micromanipulator screw on the injection mount **[1]** and confirm the needle tip is visible in the ultrasound field **[2]**.

2.8.1. Talent turning the inject screw to advance the guide needle toward the skin.

2.8.2. SCREEN: 68775_screenshot_8.mp4 *Video editor: Please use markedscreenrecordings.pptx to annotate 68775_screenshot_8.mp4*

2.9. Then, activate the **needle guide** feature in the ultrasound software to confirm the planned trajectory of the guide needle **[1]**. Advance the guide needle toward the selected injection site, stopping with the bevel 1 to 2 millimeters from the left ventricular anterior wall **[2]**.

2.9.1. SCREEN: 68775_screenshot_9.mp4

2.9.2. Display real-time advancement of the guide needle toward the LVAW, with the bevel stopping 1 to 2 millimeters from the wall.

2.10. Once the needle is in position, instruct one operator to stabilize the guide needle at its

base to maintain constant ultrasound visualization **[1]**. Ask another operator to loosen the syringe clamp, carefully remove the syringe **[2],** and replace it with the syringe containing the injectate **[3-TXT]**.

Videographer's Note: Steps 2.10.1-2.11.4 were filmed in one take, no stops since it is too delicate

2.10.1. Talent holding the guide needle firmly in place under ultrasound guidance.

2.10.2. Talent loosening the clamp, removing the empty syringe.

2.10.3. Talent attaching the injectate-loaded syringe. **TXT: Ensure to keep the guide needle stationary**

Videographer's Note: Same as 2.11.1

2.11. Next, attach an 88 millimeter long 29-gauge needle to the syringe containing the injectate.**[1]**. Secure the syringe onto the injection mount **[2]**. Manually insert the needle through the stationary guide needle **[3],** making minor adjustments with the micromanipulator screws along the x-axis, if required **[4]**. Continue to advance the needle manually until the bevel becomes visible in the thoracic cavity on ultrasound **[5]**.

2.11.1. Talent attaching the 29 gauge by 88 millimeter needle to the filled syringe.

2.11.2. Talent mounting the syringe onto the injection holder.

2.11.3. Talent making minor adjustments along the x-axis.

2.11.4. Talent beginning manual insertion through the guide needle.

2.11.5. Show ultrasound image with the 29 gauge needle bevel appearing inside the thoracic cavity.

2.12. Once the 29-gauge needle is visible on ultrasound, advance it into the myocardium of the left ventricular anterior wall using the micromanipulator screws for precise control **[1]**. Ensure the full length of the needle bevel is embedded in the myocardium **[2]**.

2.12.1. Talent turning the micromanipulator screws to guide the needle into the myocardium.

2.12.2. SCREEN: 68775_screenshot_10.mp4 *Video editor: Please use markedscreenrecordings.pptx for annotation*

2.13. Slowly inject the injectate into the myocardium **[1]**. Confirm a successful injection by observing a bright, dense echogenic spot at the injection site that moves with the wall motion of the left ventricular anterior wall **[2-TXT]**.

2.13.1. Talent pressing the syringe plunger slowly to administer the injectate.

2.13.2. SCREEN: 68775_screenshot_11mp4. **TXT: Wait for 10 s post-injection to prevent backflow** *Video editor: Please use markedscreenrecordings.pptx for annotation*

2.14. Finally, remove the syringe and needles from the injection mount to prevent accidental needle sticks **[1]**. Carefully lift the rat off the animal platform and place it on a heating pad for recovery **[2-TXT]**.

2.14.1. Talent unfastening the syringe from the mount and removing it with the needle.

2.14.2. Talent gently lifting the rat from the platform and placing it on a heating pad. **TXT: Monitor rat's vital signs until complete recovery**

Results

3. Results

3.1. Bioluminescence imaging revealed partial leakage of the injected stem cells due to premature needle withdrawal, indicated by diffuse luminescent signal in the thoracic cavity **[1].** A successful intramyocardial injection was confirmed by a concentrated luminescent signal at the left ventricular mid-apex region **[2].**

3.1.1. LAB MEDIA: Figure 3. *Video editor: Highlight panel 3A and show the small blue-green fluorescence toward the left side!*

3.1.2. LAB MEDIA: Figure 3. *Video editor: Highlight panel 3B and show the circular, bright luminescent area in the center*.

