

Video Article

Ultrasound-guided Transthoracic Intramyocardial Injection in Mice

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Abstract

Murine models of cardiovascular disease are important for investigating pathophysiological mechanisms and exploring potential regenerative therapies. Experiments involving myocardial injection are currently performed by direct surgical access through a thoracotomy. While convenient when performed at the time of another experimental manipulation such as coronary artery ligation, the need for an invasive procedure for intramyocardial delivery limits potential experimental designs. With ever improving ultrasound resolution and advanced noninvasive imaging modalities, it is now feasible to routinely perform ultrasound-guided, percutaneous intramyocardial injection. This modality efficiently and reliably delivers agents to a targeted region of myocardium. Advantages of this technique include the avoidance of surgical morbidity, the facility to target regions of myocardium selectively under ultrasound guidance, and the opportunity to deliver injectate to the myocardium at multiple, predetermined time intervals. With practiced technique, complications from intramyocardial injection are rare, and mice quickly return to normal activity on recovery from anesthetic. Following the steps outlined in this protocol, the operator with basic echocardiography experience can quickly become competent in this versatile, minimally invasive technique.

Video Link

The video component of this article can be found at <http://www.jove.com/video/51566/>

Introduction

Heart disease is the leading cause of death for both men and women in the United States, accounting for 600,000 deaths annually¹. Murine models of cardiovascular disease are critically important for investigating pathophysiological mechanisms and for exploring potential therapies. Myocardial delivery of gene therapy vectors, stem cells, modified RNAs, and other therapeutic agents permits investigation of their therapeutic potential for heart disease²⁻⁷. Currently, there are limited options for myocardial delivery of therapeutic agents in mouse models⁸. Intramyocardial injection under direct visualization is commonly used, but requires a sternotomy or thoracotomy and is limited to the exposed region of the heart. While convenient when performed at the time of another experimental manipulation such as LAD ligation, the need for an invasive procedure for intramyocardial delivery limits potential experimental designs and introduces additional effects from the procedure (e.g., fibrosis due to thoracotomy). Percutaneous pericardial delivery of viral vectors has been reported, but the site and distribution of therapeutic agent is not homogeneous and is difficult to control⁹. Percutaneous coronary injection results in more homogenous distribution of injected material, but efficient and reproducible coronary delivery is challenging in murine models.

Here, we describe a closed chest intramyocardial injection technique that allows minimally invasive, operator controlled targeting of therapeutic agents under ultrasound guidance. The technique is easy to learn, obviates the need for thoracotomy or sternotomy and their attendant experimental complications, and provides greater flexibility on the timing and sites of intramyocardial injection. Thus, echocardiography-assisted intramyocardial injection represents a technically simple and highly effective method of manipulating the myocardium in murine experimental models.

Protocol

All described steps were performed under protocols approved by the Institutional Animal Care and Use Committee of Boston Children's Hospital.

1. Preparation

1. Perform baseline cardiac anatomical and functional assessment by echocardiography prior to beginning the injection protocol, as the optimal fixed transducer position for intramyocardial injection may not yield the optimal standard views for delineating anatomy and assessment of function.
2. The injection setup is shown in **Figures 1A-1C**. Place an empty syringe with a sheathed needle, bevel oriented upwards, in the syringe clamp (see **Figure 1C**) and secure the ultrasound transducer probe in the scan-head clamp (see **Figure 1B**). Loosen the scan-head clamp ball lock

joint (**Figure 1B**) and manipulate the transducer orientation so that it is aligned parallel to the axis of the needle. Fix the scan-head position by tightening the scan-head clamp ball lock joint.

NOTE: For injection in adult mice, a 30 G needle with a 1 in/2.5 cm length is optimal. A 1 ml syringe can be used for larger volumes, while a gastight syringe can be used for more precise control of smaller volumes (5-10 μ l).

3. Apply ultrasound gel liberally to the transducer tip with a spatula to cover the head along its entirety. Carefully unsheath the needle and use the needle mount controls to advance the needle directly under the transducer and within the ultrasound gel for visualization. Make minor adjustments using the needle mount control so that the needle is visualized clearly along its length on the ultrasound image. If the transducer was properly aligned parallel to the needle in step 1.4, then the needle should remain within the imaging plane as it is advanced and withdrawn with the injection control knob (**Figure 1C**).
4. In subsequent steps, do not disturb the needle/transducer horizontal alignment by moving either in the horizontal axis. Rather, target specific areas of the heart for injection by changing the vertical (y-axis) position of the needle mount and by moving the animal platform.
5. Move the transducer superiorly from the animal platform using the scan height control (**Figure 1B**) to allow subsequent placement of the anesthetized mouse onto the animal platform. This will not disrupt the x-axis alignment of the transducer to the long axis of the needle.
6. Remove the syringe that was used for alignment from the syringe clamp and discard carefully. Load the new needle and syringe with the injectate to the final target volume, allowing for dead space in the syringe tip. Be careful to remove air bubbles. Place the syringe into the syringe clamp without adjusting its x-axis alignment. Fully retract the syringe using the injection control.
NOTE: For initial training purposes, the use of Evan's blue dye (1%), Trypan blue stain (0.4%) or a suspension of fluorescent microspheres as the injectate can assist the operator in confirming competency and success of targeted injection.

