Background—Magnetic resonance imaging (MRI) permits 3-dimensional (3D) cardiac imaging with high soft tissue contrast. X-ray fluoroscopy provides high-resolution, 2-dimensional (2D) projection imaging. We have developed real-time x-ray fused with MRI (XFM) to guide invasive procedures that combines the best features of both imaging modalities. We tested the accuracy of XFM using external fiducial markers to guide endomyocardial cell injections in infarcted swine hearts.

Methods and Results—Endomyocardial injections of iron-labeled mesenchymal stromal cells admixed with tissue dye were performed in previously infarcted hearts of 12 Yucatan miniswine (weight, 33 to 67 kg). Features from cardiac MRI were displayed combined with x-ray in real time to guide injections. During 130 injections, operators were provided with 3D surfaces of endocardium, epicardium, myocardial wall thickness (range, 2.6 to 17.7 mm), and infarct registered with live x-ray images to facilitate device navigation and choice of injection location. XFM-guided injections were compared with postinjection MRI and with necropsy specimens obtained 24 hours later. Visual inspection of the pattern of dye staining on 2,3,5-triphenyltetrazolium chloride–stained heart slices agreed (0.69) with XFM-derived injection locations mapped onto delayed hyperenhancement MRI and the susceptibility artifacts seen on the postinjection T2*-weighted gradient echo MRI. The distance between the predicted and actual injection locations in vivo was 3.2±2.6 mm (n=64), and 75% of injections were within 4.1 mm of the predicted location.

Conclusions—Three-dimensional to two-dimensional registration of x-ray and MR images with the use of external fiducial markers accurately targets endomyocardial injection in a swine model of myocardial infarction. (Circulation. 2006;114:2342-2350.)

Key Words: catheterization ■ interventional magnetic resonance imaging ■ mapping ■ myocardial infarction ■ stem cell therapy ■ stereotaxic techniques ■ radiography

Transcatheter endomyocardial injection may be an important route for delivering emerging biological therapies such as cellular products,1–8 plasmid,9–14 and virus.10 This route may be particularly important for treating myocardium supplied by an occluded epicardial coronary artery. The ability to target endomyocardial injections may facilitate local delivery of therapies to those myocardial regions where chances of efficacy are greatest and minimize the risk of injections into regions with thin myocardial walls.

Clinical Perspective p 2350

Cardiac magnetic resonance imaging (MRI) provides precise noninvasive assessment of global and regional wall motion15 and myocardial viability.16 We have developed a novel method to register MR images of the heart with x-ray fluoroscopy using external fiducial markers.17 In this study, we describe the use of this technique of x-ray fused with MRI (XFM) to perform targeted endomyocardial injections of iron-labeled mesenchymal stromal cells (Fe-MSC) in hearts of swine with prior myocardial infarction.

Methods

Animal Procedures

Animal procedures were approved by the National Heart, Lung, and Blood Institute Animal Care and Use Committee. Twelve Yucatan miniswine (weight, 33 to 67 kg; 5 male and 7 female) received...
aspirin, clopidogrel, and atenolol for 3 days before myocardial infarction. Anesthesia was induced with the use of atropine, butorphanol, ketamine, and xylazine and maintained with the use of inhaled isoflurane and mechanical ventilation. After transfemoral arterial and venous access, animals received intravenous heparin (50 U/kg), amiodarone (300 mg), and lidocaine (0.5 to 1 mg/kg bolus plus 0.5 to 1 mg/min infusion). The goals of this study were to test the safety and accuracy of XFM-guided targeted endomyocardial injection in and around infarcted myocardium of varying size, age, and location. Myocardial infarction was induced by 45- to 90-minute balloon occlusion of either the left anterior descending (n=8) or left circumflex (LCX) (n=4) coronary arteries. Endomyocardial injection procedures were performed 3 to 74 days (median, 17.5) after the myocardial infarction procedure.

Preparation and Labeling of Mesenchymal Stromal Cells
Fe-MSC were prepared from bone marrow aspirates taken from 2 additional Yucatan miniswine. Femuroxides (Feridex, Berlex Pharmaceuticals, Montville, NJ) labeling was facilitated by protamine sulfate. Fe-MSC were resuspended at a final concentration of 2 to 4×10^6/mL in phosphate-buffered saline for injection.