3.2. *Ex vivo* imaging of sectioned hearts showed the strongest bioluminescent signal in the mid-apex slice **[1],** with weaker signals in the apex **[2]** and mid-ventricle sections **[3].**

3.2.1. LAB MEDIA: Figure 3. *Video editor: Highlight panel 3C and emphasize the middle circular section labeled "mid-apex" with the most intense glow*.

3.2.2. LAB MEDIA: Figure 3C. *Video editor: Highlight panel 3C and emphasize the right section labeled "apex" with the dimmer glow*.

3.2.3. LAB MEDIA: Figure 3C. V*ideo editor: Highlight panel 3C and emphasize the left section labeled "mid" with the faint luminescence.*

3.3. Post-mortem imaging of the heart showed a visible purple hydrogel adjacent to the infarct zone, confirming targeted delivery **[1].** Fluorescence imaging of heart tissue showed that the red tracer was confined to the peri-infarct zone, confirming localized delivery **[2].**

3.3.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the region marked with white arrows.*

3.3.2. LAB MEDIA: Figure 4B. *Video editor: Highlight the bright red cluster in the lower-left region of the blue-stained tissue.*

3.4. Sirius Red staining confirmed that the tracer was delivered to a region of intermediate wall thickness, between the infarct core and healthy myocardium **[1].**

3.4.1. LAB MEDIA: Figure 4C. *Video editor: Highlight the red-stained fibrotic areas*

surrounding the open central cavity of the heart slice.

3.5. Color Doppler imaging confirmed that the needle was correctly placed inside the myocardium with no visible blood flow before injection **[1].** During injection, the anterior wall remained intact with no external blood flow detected **[2].** After needle withdrawal, the retained injectate was visible within the myocardium, and no hemorrhage was observed **[3].**

3.5.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the area with red and yellow arrows*

3.5.2. LAB MEDIA: Figure 5B. *Video editor: Highlight the area with yellow arrows and circle*

3.5.3. LAB MEDIA: Figure 5C. *Video editor: Highlight the area with yellow arrows and circle*

Pronunciation Guide:

1. Echocardiography
Pronunciation link:
https://www.merriam-webster.com/medical/echocardiography
IPA: /ˌɛkoʊˌkɑrdiˈɑgrəfi/
Phonetic Spelling: EK-oh-kar-dee-OG-ruh-fee

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2. Intramyocardial
Pronunciation link:
https://www.merriam-webster.com/medical/intramyocardial
IPA: /ˌɪntrəˌmaɪ.oʊˈkɑrdiəl/
Phonetic Spelling: in-truh-MY-oh-KAR-dee-uhl

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3. Myocardial
Pronunciation link:
https://www.merriam-webster.com/dictionary/myocardial
IPA: /ˌmaɪ.oʊˈkɑrdiəl/
Phonetic Spelling: MY-oh-KAR-dee-uhl

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4. Bioluminescence
Pronunciation link:
https://www.merriam-webster.com/dictionary/bioluminescence
IPA: /ˌbaɪ.oʊˌluː.məˈnɛsəns/
Phonetic Spelling: BY-oh-loo-muh-NES-uhns

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5. Fluorescence
Pronunciation link:
https://www.merriam-webster.com/dictionary/fluorescence
IPA: /flʊˈrɛsəns/
Phonetic Spelling: floo-RES-uhns

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6. Injection
Pronunciation link:
https://www.merriam-webster.com/dictionary/injection
IPA: /ɪnˈdʒɛkʃən/
Phonetic Spelling: in-JEK-shun

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7. Myocardial Infarction
Pronunciation link:
https://www.merriam-webster.com/medical/myocardial%20infarction
IPA: /ˌmaɪ.oʊˈkɑrdiəl ɪnˈfɑrkʃən/
Phonetic Spelling: MY-oh-KAR-dee-uhl in-FARK-shun

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8. Ultrasound
Pronunciation link:
https://www.merriam-webster.com/dictionary/ultrasound
IPA: /ˈʌltrəˌsaʊnd/
Phonetic Spelling: UL-truh-sownd

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9. Depilatory
Pronunciation link:
https://www.howtopronounce.com/depilatory
IPA: /dɪˈpɪləˌtɔri/
Phonetic Spelling: dih-PIL-uh-tor-ee

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10. Syringe
Pronunciation link:
https://www.merriam-webster.com/dictionary/syringe
IPA: /səˈrɪndʒ/ or /ˈsɪrɪndʒ/
Phonetic Spelling: suh-RINJ or SIR-inj