2. Injection

1. Turn on the integrated warmer of the heating platform and set it for 37 °C. Place the animal platform 180° from the usual imaging orientation, with the anesthesia hose clamp and head of the animal closest to the operator. This allows the heart (in the left side of the chest) to be ipsilateral to the syringe clamp and needle. Note: Additional minor clockwise rotational adjustments to the animal platform may be necessary to orient the heart correctly for the parasternal short axis imaging plane that will be used for injection (**Figure 2A**).
2. Prepare mice for echocardiography as previously reported⁹. Anesthetize the mouse in an induction chamber with 2% isoflurane. Remove chest hair with hair removal cream and apply lubricating gel to both eyes to prevent drying of the sclera.
3. Raise the transducer using the scan head height control (**Figure 1B**). Place the anesthetized mouse supine atop the heated animal platform with the snout within a nose cone delivering 1-3% isoflurane (**Figures 2A-2B**). Gently insert a rectal probe and tape the four paws to the ECG electrodes, applying electrode gel for electrical contact.
NOTE: An appropriate level of anesthesia must be ensured for the humane treatment of the animal. There should be no change in heart rate and no response to placement of the needle through the chest wall. The animal platform's integrated homeostatic temperature controls should be used to maintain normothermia (37 \pm 0.5 °C), as hypothermia will result in relative bradycardia, ventricular dilatation, and possible discomfort.
4. Once the mouse is secure on the animal platform, lower the transducer onto the depilated chest using the scan head height control (**Figure 1B**). The optimal ultrasound setup for injection is for the heart to be visualized in the parasternal short axis orientation, as per standard echocardiographic technique. Rotate the animal platform 20-30° clockwise to obtain the optimal acoustic window for injection in the short axis imaging plane (**Figures 2A-2B**). Note: Alternatively, injection can be performed from a parasternal long axis orientation by counter-clockwise rotation of the animal platform (**Figure 2C**).
5. Use the animal platform adjustment controls to adjust the field of view and to target any desired injection site in the left ventricular myocardium. Pan back-and-forth from the apex to the base of heart to target the desired injection location in the left ventricular myocardium (**Figures 3A-3C**). Note: The midpapillary parasternal short-axis view (**Figure 3A**) offers reproducible landmarks that permit followup imaging of the injection site. Note: Alternatively, the parasternal long axis view can be used to target a predefined injection site (**Figure 3D**).
6. Starting with the syringe in the fully retracted syringe clamp, slowly advance the syringe towards the animal's chest by turning the injection control clockwise (**Figure 4A**). To permit clear ultrasound visualization of both the heart and the needle tip as it approaches the chest, use plenty of ultrasound gel over the left side of the chest and optimize the acoustic window by setting a wide field of view on the echocardiography controls. Set the focal point/zone at the target site for injection. Minor adjustments to the needle mount controls can optimize the image of the needle along its length.
NOTE: Some ultrasound machines have a needle guide software function to digitally extend a line along the long axis of the needle through to the target myocardium (**Figure 4B**). Such a software tool can be helpful but is not essential.
7. With the animal appropriately sedated (1-3% isoflurane mixed with 0.5-0.8 L/min 100% oxygen), advance the needle through the chest wall of the mouse and into the myocardium, carefully observing the position of the beveled needle tip at all times. Stop advancing when the needle tip is within the target myocardium (**Figure 4C**). The whole beveled tip should be securely within the myocardium, to avoid injectate leak into the pericardial space.
8. When the tip is in the desired location, deliver the injectate by pushing on the syringe plunger. Deliver the injectate slowly, over 5-30 sec (depending on the volume being delivered). Up to 50 μ l of injectate can be delivered without compromising ventricular function. A transient echobright appearance to the injected myocardial region may be evident after successful injection. A brief (seconds) period of relative bradycardia is occasionally noted with injection into the myocardium and quickly resolves.
9. Once the injectate has been administered, promptly withdraw the needle by counter-clockwise rotation of the injection control knob. The mouse should be kept under anesthesia for several minutes of echocardiographic observation to confirm preserved ventricular function and no postprocedural complications. If indicated, multiple regions of myocardium can be serially injected by repositioning of the needle's angle of approach by adjustment of the animal platform. After intramyocardial injection, the mouse is placed in a cage on its own and allowed to recover from anesthesia under observation.
10. Do not leave an animal unattended until it has regained sufficient consciousness to maintain sternal recumbency. Do not return an animal that has undergone intramyocardial injection to the company of other animals until fully recovered. The cage should be placed on a thermoregulated pad with ready provision of water and mouse diet. Postprocedural discomfort is not expected and failure to appropriately resume normal behavior shortly after intervention suggests a potential complication (see Discussion).

- Carefully discard the needle immediately after completion of intramyocardial injection to minimize the risk of sharps injury to the operator or bystanders. Reusing a needle can result in blunting of the tip making it more challenging to pierce the myocardium and resulting in a higher risk of complication.

