MYOCARDIAL REGENERATION

MRI Evaluation of Local Myocardial Treatments: Epicardial Versus Endocardial (Cell-Fix Catheter) Injections

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Aims: We compared two procedures for local myocardial treatment: transepicardial versus transendocardial catheter injection. Transepicardial injections were performed under direct surgical visualization whereas transendocardial injections were performed using electrophysiological guidance.

Methods: A left ventricle (LV) myocardial infarction (MI) was surgically created in 14 sheep. At 3 months, gadolinium was injected IV followed by the injection of super paramagnetic iron oxide (SPIO) into MI. Animals were divided into two groups: transepicardial injection (Group I) versus transendocardial (Group II) using “Cell-Fix” catheter injection. This catheter was developed to identify by electrophysiology the infarcted area and to stabilize injections suctioning the device to the endocardium. Postgadolinium delayed-enhancement magnetic resonance imaging (MRI) was performed to stain the infarct size. SPIO injections were used to assess the magnitude of the treated area. The ratio between SPIO black stained treatment areas and white gadolinium stained infarcted areas was calculated using MRI.

Results: The electrophysiological recordings by the catheter for the MI versus normal LV wall were: R wave amplitude 4.16 versus 12.08 mV (P = 0.003), slew rate (slope of the signal) 0.36 V/s versus 1.04 V/s (P = 0.008). The ratio of the SPIO diffusion into the MI was 41.2 ± 8.1% for surgical and 63.7 ± 8.2% for percutaneous endocardial injections (P = 0.0132).

Conclusion: MRI allows evaluation of the extent of local myocardial treatments. The differences shown between epicardial and endocardial injections concerning the distribution of SPIO can be justified by the methodology of injection and by a more precise MI detection by electrophysiology. In conclusion, electrophysiological recordings to guide injections is superior to direct surgical visualization in terms of injecting into infarcted tissue. (J Interven Cardiol 2007;20:188–196)

Introduction

Several promising therapies for myocardial regeneration in ischemic heart disease have been developed, including cell transplantation, delivery of growth factors and genes, and cardiac tissue engineering. New technologies for cell implantation and local molecular release derived from interventional cardiology procedures are emerging. Intracoronary, endoventricular, and thoracoscopic transepicardial cell delivery procedures for therapeutic angiogenesis and myogenesis have been performed. Periodically repeated injections could be necessary to progressively reduce the infarct scars.

Local treatments for myocardial regeneration highlight the need for efficient and practical delivery methods to the heart. Whatever the transmyocardial route, whether via the epicardium or endocardium, one must mention the lost and high mortality of cells. This mortality is probably linked to the injection itself and the...
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Table 1. Methods of Stem Cell Implantation

<table>
<thead>
<tr>
<th>Cell delivery</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tr>
<td>Epicardial (surgical)</td>
<td>Accuracy of injection</td>
<td>Surgical and anesthetic risks</td>
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<tr>
<td>Endocardial (endoventricular catheter)</td>
<td>Less invasive</td>
<td>Difficulty in localizing the target area</td>
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<td>Intra coronary</td>
<td>Periodical injections</td>
<td>Loss of cells</td>
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<tr>
<td></td>
<td>Easy</td>
<td>Migration of cells to the myocardium unknown</td>
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<tr>
<td></td>
<td>Low cost</td>
<td>Risk of microembolizations</td>
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poor vascular supply of chronic postinfarct scars. For this reason it is important to deliver the maximum of available cells. Surgical cell implantation was proposed associated with other procedures (e.g. CABGs); this approach is performed into well-exposed ischemic areas, permitting multiple injection points within and principally around the infarct. Video-assisted thoracoscopic and robotic cell implantation is currently being investigated. Advantages and drawbacks of cell delivery approaches are summarized in Table 1.

