Molecular Magnetic Resonance Imaging of Myocardial Angiogenesis After Acute Myocardial Infarction

Marlies Oostendorp, PhD; Kim Douma, PhD; Allard Wagenaar, BSc; Jos M.G.M. Slenter, BEng; Tilman M. Hackeng, PhD; Marc A.M.J. van Zandvoort, PhD; Mark J. Post, MD, PhD; Walter H. Backes, PhD

Background—Angiogenesis is a natural mechanism to restore perfusion to the ischemic myocardium after acute myocardial infarction (MI). Therapeutic angiogenesis is being explored as a novel treatment for MI patients; however, sensitive, noninvasive in vivo measures of therapeutic efficacy are lacking and need to be developed. Here, a molecular magnetic resonance imaging method is presented to noninvasively image angiogenic activity in vivo in a murine model of MI with cyclic Asn-Gly-Arg (cNGR)–labeled paramagnetic quantum dots (pQDs). The tripeptide cNGR homes specifically to CD13, an aminopeptidase that is strongly upregulated during myocardial angiogenesis.

Methods and Results—Acute MI was induced in male Swiss mice via permanent ligation of the left anterior descending coronary artery. Molecular magnetic resonance imaging was performed 7 days after surgery and up to 2 hours after intravenous contrast agent administration. Injection of cNGR-pQDs resulted in a strong negative contrast that was located mainly in the infarcted myocardium. This negative contrast was significantly less in MI mice injected with unlabeled pQDs and in sham-operated mice injected with cNGR-pQDs. Validation with ex vivo 2-photon laser scanning microscopy revealed a strong colocalization of cNGR-pQDs with vascular endothelial cells, whereas unlabeled pQDs were mostly extravasated and diffused through the tissue. Additionally, 2-photon laser scanning microscopy demonstrated significant microvascular remodeling in the infarct/border zones compared with remote myocardium.

Conclusions—cNGR-pQDs allow selective, noninvasive detection of angiogenic activity in the infarcted heart with the use of in vivo molecular magnetic resonance imaging and ex vivo 2-photon laser scanning microscopy. (Circulation. 2010;121:775-783.)

Key Words: angiogenesis ■ magnetic resonance imaging ■ microcirculation ■ myocardial infarction

Neovascularization through angiogenesis provides a natural repair mechanism to restore perfusion of ischemic tissue after myocardial infarction (MI) and is a determinant of postinfarct remodeling and patient prognosis. Consequently, stimulation of angiogenesis (eg, via administration of growth factor genes or proteins) seems an attractive option to reduce ischemic injury in the myocardium after MI, especially in patients in whom traditional revascularization failed. Although promising results were obtained with therapeutic angiogenesis in animal models of MI, double-blind placebo-controlled clinical trials have been disappointing. These contradictory outcomes may be related to the choice of therapeutic agent, route of administration, selection of clinical trial populations, and absence of a sensitive, validated in vivo (surrogate) marker of therapeutic efficacy. Readout tools to evaluate the efficacy of angiogenic therapy should be noninvasive, repeatable, and sensitive to early therapeutic responses. Traditional noninvasive imaging techniques, including nuclear imaging, echocardiography, or magnetic resonance imaging (MRI), detect the physiological consequences of angiogenesis (ie, improved myocardial perfusion or global cardiac function) that occur relatively late during the angiogenic process. Therefore, their applicability to the evaluation of myocardial angiogenesis remains to be investigated. In contrast, molecular imaging potentially permits early detection of myocardial angiogenesis (ie, before changes in perfusion and/or function become apparent) via specific detection of upregulated disease markers with tar-
geted contrast agents. Only a few reports are available that describe molecular imaging of cardiac angiogenesis with nuclear or optical imaging.

We investigated the applicability of molecular MRI for detecting angiogenic activity noninvasively in vivo in the mouse heart after acute MI. Clinical advantages of MRI include excellent soft tissue contrast and spatial resolution and the absence of ionizing radiation. Compared with nuclear and optical imaging, the detection limit for molecular markers is significantly higher for MRI.