Representative Results

Murine Intramyocardial Injection with Blue Dye or Fluorescent Microspheres

Injection of Evan's blue dye is useful for training purposes. Soon after injection, euthanize the mouse and remove the heart to visualize the location of the injected blue dye. **Figure 5** shows an example of a successful injection, with blue dye infiltrating the myocardium at the mid-papillary muscle level (**Figure 5A**, region enclosed by dotted line). Evans blue dye will wash out of the tissue within a few hours. For more permanent marking of the injection site, fluorescent microspheres that remain indefinitely at the injection site can be used. **Figures 5C-5F** illustrates multiple injection targets in the anterior and posterior left ventricular walls.

Adenovirus Injection into Myocardium

To visualize the delivery of adenovirus to myocardium by ultrasound guided injection, we injected an adenovirus in which the cardiomyocyte specific rat troponin T promoter¹⁰ drives expression of mammalian codon-optimized Cre¹¹. The domain of Cre-mediated recombination was determined using Rosa26^{mTmG} mice, in which Cre recombination turns off red fluorescent protein (RFP) and turns on green fluorescent protein (GFP). A single injection of 50 μ l virus (2.8×10^9 infectious units/ml) regionally activated GFP expression within 7 days of injection (**Figure 6**). Some regional myocardial infiltration along tissue planes beyond the initial site of injection is appreciated in the sectioned images.

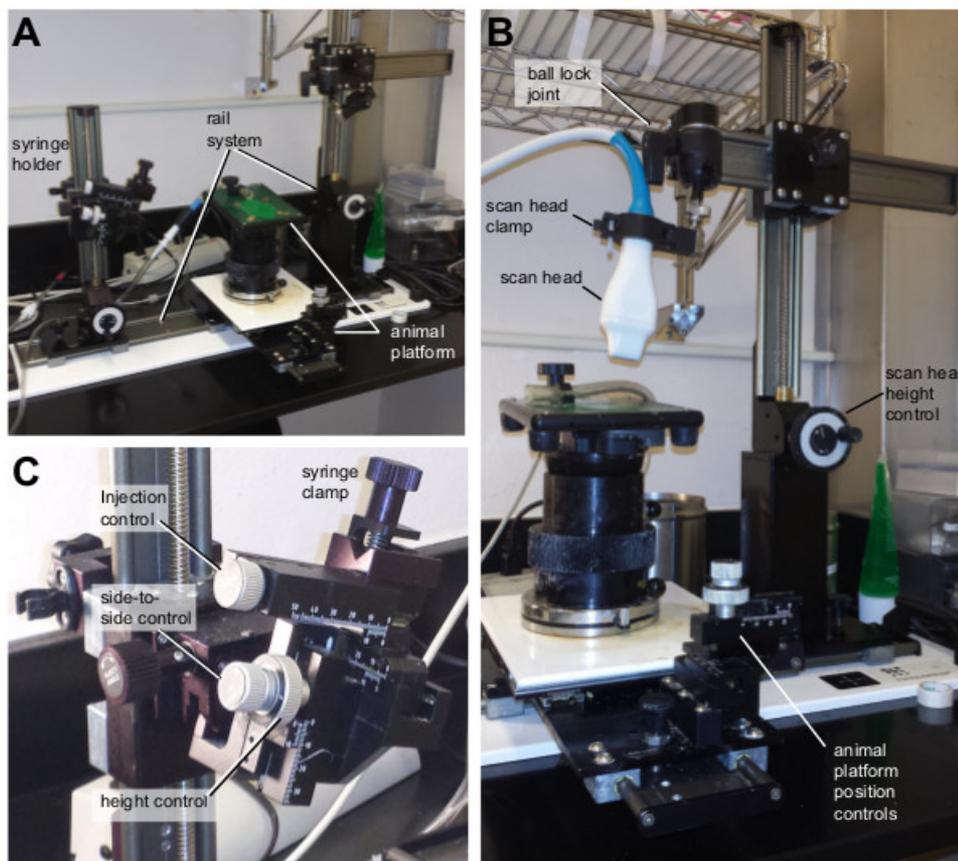


Figure 1. Ultrasound-guided injection setup. **A)** Overview of integrated rail system, which permits alignment of injection syringe and ultrasound scan head. **B)** Ultrasound scan head controls. The scan head is locked in position, aligned to the injection syringe. The field of view is adjusted by moving the animal platform. **C)** Injection syringe controls.

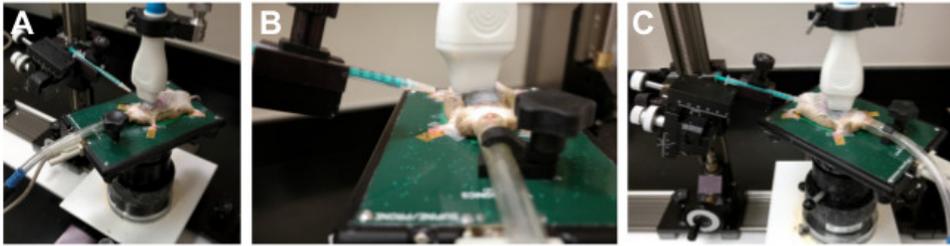


Figure 2. Optimal positioning of mouse on the animal platform for ultrasound-guided needle injection. **A)** Orientation of the animal platform, needle and transducer for intramyocardial injection from the short-axis imaging plane. **B).** Closeup of the needle's path under the transducer's field of view through a generous amount of ultrasound gel as it passes into the thoracic cavity. **C)** Orientation of the animal platform, needle and transducer for intramyocardial injection in the parasternal long-axis imaging plane.