Magnetic resonance imaging (MRI) techniques can be used to assess accurately infarcted myocardial areas. Acute and chronic myocardial infarctions (MI) have been demonstrated as hyperenhanced regions on delayed contrast-enhanced MRI (DE-MRI). It has been proposed that the combined use of extracellular MR contrast-media agents and iron oxide particles can be used to monitor the distribution of locally injected therapeutic agents. The former (gadolinium) produces hyperenhancement on the scar tissue (high signal in white), while the iron particles (SPIO) cause hypoenhancement (signal void in black).

In the present study, we performed a surgical model of coronary artery occlusion in sheep. We focused our attention onto two subjects: (1) the feasibility of targeted myocardial injections of iron oxide particles into the infarcted myocardium, using a transendocardial catheter versus surgical transepicardial injections, and (2) the feasibility of DE-MRI to evaluate the size of the infarcted area and the distribution of locally injected agents.

Methods

Animal Model. In 14 Rambouillet sheep weighing 25–38 kg (mean 32 ± 3.2 kg), a left ventricle (LV) myocardial infarct was surgically created. All animals received care in compliance with the European Convention on Animal Care (Journal of the European Community L 358/1, November 24, 1986). After preoperative medication with Vetranquil 1 mg/kg (Acepromazine, Ceva, Libourne, France) and induction of anesthesia with Diprivan 6 mg/kg (Propofol, AstraZeneca, Rueil-Malmaison, France), animals were intubated and mechanically ventilated with an Aestiva/5 system (Datex-Ohmeda, Helsinki, Finland). Anesthesia was maintained with oxygen inhalation of Forene 2–3% (Isoflurane, Abbot, Rungis, France). The electrocardiogram was monitored during operation. A central venous line was placed through the external jugular vein for administration of fluid and drug infusions.

Left thoracotomy was performed at the level of the 5th intercostal space, and the heart was exposed. To reduce the risk of ventricular fibrillation, a continuous IV perfusion (2 mg/kg per hour) of Xylocaine 1% (Lidocaine, AstraZeneca) was performed during the entire surgical procedure. In all animals the distal left anterior descending and second diagonal coronary arteries were surgically ligated using a 3/0 coated braided polyester suture. Afterwards, the apex and the distal 1/3 of the anterior wall of the LV appeared as a pale area. At the end of the operation, a chest drain was placed into the left hemithorax and the wound was closed. Serum troponine I levels were checked 48 hours after the MI. Postoperative sheep treatment consisted in cefazolin injected intramuscularly at 1 g per day over 5 days.

Treatment Groups. Fourteen to 16 weeks after myocardial infarction the surviving animals were randomized in two groups, to receive 1% super paramagnetic iron oxide (SPIO) solution (Endorem; Guerbet, Aulnay-sous-Bois, France), using either surgical epicardial injections (Group I, n = 5) or percutaneous endocardial injections (Group II, n = 5). The density of the SPIO solution was compared to the density of a control myoblast cell culture solution (100 million cells in 1 ml of culture medium) and to the density of a control VEGF-isoform 165 solution (200 µg diluted in 1 ml of PBS, phosphate-buffered saline). Intravenous gadolinium solution (Dotarem; Guerbet, Aulnay-sous-Bois, France) was administered to all the sheep at the dose of 0.4 ml/kg 10–15 minutes prior to the SPIO administration. Thereafter animals were sacrificed right after SPIO injections, by lethal IV injection of 20 cc of a 10% KCl solution. Heart harvesting was the last surgical procedure (Fig. 1), followed by heart freezing.
at a temperature between –20 and –25 °C. Ex vivo MRI studies were then performed.

**Epicardial Injection of SPIO.** In Group I animals, at 3 months of infarction a median sternotomy was performed under general anesthesia as previously described. Pericardial adhesions were gently divided; during this redo surgery the infarcted area was not clearly visualized. Intravenous gadolinium was injected, and 15 minutes later the SPIO solution was locally injected in the scar center and periphery, directly with a syringe and a 27G needle. In the beating heart, five 0.3 cc injections of the 1% SPIO solution were administered at a manual pressure (without using pressure gauges), trying to space more than 5 mm between injections. Criteria to guide the epicardial injections were the ventricular surface discoloration and hypokinesia. In order to avoid regurgitation of the SPIO solution (channel leakage) we used finger compression for 1 minute; however, some liquid may have been lost during this maneuver. Finally, a lethal IV injection of KCl was performed and the hearts were harvested and frozen.