Previously developed Gd-labeled paramagnetic quantum dots (pQDs) conjugated with the cyclic tripeptide Asn-Gly-Arg (cNGR) were chosen as the contrast agent to selectively image the myocardial neovasculature. cNGR homes specifically to CD13, a membrane-bound aminopeptidase that is strongly upregulated on endothelial cells of angiogenic vessels to CD13, a membrane-bound aminopeptidase that is

Contrast Agent

Streptavidin-coated cadmium-selenium–based QDs with 585-nm emission wavelength (1 μmol/L in borate buffer, pH 8.3) were purchased from Invitrogen (Breda, the Netherlands). cNGR-labeled pQDs were freshly prepared for each experiment by mixing 100-μL QDs with biotinylated Gd-wedge, a polylysine dendrimer comprising 8 Gd-DTPA moiesies, and biotinylated cNGR peptide in a molar ratio of 1:24:6 as described previously. Biotinylated Gd-wedge and cNGR were synthesized with TBMW solid-phase peptide synthesis. Unlabeled pQDs were prepared similarly but without cNGR. The maximum number of cNGR ligands and Gd-DTPA groups per cNGR-pQD particle was 6 and 192, respectively, resulting in a total administered dose of ~0.5 μmol Gd/kg body weight. At 7 T, the intrinsic longitudinal and transverse relaxivities were 7.1 and 49 (mmol/L Gd)−1·s−1, respectively. Furthermore, the susceptibility shift was 3.1×10−7 ppm per 1 mmol/L Gd (see the Methods section in the online-only Data Supplement).

The plasma half-lives of cNGR-labeled and unlabeled pQDs were 7.1±1.5 minutes and 7.1±1.4 minutes, respectively (P=0.5), as estimated in tumor-bearing mice with dynamic MRI (not shown). Similar systemic behavior is expected in the present animal model of MI. Both contrast agents are cleared from the circulation mainly by the liver and spleen.

Animal Model

All of the experiments were approved by the institutional ethics committee on animal welfare. MI was induced in 12-week-old male Swiss mice via permanent occlusion of the left anterior descending coronary artery (see the Methods section in the online-only Data Supplement). MI mice were divided into 2 groups and received either cNGR-pQDs (n=6) or unlabeled pQDs (no targeting ligand; n=4). An additional group of sham-operated mice was injected with cNGR-pQDs (n=5). MRI experiments were performed 7 days after surgery because the level of angiogenic activity is maximal at this time point.

Before MRI, mice were anesthetized with 1% to 2% isoflurane (Abbott Laboratories Ltd, Queensborough, UK) in medical air. The left jugular vein was exposed, and contrast agent was injected with a 30-gauge needle. Next, the wound was closed and the mouse was transferred to an animal bed with a built-in anesthesia mask. Neonatal ECG electrodes (3M, St Paul, Minn) were placed on the paws of the right front leg and left hind leg and connected to an MR-compatible small-animal monitoring system (SA Instruments, Stony Brook, NY). Temperature and respiratory rate were also continuously monitored.

MRI Protocol

MRI was performed on a 7-T Bruker Biospec 70/30 USR (Bruker Biospin GmbH, Ettlingen, Germany) with the BGA12-S mini-imaging gradient (maximum gradient strength, 720 mT/m·s; slew rate, 6000 T/m·s−1) interfaced to an AVANCE II console. Images were acquired with a quadrature volume resonator with a 3.5-mm inner diameter. First, a bright-blood cine image with 10 cardiac phases was recorded in the horizontal long-axis view with a retrospectively self-gated protocol (IntraGate, Bruker Biospin GmbH; see Movies I and II in the online-only Data Supplement). Second, a short-axis, apical self-gated cine image was recorded, passing through the infarction as seen on the long-axis view (Figure 1). This short-axis orientation was applied for all subsequent images. Next, ECG-triggered, respiratory-gated, end-diastolic bright-blood gradient-echo images were recorded as follows: repetition time, 15
ms; echo time, 2.9 or 6.0 ms; flip angle, 50°; slices, 1; thickness, 1 mm; signal average, 4; field of view, 4×4 cm²; matrix, 256×256; in-plane resolution, 0.16×0.16 mm²; and acquisition time, ~3 minutes. Image acquisition was started 30 minutes after contrast agent administration and was repeated every 10 minutes up to 2 hours after contrast, after which point mice were euthanized by cervical dislocation. Fifteen minutes after death, mice were repositioned in the MRI scanner, and postmortem short-axis spin-echo images were recorded (repetition time, 1300 ms; echo time, 9.1 ms; slices, 5; thickness, 1 mm; field of view, 4×4 cm²; matrix, 256×256), covering most of the left ventricle (LV). These images allowed more accurate infarct size determination than in vivo images because the healthy myocardium significantly thickens postmortem, whereas the infarcted myocardium stays thin. Furthermore, the T1, T2, and T2* relaxation times were quantified postmortem (see the Methods section in the online-only Data Supplement). Finally, hearts were excised, embedded in optimal-cutting-temperature compound (Sakura Finetek Europe, Zoeterwoude, the Netherlands), snap-frozen in ice-cold 2-methylbutane (Acros Organics, Geel, Belgium), and transferred to liquid nitrogen. Tissues were stored at −80°C until TPLSM measurements.