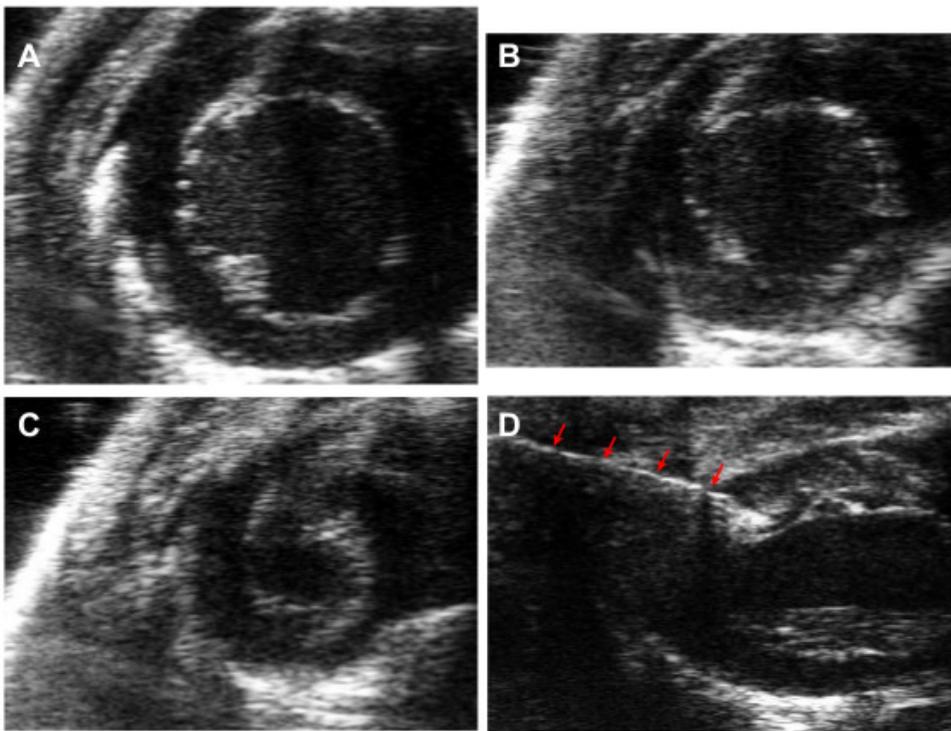


Figure 3. Representative ultrasound images of the myocardium for targeting in the parasternal short axis and long axis planes. **A-C)** Short axis images of the left ventricle at the level of the mid-papillary muscles (A), beneath the papillary muscles (B) and apex of the heart (C). **D)** Long axis image demonstrating intramyocardial injection in the anterior left ventricular wall. Arrows highlight the position of the needle shaft.