**Cell-Fix Catheter Procedure and Percutaneous Injection of SPIO.** A new endoventricular catheter called “Cell-Fix” was evaluated (European Patent 04292756.6, U.S.A. Patent A20050113760). This device allows simultaneously infarct detection and cell and/or growth factors delivery.

In Group II animals, at 3 months of infarction, access was obtained through the femoral artery. The Cell-Fix catheter was inserted and placed into the LV cavity 100 IU/kg heparin was given. After a biplane LV angiogram (left anterior oblique [LAO] 60° and right anterior oblique [RAO] 30°) was obtained, an outline of the LV chamber was drawn on transparent tabloids that were taped to the fluoroscopy monitors. Then a unipolar electrophysiologic map of the akinetic infarcted LV region was obtained using the Cell-Fix catheter needle, which was coupled to an AV Pacing System Analyzer (Model 5311, Medtronic, Minneapolis, USA). The measurements of the local electrical conductivity of the tissue were used to determine the potential activity of the myocardium. The measured conductivity was referred as the “myocardial depolarization signal.” An infarcted area was considered to be diagnosed if the two following conditions were simultaneously satisfied: (a) an endocavity ventricular electrogram depolarization slew rate of less than 0.5 volts per second and (b) an endocavity ventricular electrogram amplitude of less than 5 millivolts, which constitutes a “microvoltage.” The gradient “slew rate” is the speed rate of ascent or descent of a gradient from zero to its maximum amplitude, either positive or negative. To avoid the risk of ventricular fibrillation of the sheep infarcted hearts, we did not perform pacing tests. In fact, these animals are prone to irreversible ventricular arrhythmias during cardiac surgery.

The mapping of the LV was done by advancing the catheter tip into the heart chamber until contact was made with the heart wall. The needle was inserted and the position was recorded and saved. The catheter tip was then moved to another position in contact with the heart wall and again the position was recorded and saved. The needle was retracted during insertion of the catheter into the heart and removal therefrom as well as while the catheter was being navigated from point to point within the heart, but extended out of the distal end of the catheter for electrophysiological assessments and to deliver the SPIO inside the ventricular wall. Healthy heart tissue was identified by strong electrical signals in combination with strong fluoroscopic and echocardiographic displacement of the catheter and LV wall. Ischemic areas to be treated were marked on the map to be injected with the SPIO solution.

With the 8F Cell-Fix injection catheter, transendocardial SPIO injections were made. The catheter ends in a 27-gauge retractable needle (Fig. 2). Depending on the average wall thickness of the target region (checked by transthoracic echocardiography performed during the case), the needle length was set at 3–5 mm when the catheter tip had a 90° curve. The Cell-Fix catheter comprises a tip deflection mechanism for steering and
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Figure 2. Cell-Fix catheter in the unfolded position. The catheter includes a retractable needle (A) and a fixing “sucker” system in the form of a polyurethane umbrella (α) to be fixed by vacuum (G) to the endocardium.

Figure 3. Cell-Fix catheter in the folded position.

navigating the distal end (this system is operated by pull-wires) and a fixing “sucker” system to the endocardium, in the form of a suction cup. This “umbrella” can be retracted inside the exterior tube of the distal part of the catheter (Fig. 3). The suction cup has the advantage to be mobile between folded and unfolded positions with the injection needle in retracted position; this feature also facilitates extraction of the catheter on completion of the intervention. The catheter comprises an evacuation conduit opening into the interior of the suction cup, adapted to be connected to a vacuum system (Figs. 2 and 3). After fixing the suction cup to the endocardium of the infarcted zone, a high suction force increases the stability of the catheter in this position.