MRI Analysis
All of the data processing was performed in Matlab (MathWorks, Natick, Mass) unless stated otherwise. Regions of interest were drawn manually in MRicro.21

Ejection Fraction
Orthogonal long- and short-axis cine images were used to determine the LV ejection fraction (EF). Therefore, end-diastolic and end-systolic volumes were estimated with the biplane ellipsoid model,22 and the EF was calculated as follows: (EDV−ESV)/EDV×100%, where EDV is end-diastolic volume and ESV is end-systolic volume.

Infarct Size
The infarct size was defined as the percentage of the LV surface that was infarcted and was determined as follows. The LV circumference was measured in each slice of the postmortem images with ImageJ (National Institutes of Health, Bethesda, Md) and multiplied by the slice thickness to generate the total LV surface. The size of the infarction was measured analogously. Next, the infarct area was divided by the LV area to obtain the infarct size.

Thickness of the Septum
To investigate the development of myocardial hypertrophy, the thickness of the septum during end systole was determined on the long-axis cine images. Measurements were performed manually in ImageJ at the thickest part of the septum.

Myocardial Segmentation
To estimate local cardiac function, the LV myocardium was divided into 8 radial segments13 (Figure 1B). In each segment, the contractile function was assessed via the endocardial radial shortening.23 To this extent, endocardial contours were drawn on the end-systolic and end-diastolic short-axis cine frames and the radius (r) was defined as the distance between the endocardial border and the LV center. The endocardial radial shortening was then calculated as (rED−rES)/rES×100% and plotted as function of segment number (Figure 1C). Segments with reduced endocardial radial shortening were categorized as infarct/border zone by 2 readers in consensus. The remaining segments were considered remote myocardium. Segmentation was performed for each MI mouse individually because infarct size and location varied between animals. No segmentation was performed for sham-operated mice.

Hypointense Area
The size of the hypointense area was measured in each segment by counting the number of voxels with signal intensity below a threshold value and multiplying this number by the voxel size. Thresholds were defined for each image individually as the mean signal intensity in a reference region minus 2 times the SD in this region. Reference regions were drawn manually outside the heart in (nonangiogenic) skeletal muscle tissue. Additionally, contrast-to-noise ratios were calculated for infarct/border and remote myocardium versus skeletal muscle. Noise was measured outside the mouse (air).

TPLSM Protocol
Hearts were thawed and washed in Hank’s balanced salt solution (pH 7.4) to remove excess optimal-cutting-temperature compound. Next, vascular endothelial cells were stained with anti–CD31-FITC (0.5 mg/mL, BD Biosciences Pharmingen, Alphen a/d Rijn, the Netherlands) diluted 20 times in Hank’s balanced salt solution. Hearts were embedded in 2% (wt/vol) agarose gel (Invitrogen), and images were recorded in the infarction (identified visually by the pale color of the myocardium and the location of the ligature), border zone (0.5 to 1.0 mm proximal to the infarction), and remote myocardium (basal part of the heart). In-depth TPLSM images were acquired as described previously14 (see the Methods section in the online-only Data Supplement).

TPLSM Analysis
TPLSM images were analyzed semiquantitatively with ImageJ. The following parameters related to microvascular structure and pQD distribution were considered, with their scores indicated in parentheses: number of vessels (0 = little, 3 = many), vessel size (0 = small, 2 = large), vessel structure (0 = parallel), number of QDs (0 = absent, 3 = many), intravascular QDs (0 = no, 1 = yes), colocalization QDs with endothelial cells (0 = no, 1 = yes), and QD extravasation (0 = no, 1 = yes).

Statistics
Values are presented as median with 25th to 75th percentiles indicated in parentheses unless stated otherwise. Statistical analysis was performed with SPSS 17.0 (SPSS, Inc, Chicago, Ill). Independent and related samples were compared through the use of a nonparametric Mann–Whitney U test and Wilcoxon signed-rank test, respectively. Values of P<0.05 were considered significant. A Kruskal-Wallis test with multiple comparisons for the 3 groups was performed for the whole myocardium. For the time course of the hypointense area, the difference between the 2 groups was tested with a random-effects model to correct for the correlation of the time series within the same mouse.