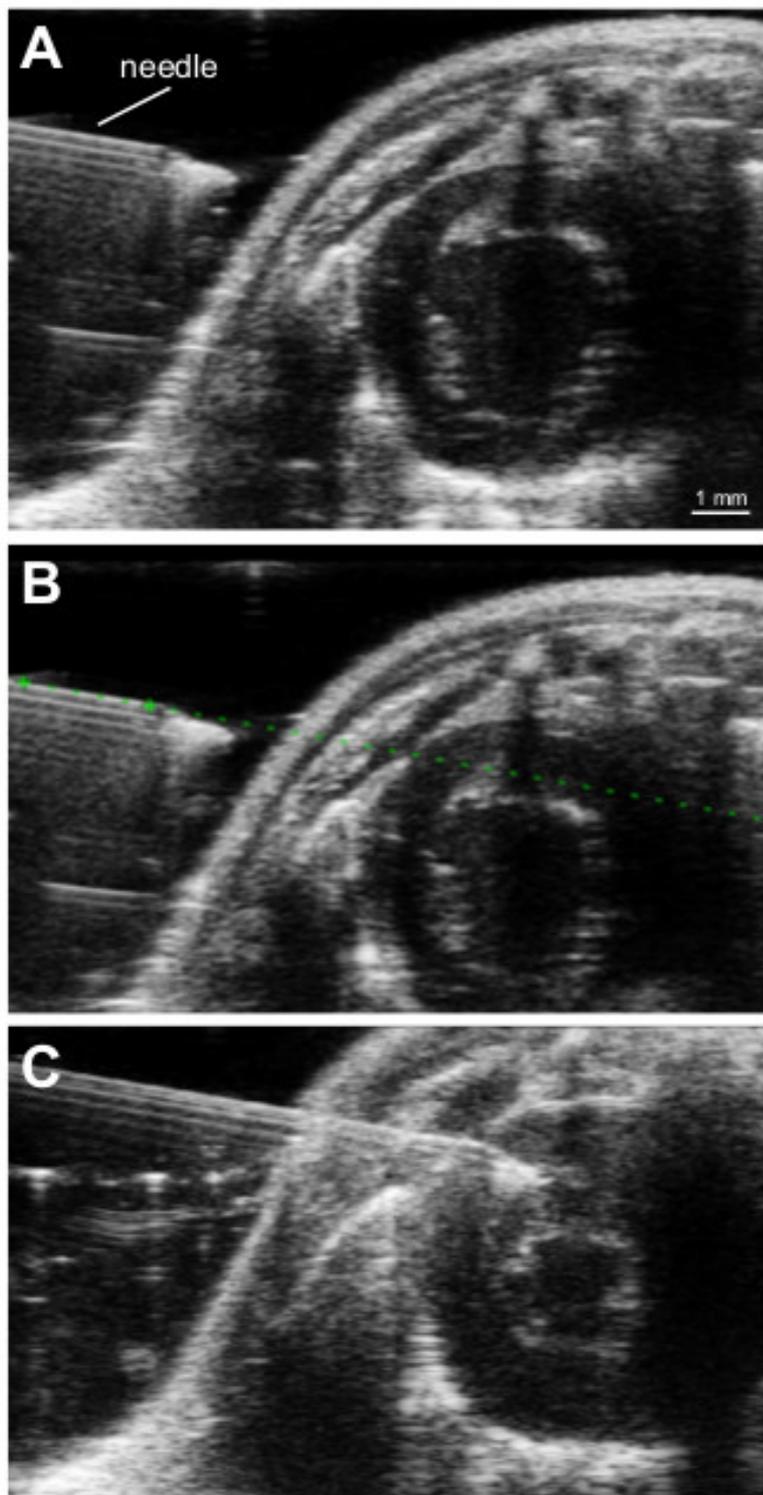


Figure 4. Representative 2D Echocardiography images. A) 30 G, 1 in/2.5 cm needle (bevel up) at a 20° downward angle prior to piercing the chest wall. The wide field-of-view displays the needle along the length of its long axis. **B)** Needle guide overlay function aligned along the long axis of the needle and predicting a course (green dotted line) through the anterior myocardium. **C)** Injected myocardium with bevel of needle buried within the anterior myocardial wall.