By connecting a 1-ml syringe to the injection port, the Cell-Fix catheter was preloaded with the SPIO solution. To establish a stable endomyocardial contact on the distal site of the catheter, the scar was stabilized by vacuum at the moment of the injection. Whenever a regular ECG rhythm resumed with persisting stable endomyocardial contact, the unipolar electrophysiologic measurements and five 0.3 cc injections of 1% SPIO solution were performed. Spacing between injection sites was approximately 5–10 mm, according with mapping. To obtain a perpendicular catheter tip position to the endocardial surface, we attempted to determine orientation based upon fluoroscopy.

MRI Protocol. All 10 ex vivo hearts were defrosted to 20 °C prior to MR imaging. MRIs were performed using a 1.5 T scanner (Signa, General Electric Healthcare, Milwaukee, USA), using a head coil. Inversion recovery (IR) sequences were used to null the MR signal of the normal left ventricular (LV) myocardium. The acquisition parameters were the following: repetition time (RT): 2000 ms, echo time (ET): 102 ms, inversion time (IT): 200–250 ms, slice thickness: 2 mm, spacing (slice gap): 0.0, echo train length: 18, matrix: 250 × 250, number of excitation (NEX): 1.00, field of view (FOV): 18 × 18 mm².

Prior to this protocol, ex vivo sheep heart MRI studies helped us in choosing the appropriate TI to null the LV normal myocardium signal in such a model. We
also tested the effect of different SPIO concentrations with these IR sequences to choose the appropriate SPIO concentration.

**Analysis of MRI Data.** Infarct scars enhance vividly 10–15 minutes after the administration of intravenous gadolinium. This enhancement represents the accumulation of gadolinium in the extracellular space, due to the loss of membrane integrity in the infarcted tissue. In our study, myocardial infarction was defined as the finding of hyperenhancement in the myocardium on MRI. At each target site, the injected SPIO created a susceptibility artifact and appeared dark (Fig. 4).

Hypoenhancement within the hyperenhancement area was first scored independently in a blinded fashion by one experienced radiologist (AA) and one cardiac surgeon (MES) on the short axis views. Both hyper- and hypoenhancement areas were then measured by manual planimetry slice by slice (Figs. 5 and 6). The volumes in one slice were obtained by multiplying the corresponding average by the slice thickness (2 mm). The total volumes were calculated by summation of all slice volumes of one heart, in order to evaluate the distribution volume of the SPIO within the scar volume. By dividing the sum of the hypoenhancement volumes by the sum of infarct volumes we obtained a result that could represent the SPIO diffusion average ratio for each infarct model.
**Statistical Analysis.** The results were expressed as a percentage or a median ± deviation from the median. The comparison between two groups was carried out through a Student’s t-test. The differences were considered significant when \( P < 0.05 \).

**Results**

During the creation of the infarction three sheep died, due to ventricular fibrillation refractory to pharmacological and electric treatments; one sheep died at one month due to sudden death. Postinfarct (48 hours) serum troponin levels were 56 ± 7 ng/mL. The density of the injected SPIO solution was equivalent to the density of the control skeletal myoblast cell culture solution and to the density of the control VEGF solution (1.001 vs 1.003 vs 1.001; density of water 0.994).

The electrophysiological studies of the infarcted and healthy cardiac muscle showed the discrepancy between these tissues. Electrical recordings by the Cell-Fix catheter for the infarcted areas versus normal LV wall were: R wave amplitude 4.16 ± 1.6 mV versus 12.08 ± 3.2 mV (\( P = 0.003 \)), slew rate 0.36 ± 0.08 V/s versus 1.048 ± 0.31 V/s (\( P = 0.008 \)) (Table 2).

The MRI studies are summarized in Table 3. The median values of SPIO diffusion in the infarcted area was 41.2 ± 8.1% for the transepicardial injections (Group I), and 63.7 ± 8.2% for the transendocardial catheter injections (Group II) (\( P = 0.0132 \)).