Results
LV Function and Infarct Size
No significant difference in body weight was observed between MI mice and sham-operated mice (Table 1). MI mice showed a strongly dilated LV on horizontal long-axis cine...
images (Figure 1A). The infarction was clearly visible as a hypokinetic or akinetic apical region with a thin myocardial wall (Movie I in the online-only Data Supplement). Short-axis cine images were recorded perpendicular to the long-axis plane and transected the infarction (Figure 1B). Here, an impaired contractile function was also clearly observed. In contrast, sham-operated mice showed normal LV contractility and wall thickness on long- and short-axis cine images (Movie II in the online-only Data Supplement). No significant differences were found in heart rate and respiration rate between the 3 experimental groups (Table 1). Furthermore, MI mice showed an increased thickness of the septum compared with sham-operated mice, indicating development of myocardial hypertrophy in response to MI.

Global LV function was assessed by the EF, as determined with the biplane ellipsoid model. The validity of the model was confirmed by the EF of sham-operated mice (Table 1), which corresponded with previously published values for healthy mice. In contrast, a significantly lower EF was found for both infarct groups (Table 1). No differences in EF were found between the MI groups, indicating similar global LV function. In addition, the infarct size was not significantly different between the MI groups (Table 1).

### Contrast Agent Uptake on Molecular MRI

Figure 2 shows short-axis gradient-echo images at 60 minutes after contrast administration for a representative mouse from each experimental group. A large hypointense area was observed in the myocardium of MI mice injected with cNGR-pQDs, which was much smaller or absent in MI mice injected with unlabeled pQDs or in sham-operated mice injected with cNGR-pQDs. Any blood pool effect of circulating contrast agent was excluded on the basis of the similar plasma half-life.

Images recorded with the shortest echo time of 2.9 ms allowed good visualization and delineation of the LV myocardium, whereas images recorded with an echo time of 6.0 ms were more suitable to determine the size of the hypointense area. In addition, gradient-echo images with 2 echo times allowed estimation of the apparent transverse relaxation time T2*, which was \(\approx 10\) ms in healthy myocardium and corresponded with the postmortem results (see the Methods section in the online-only Data Supplement).

To link local cardiac function with the molecular MRI response, the myocardium was divided into 8 radial segments and categorized as infarct/border zone or remote myocardium (Table 2). Next, the size of the hypointense area was quantified for each segment. For MI mice injected with cNGR-pQDs, the hypointense area was located mostly in the infarct/border zone and to a much lesser extent in the remote area (Table 2). Furthermore, the hypointense area in the infarct/border zone was significantly larger for MI mice injected with cNGR-pQDs compared with unlabeled pQDs, indicating a higher local contrast agent concentration in the former. No significant differences were found between the 2 groups in the remote myocardium. For comparison with sham-operated mice, the whole myocardium was considered because no segmentation was performed in these mice. A larger hypointense area was found for MI mice injected with cNGR-pQDs than for sham-operated mice (Table 2). Additionally, the

### Table 2. Average Size of the Hypointense Area as Measured at 60 min After Contrast Administration on Gradient-Echo Images With an Echo Time of 6.0 ms for the Infarct/Border Zone, Remote Area, and Whole Myocardium

<table>
<thead>
<tr>
<th>Group</th>
<th>Infarct</th>
<th>Remote Area</th>
<th>Whole Myocardium</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI + cNGR-pQDs</td>
<td>1.68 (0.80–2.80)*</td>
<td>1.08 (0.48–1.38)*</td>
<td>2.89 (2.27–3.69)*†</td>
</tr>
<tr>
<td>MI + unlabeled pQDs</td>
<td>0.14 (0.05–0.31)</td>
<td>0.35 (0.21–0.77)</td>
<td>0.69 (0.34–1.21)</td>
</tr>
<tr>
<td>Sham + cNGR-pQDs</td>
<td>NA</td>
<td>NA</td>
<td>1.72 (1.69–1.77)</td>
</tr>
</tbody>
</table>

*The infarct/border zone and remote area were defined as explained in Figure 1. The whole myocardium was considered for comparison with sham-operated mice because no segmentation was performed in these mice. A Kruskal-Wallis test with multiple comparisons for 3 groups revealed a \(P\) value of 0.024 in the whole myocardium. NA indicates not applicable.

Results of pairwise comparisons are \(\ast P<0.05\) vs unlabeled pQDs; †\(P<0.05\) vs sham-operated mice.
contrast-to-noise ratio was calculated for infarct/border and remote myocardium versus skeletal muscle (Table 3). The most negative contrast-to-noise ratio, indicating lower signal intensity for the myocardium, was found for the infarct/border zone of MI mice injected with cNGR-pQDs, whereas a considerably higher contrast-to-noise ratio was found in sham-operated mice.