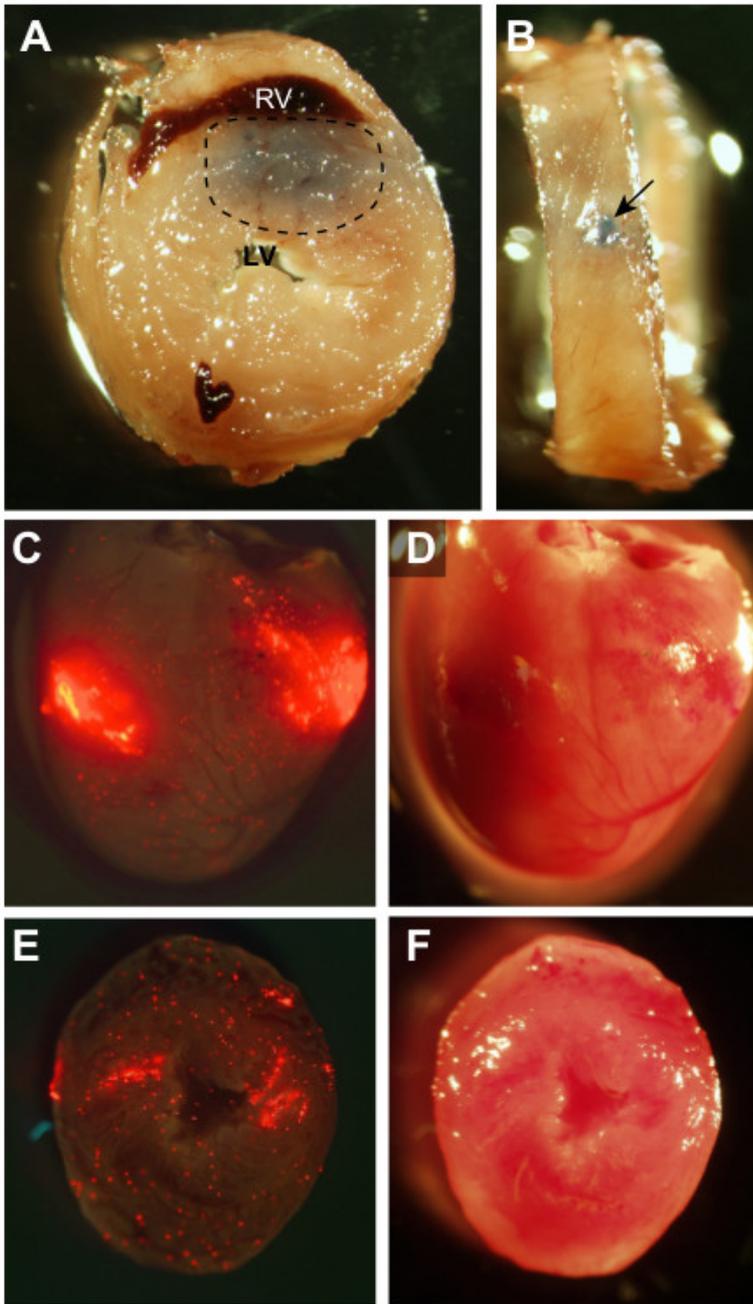


Figure 5. Marking the injection site with Evan's blue dye or fluorescent microspheres. Intramyocardial injection of blue dye into the anterior myocardial wall. **A)** Transverse section of the heart at the level of the midpapillary muscles, demonstrating the infiltration of the dye 30 min after intramyocardial delivery to the left ventricle. **B)** Visible puncture site from the needle on the left lateral surface of the myocardium. **C,D)** Murine myocardium demonstrating red fluorescent microbeads 7 days after separate intramyocardial injection in the anterior and posterior left ventricular walls. **E,F)** Left ventricular myocardium sectioned at the mid-papillary muscle in the transverse plane of the heart demonstrating deposition of red fluorescent microbeads. Scattered microbeads across the cut surface were due to dispersion into the buffer during and after cutting the heart with a knife blade.

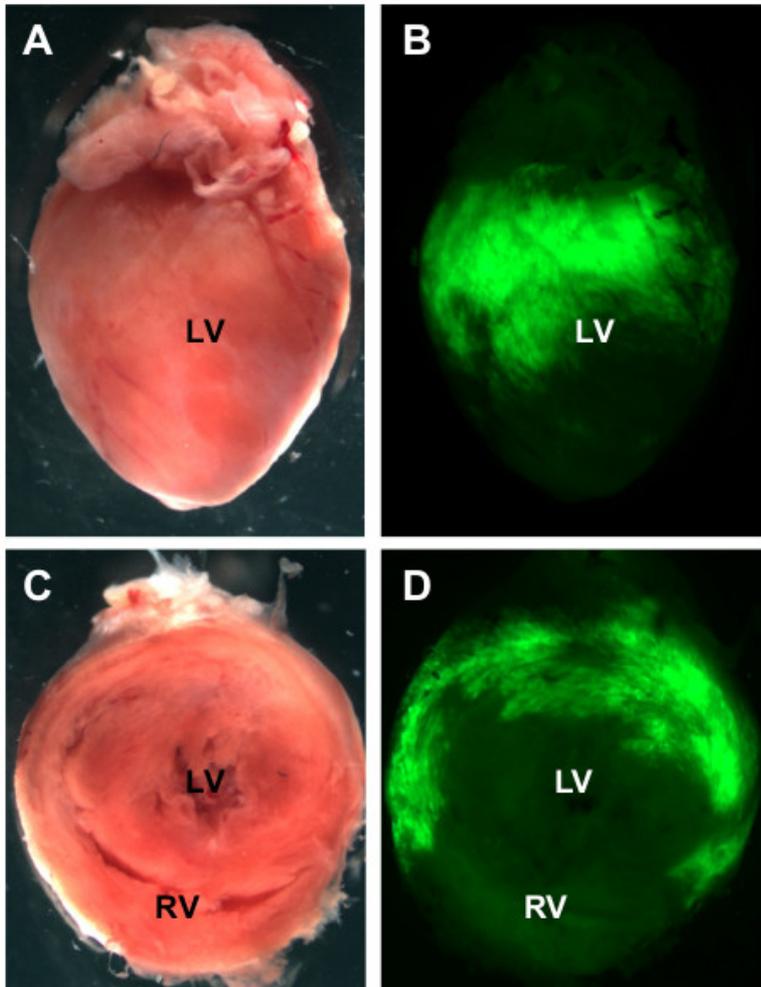


Figure 6. Fluorescent labeling of the anterior myocardial wall of a *Rosa26^{mTmG}* heart after single intramyocardial injection of a TNT-iCre Adenovirus. A) Light microscopy of the anterior wall of a murine whole heart 7 days after regional adenoviral injection. **B)** Fluorescent image (EGFP) in the same heart demonstrating successful delivery and myocardial transduction by an adenoviral vector. **C,D)** Transverse section at the mid-papillary muscle level, demonstrating the extent of regional transfection from a single intramyocardial injection of adenovirus (50 μ l of 2.8×10^9 infectious units/ml).