In some animals treated by surgical epicardial injections (Group I) we found SPIO product outside the infarcted area (in normal myocardium) (Fig. 7). This can be explained by the fact that we used a surgical model of myocardial infarction. It meant that we needed a second intervention for the epicardial injections of SPIO solution. During this redo surgery the infarcted area was not clearly visualized for the injections. This extrainfarct SPIO volume was not measured.

The better results in Group II can also be explained by the efficiency of the electrophysiological method used for the detection of the infarcted area by the Cell-Fix catheter.

**Table 3.** SPIO Diffusion Inside the Delayed-Enhancement Areas (i.e., the infarcted areas). A Ratio of the Locally Injected Agent Diffusion in the Scar Was Obtained by Division of the Hypoenhancement Volume (SPIO) within the Hyperenhancement Infarcted Volume (Gadolinium)

<table>
<thead>
<tr>
<th></th>
<th>Gadolinium stained infarct volume (mm³)</th>
<th>SPIO diffusion volume inside the infarct (mm³)</th>
<th>Vol. SPIO/Vol. infarct (mean diffusion %)</th>
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<tbody>
<tr>
<td>Group I</td>
<td>374.4 ± 35.4</td>
<td>156.4 ± 14.8</td>
<td>41.2 ± 8.1</td>
</tr>
<tr>
<td>Group II</td>
<td>316.8 ± 21.3</td>
<td>201.6 ± 12.4</td>
<td>63.7 ± 8.2</td>
</tr>
<tr>
<td>P</td>
<td>0.0765</td>
<td>0.0216</td>
<td>0.0132</td>
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**Discussion**

Investigators are facing significant technological challenges in the development of novel biologic strategies and improved local delivery systems for the treatment of ischemic HF. Local myocardial treatments using cells, growth factors, and genes for myocardial regeneration have been developed.10–14 New
technologies for cell implantation derived from interventional cardiology procedures are emerging. Intracoronary and endoventricular catheter-based cell delivery procedures for therapeutic angiogenesis and myogenesis have been performed.15,16

Percutaneous selective coronary artery cannulation for cell injection can be used for myocardial regeneration.4 This intravascular treatment is based on the potential migratory properties of some cells which retain their ability to cross the basal vascular lamina. The coronary artery delivery approach should be restricted to patients with a patent artery branch to the infarcted area and exclusively for the delivery of mononuclear bone marrow cells, since intracoronary delivery of skeletal myoblasts and bone marrow mesenchymal cells could provoke microemboli. In fact, the mesenchymal cell and myoblast sizes (length 25–30 µm) and the myoblast shape (irregular, stellar, and spindle-shaped) are more prone to embolizations than bone marrow mononuclear cells (spherical and disc-shaped, diameter 8–18 µm).12,13 In addition, because the core of the infarct area is nearly avascular and the infarct border zone has a relatively poor vascular network, these regions can not be reached by cells delivered by intracoronary approach. Importantly, following vascular delivery a big number of cells have been localized in other organs such as the lungs, the spleen, or the liver.17,18

Another approach is to reach the infarcted area through the endocardium using an endoventricular catheter approach. The real-time mapping of the diseased myocardium and the stability and accurate targeting by delivery systems during transcatheter treatment represent a challenge.5 The main difficulty of endoventricular delivery using the presently available systems is the instability of the distal tip during electric mapping and cell delivery. The quantity of the injected cells in the target infarcted area is unknown, and the success is largely dependent on many technical considerations, namely, the risk of cell loss at the moment of injection and the precise localization of the postischemic scar and the peri-infarct areas. To address these problems, a new device was developed including a distal vacuum system to stabilize the treated area, and a system of simultaneous mapping and injection. The Cell-Fix catheter offers the possibility to measure in real time endocardial electrical potentials, in order to distinguish healthy from pathologic myocardium.