Preinjection MRI Protocol
MRI was performed at 1.5 T ( Sonata, Siemens, Erlangen, Germany) with an 8-channel phased-array surface coil (Nova Medical Inc, Wilmington, Mass). Images were acquired immediately preceding injection. Dual-modality x-ray and MRI–conspicuous fiducial markers (Beekley Corp, Bristol, Conn) were prepared containing fiducial markers were secured to the thorax with adhesive dressings. All images were acquired during endexpiration. Endocardial and epicardial borders were defined from multiple, breath-held, ECG-gated cine steady state free precession (SSFPI) MRI with the following parameters: TR/TE, 11.4/4.5 ms; flip angle, 30°; inversion time, 300 ms; FOV, 244 mm; matrix, 256×127 pixels; slice thickness, 8 mm; bandwidth, 1085 Hz per pixel. Infarcts were visualized on delayed hyperenhancement (DHE) MRI images acquired 15 minutes after intravenous Gd-DTPA 0.2 mmol/kg with the use of a phase-sensitive inversion recovery sequence 19 with the following parameters: TR/TE, 11.4/4.5 ms; flip angle, 30°; inversion time, 300 ms; FOV, 350×241 mm; matrix, 256×141 pixels; slice thickness, 8 mm; bandwidth, 140 Hz per pixel. Finally, a 3-dimensional (3D) T1-weighted gradient echo scan was performed to locate the external fiducial markers with the following parameters: TR/TE, 2.37/1.18 ms; flip angle, 17°; FOV, 400×300×230 mm; matrix, 256×192×61 voxels; bandwidth, 1300 Hz per pixel. Images were transferred to a Leonardo workstation (Siemens) for manual segmentation of endocardial and epicardial borders at end-diastole and end-systole from SSFP images; infarct borders from DHE images; and external fiducial markers from the 3D, T1-weighted gradient echo images. MRI gradient warping distortion was corrected with the use of a Siemens-supplied algorithm.

XFM Registration Techniques
After MRI, the animals were transferred for x-ray (Axiom Artis FC, Siemens) with the use of a mechanized transport table that allows bidirectional transfer between the MRI and x-ray suites (Miyabe, Siemens). Single-phase MR images acquired at end-systole and end-diastole were registered to the live x-ray acquisitions as reported previously 12 and outlined in Figure 1. Briefly, fiducial marker locations in the x-ray and MR images were segmented to provide matching points within their respective coordinate systems. These were registered by minimizing the distance between matching fiducial marker locations with the use of a nonlinear least-squares optimization that iteratively fitted the 6 parameters (3 rotations and 3 translations) of a rigid body transformation. Image intensifiers–associated distortions (pin cushion and sigmoidal magnetic field) were corrected with empirical 2-dimensional (2D) fifth-order polynomials.

B-spline tensors were fitted to the MR-derived contours to generate smoothed surfaces. 21 Myocardial thickness at end-diastole for each point in the endocardial surface was determined by computing the distance to the closest point on the epicardial surface and was displayed as a 3D surface map. The MR-derived surfaces were registered with the x-ray coordinate system as above and were then fused with live x-ray images during injections. Fusion images were presented to the operator as single-phase, end-diastolic, MR-derived surfaces superimposed on live x-ray fluoroscopy images displayed at 8 frames per second on a second display alongside the vendor’s live x-ray display.

Endomyocardial Injection Procedures
Animals received heparin (50 U/kg), supplemented to maintain an activated clotting time >250 seconds. Expiratory breath-held left ventriculograms were acquired in left anterior oblique 45° and right anterior oblique 45° or anteroposterior projections on which the MR-derived surfaces of left ventricular epicardium, endocardium, and right ventricular endocardium were superimposed (Figure 2). Three-dimensional surfaces representing myocardial thickness and infarcted myocardium were used to define targets for endomyocardial injection with presumably low perforation risk (Figure 3A to 3D). Injection locations were considered safe if the wall thickness was >6 mm. We intended injections into both the center and borders of the infarcts. Injections were performed by experienced interventional cardiologists using the Stiletto endomyocardial injection system (Boston Scientific, Natick, Mass), a spring-loaded 26-gauge injection needle that extends 3.5 mm, positioned with coaxial 7F and 9F guiding catheters. The volume of each injection was 0.4 mL, including 20% tissue dye (TMD-5, Triangle Biomedical Sciences, Durham, NC). When technically possible, the injection needle was guided to a desired location on the basis of the MRI-determined infarct location. Otherwise, the closest region with appropriate wall thickness was targeted. Two orthogonal x-ray images were acquired during each injection to reconstruct the needle position in a 3D x-ray coordinate system. These were displayed to help avoid overlap (Figure 3).