Discussion

Biologics can be delivered to the myocardium by direct intramyocardial injection, intrapericardial injection, or indirect administration via the bloodstream. Recent cell based therapy trials in myocardial infarction models have described an open thoracotomy approach to the delivery of injectate¹²⁻¹⁴. An important factor in the success of a myocardial therapeutic intervention hinges on the choice of delivery route. The highest local dose of biologic is achieved by intramyocardial delivery^{15,16}. Intramyocardial injection under direct visualization is the most straightforward method and allows for targeted administration within the myocardium¹⁷. However, this method is also the most invasive, as it requires opening of the chest cavity and pericardium to expose the target myocardium¹⁸. Portions of the heart, such as its dorsal surface, are not easily visualized and injected using this method. The invasiveness of the procedure also constrains potential therapeutic dosing schedules. Here, we describe a technically straightforward, reliable and reproducible technique to deliver biologics or drugs under ultrasound-guidance safely by percutaneous intramyocardial injection, without the morbidity and constraints of surgical thoracotomy exposure. In over 150 mice injected to date, after initial technical competency was obtained, our peri-procedural mortality rate was under 5% when less than four myocardial sites were targeted at any one time. Total injectate volumes of up to 200 μ l were surprisingly well tolerated by the murine heart. The anterior myocardium lends itself well as a target for injection, given the parallel orientation of this region of myocardium to the long axis of the needle, allowing the needle to remain in the muscle of the heart more securely. It is important that the operator visualize the entire beveled tip in the myocardium prior to injection, as this ensures full delivery of the injectate without leakage into the surrounding tissue. Care to ensure the needle tip always remains visible within the myocardium is also critical for successful myocardial injection. When practicing with Evan's blue dye, inadvertent injection into the ventricular chamber will result in the mouse rapidly developing a blue hue visible most clearly in the depilated skin. It is also worth noting that needle insertion through the chest wall is determined solely by the ultrasound image displayed of the intended target myocardium and not by any surface anatomical landmarks of the mouse. For an experienced echocardiographer, competency in intramyocardial injection can be reasonably achieved after practice on 4-5 attempts.

As the needle passes through the chest wall, it must pass through the intercostal space. It is not uncommon to have to make minor adjustments to the imaging plane once through the intercostal space to keep the beveled tip clearly within view. This is usually accomplished through minor adjustments of the animal platform or the injection syringe controls. The minor modifications should be done once the needle is through the rib cage but before it has passed into the myocardium to prevent myocardial laceration and trauma from repeated needle tip manipulation. To minimize the risk of hemopericardium, the aim should be to enter the heart as few times as are necessary to complete delivery of injectate. Although the described technique is for intramyocardial injection in the short axis plane of the heart, it is equally feasible to inject in the parasternal long axis plane of the heart through simple adjustment of the animal platform to align with this plane (**Figure 2C**) and redirecting the needle to enter the intended target of myocardium (**Figure 3D**).

Successful intramyocardial injection can be confirmed by using an indicator dye followed by immediate pathological examination of the injected mouse heart. Injection of a suspension of fluorescent microspheres will remain indefinitely at the injection site and could be administered concomitantly with another agent to mark the targeted area. The mouse is euthanized using inhaled carbon dioxide by trained personnel per institutional animal handling protocols. Examination of the heart surface will reveal the injected region, and slicing the heart with a heart matrix will yield cross sections that will demonstrate the injected region. When repeated injection and/or imaging of the same myocardial region is desired over time, it is advantageous to use standard imaging planes and to target reproducible anatomical landmarks. For example, it is convenient to use the short-axis imaging plane to target the left ventricle at the midpapillary muscle level (**Figure 3A**). The most severe potential complication is myocardial laceration/rupture and consequent hemorrhage and/or hemopericardium. The risk of this complication increases with repeated passes of the needle (four or greater) through the myocardium, rapid injection of the fluid bolus, reusing a blunted needle tip, and use of smaller mice (under 15 g) mice. If multiple targets within the myocardium are injected serially (anterior wall, lateral wall or inferior wall), it is optimal to achieve this in three passes or less of the needle through myocardium to minimize risk of procedural complications. The lateral walls of left ventricular myocardium can be injected concurrently with one pass of the needle through the chest wall (**Figures 5E** and **5F**) as they align parallel to one another but any other myocardial wall target will require re-insertion of the needle through the chest wall following needle angle adjustment. The mouse should not be oversedated as bradycardia (under 400 beats per minute) may increase the end-diastolic volume and significantly reduce the myocardial wall thickness. Our experience is for mice to recover from the procedure rapidly and to ambulate in their cages within minutes of recovery from anesthesia. Complications, when they do arise, are abrupt and occur at the time of intervention.

Although this protocol is written for the intramyocardial injection, a slight modification of final needle tip position allows the operator to target injection into the bloodstream of the left ventricular cavity for systemic arterial delivery of an agent. Pulling back on the syringe plunger can confirm whether the bevel tip is in the left ventricular chamber (oxygen rich blood aspirated) or in the myocardial muscle mass. With today's highly efficient and organ-specific viral vectors, specifically an adeno-associated virus serotype 9 vector with a cardiac specific promoter, an investigator can adapt this technique as an alternative to retro-orbital or tail vein injection to achieve secure delivery of a high viral load into the coronary circulation, with reduced first pass absorption by the liver. In the chronic postinfarct murine model, the scarred and fibrotic myocardium may not lend itself well to intramyocardial injection given the resultant thin walled target the operator has to bury the needle tip in. However, border zone regions of myocardium should be amenable to intramyocardial injection and acoustic windows after thoracotomy are more than adequate for successful ultrasound guidance.