The imaging aspect by itself stresses the importance of the use of cardiac MR imaging in guiding and assessing intramyocardial therapy.19–26 The present study evaluates the feasibility of ex vivo DE-MRI to assess the distribution of locally injected SPIO within the infarcted myocardium. Accurate noninvasive detection and measurements of infarct size using extra cellular MR-contrast media have already been reported.7,27 SPIO has also proven to be a sensitive contrast agent for magnetic resonance imaging, already used to detect in vivo iron-labeled stem cells inside the myocardium. However, current MRI techniques lack the ability to quantify the SPIO and are prone to artifacts from magnetic field inhomogeneity. The use of 1% SPIO solution and the choice of an appropriate TI for the inversion recovery sequences enabled a better quantitative detection of SPIO, minimizing the susceptibility artifacts. Dick et al.28 recently used an active catheter tracking system for injection of iron-labeled stem cells into the myocardium. But this remains experimental, on small animals, and we should keep in mind that after one decade of cell therapy experimentation and first clinical trials, there are some limiting criteria concerning the type of patient or the infarct size to apply such a new therapy.1,29,30 When used in experimental animal models, gadolinium and SPIO-MRI have demonstrated accuracy and efficacy in distinguishing infarcted from healthy areas and to label the injected cells, but only in living animals. This technique requires the availability of the MRI machine, whose main task is usually clinical diagnosis. Ex vivo MRI study offers the possibility to evaluate large-animal hearts that approximate to human cardiac size. Moreover, it allows the possibility to simultaneously study several hearts in the same session.

The combined use of gadolinium contrast-media and iron oxide particles provided an estimation of the spatial distribution of injected SPIO within the infarcted area. The obtained values demonstrated differences in the diffusion of the injected SPIO through the infarcted myocardium using the Cell-Fix catheter as compared with direct needle injection; this could be justified by the methodology of our experimental study. The infarct detection technique is one of the major determinants to effectively locally treat infarcted scars.31–34 Furthermore, a recent study related to cellular cardiomyoplasty demonstrated a massive mechanical loss of microspheres with direct intramyocardial injection in the beating heart. Mechanical leakage and washout may account for a major portion of the injected solutions through the epicardial surface.35
The diagnostic-therapeutic catheter “Cell-Fix” is a second-generation system including a method to identify by electrophysiology the infarcted area and stabilize by vacuum the scar for the delivery of cells. In addition this catheter enabled the myocardium mapping to detect the infarct zone and ensure injection in the scar. In spite of these advantages, only 63.7% of our experimental myocardial infarction was able to be treated by the catheter. The Cell-Fix device offers also the possibility to employ cells of relativey large size and to inject biomaterial scaffolds to improve cell survival and induce neovascularure formation in myocardial infarction.\textsuperscript{3,36}

**Study Limitations**

The model used to create infarct was surgical (coronary ligation). The SPIO injection technique requires a new surgical intervention only in one group, i.e., for epicardial injection. Localization of the infarct zone was not easy with this approach, resulting in less accurate injections. Some injections were performed not only into the scar center but in the periphery. The endocardial injection using the Cell-Fix catheter did not require a new surgical intervention. In future experiments and to avoid reoperating on the animals, it could be important to perform the infarction model using non-surgical approaches, e.g., catheter-based coronary occlusion with coils or sponges. We performed “ex vivo” MRI studies; this approach was not perhaps an ideal model but permitted the use of our hospital facilities to evaluate animal hearts.

The present study was performed using SPIO injections instead of cells or growth factors. The goal was to quantify by MRI the amount of the treated area. In future investigations it could be interesting to implant cells using the Cell-Fix catheter and evaluate the efficacy by histological studies. These aspects were not assessed in our study and will require further investigations.

**Conclusions**

This study demonstrates that MRI investigations are useful to evaluate and compare the efficiency of different local myocardial injection techniques. Our results reflect the efficacy of the electrophysiological method used for the detection of the infarcted area by the Cell-Fix catheter. MRI studies showed that differences in the distribution of iron oxide particles (SPIO) within the infarcted scar depend on the injection method used, i.e., transepicardial or transendocardial. The surgical model of myocardial infarction used in this study required performing a second operation for local myocardial treatment through the epicardium. During this redo the infarcted area was not clearly visualized for the injections of the SPIO solution, resulting in less accurate injections of the product.

**References**