Postinjection MRI Protocol
At the end of the injection procedure, animals were transferred for repeat MRI and to determine evidence of pericardial effusion. Diaphragmatic navigator–gated 3D T2*-weighted gradient echo (T2*W-GRE) imaging (TR/TE, 8.39/3.18 ms; flip angle, 17°; FOV, 250×250×88 mm; matrix, 256×256×88 voxels; bandwidth, 130 Hz per pixel) was performed to locate Fe-MSC. A 3D acquisition was repeated to locate external fiducial markers. Animals recovered and 24 hours later were euthanized under general anesthesia with the use of intravenous KCl. Hearts were removed, sectioned into 3- to 4-mm slices, and stained with 2,3,5-triphenyltetrazolium chloride (TTC) as described 22 to visualize infarcts. Fe-MSC injection sites were also identified by coadministered tissue dye. Slices were photographed on both apical and basal surfaces.

Data Analysis
Myocardial infarction was determined in vivo by DHE after Gd-DTPA infusion or ex vivo by pallor on TTC staining. The relationship between injection site and infarction was assessed qualitatively in 2 different ways. First, 3D locations of the injection sites from x-ray were displayed on DHE images with the inverse transformation of data used to perform XFM. This assumed that each injection volume of 0.4 mL would distribute as a uniform sphere centered on the position of the needle tip. Second, injected dye was visualized in relation to ex vivo TTC staining. Within each animal, injections formed vertices of complex polygons, analogous to individual stars within a constellation. In this way, corresponding locations of each injection were uniquely identified in necropsy and XFM images. For each approach, 2 observers independently classified injections as...
Quantitative accuracy of each injection was determined by comparing needle tip locations on x-ray images with the locations of the iron-induced susceptibility artifacts on T2*W-GRE images. The coordinates of the susceptibility artifacts were obtained by manually tracing the border of the artifact on each image and calculating the centroid of all the tracings for each injection. These coordinates were mapped into 3D x-ray space with the use of a second XFM registration transformation computed by matching external fiducial marker positions on the x-ray images and the second 3D MR marker acquisition. The target registration error,23 an in vivo measurement representing the difference between the XFM-predicted and actual injection locations, was calculated as the 3D distance of the circumferential and long-axis components of the vector between end-diastolic, XFM-derived injection sites and end-diastolic susceptibility artifact on T2*W-E images (Figure 4). Because XFM guidance can only assist the operator in determining the circumferential and long-axis placement of the injection catheter and because the radial position of the needle is dependent only on the needle length and the compliance of the myocardium at that location, we ignored the radial component of the vector when calculating the target registration error.

Data are presented as mean±SD of the targeting error for each animal, as well as in a cumulative distribution plot of the targeting error for all injections. Categorical targeting errors are analyzed in aggregate with the use of a κ statistic of intermodality agreement, weighted for multiple categories.24

The authors had full access to the data and take full responsibility for their integrity. All authors have read and agree to the manuscript as written.

Results

Survival, Procedural Details, and Complications

We performed a total of 130 injections in 12 animals with myocardial infarctions of varying size and age in both the left anterior descending and LCX territories. Relevant procedural data are summarized in Table 1. All animals survived to the study-specified euthanasia end point 24 hours after the injection procedure.