Ultrasound-guided, intramyocardial injection in animal models represents a well tolerated, efficient, minimally invasive, and technically straightforward method to delivery biological agents to the myocardium. This technique can be readily employed for the successful delivery of gene vectors or cell based therapies at any experimental time point in a murine model, including after prior open thoracotomy procedures. Acquiring familiarity and competence with the equipment and the injection procedure will ensure subsequent success in experimental models.

Disclosures

The authors have nothing to disclose.

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References

1. Kochanek, K. D. *et al.* Deaths: final data for 2009. *Natl Vital Stat Rep.* **60**(3), 1-117 (2011).
2. Strauer, B. E., & Steinhoff, G. 10 years of intracoronary and intramyocardial bone marrow stem cell therapy of the heart: from the methodological origin to clinical practice. *J Am Coll Cardiol.* **58**, 1095-1104, doi:10.1016/j.jacc.2011.06.016 (2011).
3. Cheng, K. *et al.* Intramyocardial autologous cell engraftment in patients with ischaemic heart failure: a meta-analysis of randomised controlled trials. *Heart Lung Circ.* **22**(11), 887-894, doi:10.1016/j.hlc.2013.04.112 (2013).
4. Fischer-Rasokat, U. *et al.* A pilot trial to assess potential effects of selective intracoronary bone marrow-derived progenitor cell infusion in patients with nonischemic dilated cardiomyopathy: final 1-year results of the transplantation of progenitor cells and functional regeneration enhancement pilot trial in patients with nonischemic dilated cardiomyopathy. *Circ Heart Fail.* **2**, 417-423, doi:10.1161/CIRCHEARTFAILURE.109.855023 (2009).
5. Seth, S. *et al.* Percutaneous intracoronary cellular cardiomyoplasty for nonischemic cardiomyopathy: clinical and histopathological results: the first-in-man ABCD (autologous bone marrow cells in dilated cardiomyopathy) trial. *J Am Coll Cardiol.* **48**, 2350-2351, doi:10.1016/j.jacc.2006.07.057 (2006).
6. Ladage, D. *et al.* Percutaneous methods of vector delivery in preclinical models. *Gene Ther.* **19**, 637-641, doi:10.1038/gt.2012.14 (2012).
7. Zangi, L. *et al.* Modified mRNA directs the fate of heart progenitor cells and induces vascular regeneration after myocardial infarction. *Nat Biotechnol.* **31**, 898-907, doi:10.1038/nbt.2682 (2013).

8. Laakmann, S. *et al.* Minimally invasive closed-chest ultrasound-guided substance delivery into the pericardial space in mice. *Naunyn Schmiedebergs Arch Pharmacol.* **386**(3), 227-238, doi:10.1007/s00210-012-0815-2 (2013).
9. Respress, J. L., & Wehrens, X. H. Transthoracic echocardiography in mice. *J Vis Exp.* (39), e1738, doi:10.3791/1738 (2010).
10. Wang, G. *et al.* Characterization of cis-regulating elements and trans-activating factors of the rat cardiac troponin T gene. *J Biol Chem.* **269**, 30595-30603 (1994).
11. Shimshek, D. R. *et al.* Codon-improved Cre recombinase (iCre) expression in the mouse. *Genesis.* **32**, 19-26 (2002).
12. Lichtenauer, M. *et al.* Intravenous and intramyocardial injection of apoptotic white blood cell suspensions prevents ventricular remodelling by increasing elastin expression in cardiac scar tissue after myocardial infarction. *Basic Res Cardiol.* **106**(4), 645-655, doi:10.1007/s00395-011-0173-0 (2011).
13. Herrmann, J. L. *et al.* Postinfarct intramyocardial injection of mesenchymal stem cells pretreated with TGF- α improves acute myocardial function. *Am J Physiol Regul Integr Comp Physiol.* **299**(1), R371-378, doi:10.1152/ajpregu.00084.2010 (2010).
14. Zhou, Y. *et al.* Direct injection of autologous mesenchymal stromal cells improves myocardial function. *Biochem Biophys Res Commun.* **390**(3), 902-907, doi:10.1016/j.bbrc.2009.10.074 (2009).
15. Campbell, N. G., & Suzuki, K. Cell delivery routes for stem cell therapy to the heart: current and future approaches. *J Cardiovasc Transl Res.* **5**(5), 713-726, doi:10.1007/s12265-012-9378-3 (2012).
16. Hou, D. *et al.* Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: implications for current clinical trials. *Circulation.* **112**(9 Suppl), I150-156, doi:10.1161/CIRCULATIONAHA.104.526749 (2005).
17. Dib, N. *et al.* Recommendations for successful training on methods of delivery of biologics for cardiac regeneration: a report of the International Society for Cardiovascular Translational Research. *JACC Cardiovasc Interv.* **3**(3), 265-275, doi:10.1016/j.jcin.2009.12.013 (2010).
18. Dib, N. *et al.* Cell therapy for cardiovascular disease: a comparison of methods of delivery. *J Cardiovasc Transl Res.* **4**(2), 177-181, doi:10.1007/s12265-010-9253-z (2011).