Two procedure-related complications occurred in a single animal (weight of 34 kg, below the limit of 40 kg recommended by the injection catheter manufacturer), 3 days after LCX infarction. A small pericardial effusion that was noted on preinjection MRI grew to moderate size afterward, without MRI or hemodynamic features of tamponade. Careful postmortem inspection did not reveal an obvious myocardial perforation site. This animal also suffered sustained ventricular tachycardia, which was successfully cardioverted.
Targeting of Endomyocardial Injection

Figure 3 illustrates the 3 key features of XFM-guided endomyocardial injections with the use of our method, namely, safety (Figure 3A, 3B), targeting (Figure 3C, 3D), and accuracy (Figure 3E, 3G). The percentage of injections categorized as within remote, peri-infarct, and infarct locations showed substantial agreement between the 2 modalities ($\kappa=0.69$; Table 2), whether determined by inspection of the dye staining on postmortem heart slices or determined from XFM-derived injection locations mapped onto the DHE images. Visual inspection of these distributions also compared favorably with the susceptibility artifacts seen on the postinjection T2*W-GRE imaging (Figures 4 and 5).

Quantitative in vivo measurement of the accuracy of XFM-guided injections was performed for 64 injections in those 7 animals with suitable-quality postinjection T2*W-GRE images. In 3 other animals, T2*W-GRE imaging failed to identify susceptibility artifacts because of inadequate MSC labeling with ferumoxides, and in 2 other animals Fe-MSC were not available. The overall in vivo targeting error was $3.2\pm2.6$ mm (interquartile range, 1.0 to 4.1). These data are presented for each animal and as a cumulative distribution plot in Figure 6. With the exclusion of the first animal, an outlier, the targeting error was $2.6\pm1.9$ mm (interquartile range, 1.0 to 3.6), including 58 injections.

Discussion

For clinical interventional procedures, guidance systems that facilitate delivery of therapies to targets on the basis of their physiological characteristics and anatomic location might improve procedural ease, safety, and efficacy. We have demonstrated the feasibility of accurate XFM-guided endomyocardial injections in a swine model of myocardial infarction in which DHE MRI was used to define the area of infarction. This method combines the strengths of x-ray and MRI to help operators navigate devices and deliver targeted therapy to the left ventricle based on direct assessments of regional myocardial thickness and tissue character.

Our study design allowed quantitative in vivo calculation of the target registration error, a measure of the intrinsic accuracy of our targeting method, by computing the distance between the coordinates of the susceptibility artifact due to injected Fe-MSC and the XFM-determined injection location for each injection. The average target registration error was $3.2\pm2.6$ mm, and 75% of our injections were within 4.1 mm of the XFM-predicted location. We are not aware of any comparable in vivo measurements of target registration error in the heart. We performed our measurement of target registration error by comparing injection needle positions with the location of corresponding iron-induced susceptibility artifacts on T2*W-GRE imaging rather than by comparing positions with the location of dye stains on the ex vivo TTC-stained heart slices. Ex vivo measurements of the distance of injection sites marked by dye staining to the infarct border on TTC-stained heart slices are difficult to quantify accurately for the following reasons. Myocardial wall thickness and cardiac dimensions are significantly altered postmortem. During intramyocardial injection, most
injectate disperses via venous, lymphatic, and intracavitary routes, which may cause dye dispersion away from the original injection site and produce the areas of confluent tissue dye staining observed ex vivo, confounding localization of discrete injection sites. In addition, it is difficult to precisely localize injection sites on surfaces of heart slices alone, especially in instances in which, in a given slice, the injection might span the cut surfaces. This makes it difficult to compare the target registration error data reported in the present study with previous reports; therefore, we performed the qualitative analysis summarized in Table 2.

In the present study, we fused static end-diastolic images of MRI-derived contours onto real-time x-ray throughout the cardiac cycle. Alternatively, we could “flash” overlay the ECG-gated MRI data only during the appropriate phase of the cardiac cycle during x-ray. For the purpose of demonstration, in 1 animal we fused ECG-gated, MRI-derived contours from multiple phases of the cardiac cycle onto matching phases of the cardiac cycle under x-ray (see online-only Data Supplement). This technique might further reduce targeting error.

A novel feature of XFM guidance is the use of a global map of left ventricular myocardial thickness in addition to the display of the infarct location during navigation and selection of an injection site. The thickness of the myocardial wall that can be injected safely varies according to the needle length of the injection system. The manufacturer of the injection system used in the present study suggested that myocardial thickness be at least 6 mm wherever injections are undertaken. The myocardial thickness map gives the operator a “go/no go” picture of the left ventricular endocardial surface by coloring regions with a safe myocardial thickness in green and unsafe regions in red. The safety threshold can be varied depending on the device chosen and may therefore be applied to different injection systems.

Our registration system can be implemented across vendor platforms. MR data are transferred in Digital Imaging and Communications in Medicine (DICOM) standard format. Real-time x-ray fluoroscopy data are captured with a video frame grabber or might be obtained directly from the vendor digital image chain. The only vendor-specific elements of our system

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Figure 3. XFM targeting of endomyocardial injections according to infarct location (blue surface) and regional myocardial wall thickness (colored green for wall thickness >6 mm and red for wall thickness ≤6 mm) in an animal with a chronic LCX infarct. These surfaces were displayed over x-ray in orthogonal projections (A and B). The catheter is positioned where the wall thickness is ≤6 mm (arrow); this location was therefore rejected. C and D demonstrate relocation of the injection catheter to a “safe” peri-infarct location with wall thickness >6 mm. After deployment of the needle, orthogonal x-ray views allow reconstruction of the injection location in 3D (yellow spot, numbered 4). Previous injection locations (yellow spots, numbered 1 to 3) are also displayed to help avoid overlapping injections. The 3D injection locations are also displayed superimposed on the prior DHE MRI (E and G). A postmortem TTC-stained heart slice (F), located between the MRI slices displayed in E and G, shows tissue dye-staining patterns that correlate well with the XFM-derived injection locations.
are the components that query the x-ray system for gantry and table position. These software subsystems could be customized for any vendor. In the future, our methodology, with the use of external fiducial markers to register images within a combined x-ray/MRI suite, could be applied to noncontiguous x-ray and MRI systems, as long as the fiducial markers are not displaced.

Comparison With Other MRI- or Computer Tomography–Based Methods
Our XFM system combines prior 3D data derived from MRI with real-time 2D projection data from live x-ray fluoroscopy. It has enhanced registration accuracy compared with simple superposition because it encompasses distortion correction for pincushion and sigmoidal error in the image intensifier–based x-ray images; gradient warp error in the MR images; and correction for varying x-ray FOV, gantry position, and table position. Other investigators have used less extensive multimodality image registration systems in catheter-based cardiovascular procedures.

In an imaging phantom, our system produces a target registration error of 0.99 mm. This compares favorably with target registration error measurements in a phantom made by Reddy and colleagues of 1.2 mm in a system combining prior MRI with electroanatomic-guided catheter injections and by Rhode and colleagues of 2.55 mm in a combined x-ray/MRI system using optical tracking of the patient table to register prior MRI with real-time projection, x-ray–guided injections. The experiments by Reddy and colleagues had the advantage of seeking to ablate specific myocardial targets created with prior iron injections mapped from MRI onto their electroanatomic guidance system. Their approach may be limited because natural catheter and myocardium geometries might favor initial targets to which the catheter intrinsically returns during the follow-up ablation experiment, exaggerating precision. We instead targeted infarct borders in an effort to avoid this catheter-geometry bias. The approach by Rhode and colleagues has the advantage of requiring no image segmentation or image-similarity computation to accomplish the registration. However, their method registers the imaging systems independent of the patient. Therefore, patient motion disrupts the image registration. Our system allows for updating registration due to patient movement because the registration is based on patient-specific markers. Moreover, Rhode and colleagues characterized the target registration error only for phantom experiments.

TABLE 2. Injection Location Classification Relative to Infarct as Determined by XFM and Ex Vivo Analysis in 9 Animals

<table>
<thead>
<tr>
<th>Ex Vivo Analysis</th>
<th>XFM</th>
<th>Within Infarct</th>
<th>Peri-Infarct</th>
<th>Remote</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within Infarct</td>
<td>16</td>
<td>7</td>
<td>1</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Peri-Infarct</td>
<td>4</td>
<td>29</td>
<td>4</td>
<td>37</td>
<td></td>
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<tr>
<td>Remote</td>
<td>3</td>
<td>1</td>
<td>23</td>
<td>27</td>
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<tr>
<td>Total</td>
<td>23</td>
<td>37</td>
<td>28</td>
<td>88</td>
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The weighted $\kappa$ statistic, reflecting the agreement between the 2 modalities, was 0.69.
Real-time interventional MRI\(^2\) is an attractive alternate approach for myocardial injection but is limited by the unavailability of conspicuous clinical-grade devices. This approach would otherwise be superior in that infarct and myocardial thickness can be displayed in real time rather than as a previously acquired roadmap, as in this study.

Comparison With Other Guidance Methods

Targeted endomyocardial injections have been performed with a number of guidance systems, including electroanatomic mapping\(^3,4,7,9,11–13,33,34\) and intracardiac\(^14\) and 3D surface echocardiography.\(^35\)

To date, electroanatomic mapping has provided the mainstay for targeting injection.\(^3,4,7,9,11–13,33,34\) In this technique, the 3D position of a custom catheter is located by automated analysis of the currents induced in the catheter tip as it moves within 3 intersecting magnetic fields surrounding the patient. At each point, endocardial voltage or periodic motion of the catheter tip can be recorded. From a fluoroscopically determined anatomic reference point, the catheter is moved along the endocardial surface to record multiple points. In general, multiple measurements are required to provide an adequate interpolated 3D map, usually color coded according to the measured unipolar voltage. This can make electroanatomic mapping time-consuming, even in experienced hands, although newer iterations may make the process less laborious. Electroanatomic mapping does not provide direct assessment of myocardial wall thickness to the operator during the procedure. Furthermore, thresholds of unipolar voltage thought to represent myocardial viability are controversial.\(^36,37\) The ability of this technique to identify areas of hypoperfusion and myocardial viability does not consistently compare well with conventional imaging techniques.\(^38,39\) Consequently, considerable expertise and experience are required to interpret these maps reliably. Mapping confers incremental risk,\(^9\) requires multiple steps, and appears to provide less structural information than MRI.

Both intracardiac\(^14\) and 3D\(^35\) echocardiographic techniques have been used to determine the success of endomyocardial injections. Both have been used in conjunction with x-ray. Apposition of the catheter to the endocardium in real time can be visualized directly, and mixing the injectate with echo contrast can provide direct confirmation of the success of injection. In addition, the temporal resolution offered by echocardiography provides real-time determination of the myocardial wall thickness at injection sites and immediate identification of injection-related complications. These approaches are promising but are currently limited by their inability to provide complete volumetric imaging of the heart.

Conclusion

We report the successful application of external fiducial marker–based 3D to 2D registration and fusion of x-ray and MR images for targeted endomyocardial injection in a model of myocardial infarction. The target registration error was 3.2±2.6 mm, with 75% of injections within 4.1 mm of the XFM-predicted location. Our approach likely can be applied without the need for a combined x-ray/MRI suite, without additional optical tracking or electroanatomic mapping hard-
ware, and does not restrict operators in their choice of catheters or devices. It is potentially generalizable to other endovascular applications and can be combined with electro-anatomic mapping, if desired. The present data suggest that the safety and efficacy of this approach for performing targeted endomyocardial delivery should be evaluated further in the clinical setting.

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Disclosures
None.

References


CLINICAL PERSPECTIVE

Magnetic resonance imaging (MRI) provides superb imaging of heart function and pathology, but MRI-guided interventional procedures remain technically challenging for want of clinical-grade catheter devices. X-ray fluoroscopy, despite its high temporal and spatial resolution, provides only limited depiction of myocardial viability and function and appears inadequate to guide spatially precise myocardial injections. With the use of skin markers to register image data from the 2 separate imaging modalities and to correct for patient movement, this work combines 3-dimensional features of interest with real-time 2-dimensional x-ray imaging, called x-ray fused with MRI (XFM). This report demonstrates the feasibility of accurate XFM-guided endomyocardial injections in a pig model of myocardial infarction. The x-ray permits simple manipulation of injection catheters; the superimposed MRI data provide information about the spatial position of the myocardial infarcts and about the location of “danger zones” where the myocardium is considered too thin for safe injection. This method combines the strengths of x-ray and MRI to help operators navigate devices and deliver targeted therapy to the left ventricle. XFM may have other applications in enhancing the x-ray–guided catheter treatment of heart disease.
X-Ray Fused With Magnetic Resonance Imaging (XFM) to Target Endomyocardial Injections: Validation in a Swine Model of Myocardial Infarction
Ranil de Silva, Luis F. Gutiérrez, Amish N. Raval, Elliot R. McVeigh, Cengizhan Ozturk and Robert J. Lederman

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