**Contrast Agent Retention**

The retention of cNGR-pQDs and unlabeled pQDs in the infarct/border zone of the heart was evaluated by measuring the size of the hypointense area as a function of time after contrast administration (Figure 3). For cNGR-pQDs, an increase was observed, indicating contrast agent accumulation, which peaked at ≈1 hour after contrast injection. For unlabeled pQDs, the hypointense area was smaller than for cNGR-pQDs at all of the investigated time points. There was a slight peak at 40 minutes after contrast, after which the image contrast remained more or less constant. Using a random-effects model, statistical analysis of the time course revealed a trend (P = 0.08) that the hypointense area remained larger for cNGR-pQDs compared with unlabeled particles over time. Taken together, the time-series data show retention of cNGR-pQDs in the infarct/border zone up to 2 hours after contrast, which was considerably less for unlabeled pQDs and corresponds with previous work.13

**Microvascular Structure and Remodeling**

TPLSM images recorded in the infarct area, border zone, and remote myocardium showed remarkable differences in microvascular morphology (Figure 4). In remote regions, numerous small, parallel-oriented vessels were found, following the orientation of the cardiomyocytes.13 In contrast, the number of vessels was 2-fold lower in the infarcted area (Table III in the online-only Data Supplement), which appeared rather heterogeneous and contained avascular regions. Furthermore, vessels in the infarcted area lacked a distinct organization and had a 2-fold larger diameter than in the remote region. In the border zone, a mixed morphology was observed: Both small and larger vessels were found, and the organization was less structured compared with remote regions. Taken together, these findings clearly reveal microvascular remodeling in the infarction and border zones in response to myocardial ischemia.

**Contrast Agent Localization**

Both cNGR-labeled and unlabeled pQDs were located in the infarct and border zones and were detected only sparsely in the remote myocardium (Figure 5). Infarct and border zone could not be distinguished by QD contrast or localization but were rather apparent from vascular morphology. Therefore, these 2 regions were grouped in the subsequent analysis.

In the infarct/border zone, cNGR-pQDs were found mainly to colocalize with vascular endothelial cells (Figure 5), which concurs with earlier results.13 As an example, Movie III in the online-only Data Supplement shows a 3-dimensional TPLSM imaging stack obtained at increasing depths, with abundant colocalization of cNGR-pQDs with endothelial cells in the infarct area. Although intravascular and extravascular cNGR-pQDs were also observed, they were 3 times less frequent. In contrast, unlabeled pQDs were observed mainly in the extravascular space, with intravascular or colocalized unlabeled pQDs being 2-fold less frequent. Furthermore, the amount of extravasated unlabeled pQDs in the infarct region was 2-fold less compared with the number of bound cNGR-pQDs is this area (Table III in the online-only Data Supplement). TPLSM data therefore support that cNGR-pQDs bind specifically to activated endothelial cells of angiogenic vessels in the infarct/border zone of the myocardium, whereas unlabeled pQDs do not. In sham-operated mice, considerably fewer cNGR-pQDs were found compared with MI mice. If present, cNGR-pQDs were found to be intravascular and not colocalized with endothelial cells.

---

**Table 3. Contrast-to-Noise Ratios for MI Mice Injected With cNGR-pQDs, MI Mice Injected With Unlabeled pQDs, and Sham-Operated Mice Injected With cNGR-pQDs**

<table>
<thead>
<tr>
<th>Group</th>
<th>Infarct/Border</th>
<th>Remote</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI + cNGR-pQDs</td>
<td>−15.4 (−16.9 to −8.7)</td>
<td>−5.7 (−12.9 to −1.9)</td>
</tr>
<tr>
<td>MI + unlabeled pQDs</td>
<td>−6.1 (−9.5 to −3.4)</td>
<td>−7.4 (−12.6 to −2.9)</td>
</tr>
<tr>
<td>Sham + cNGR-pQDs</td>
<td>NA</td>
<td>−1.4 (2.7 to 7.1)</td>
</tr>
</tbody>
</table>

Contrast is defined as the signal in the myocardial tissue minus that in skeletal muscle. Differences were not statistically significant. NA indicates not applicable.

---

**Figure 3. Size of the hypointense area on gradient-echo images (echo time, 6 ms) vs time postcontrast agent administration for cNGR-pQDs and unlabeled pQDs in the infarct/border zone of the heart. Data are presented as mean±SE.**
Discussion

Present Findings

Here, we demonstrated the applicability of molecular MRI for detecting myocardial angiogenesis in vivo in a murine model of acute MI. Compared with standard delayed enhancement imaging, which can only detect infarct size and location on the basis of passive extravasation and abnormal wash-in and washout of a small contrast agent in the infarcted tissue, the presented method provides unique information on the angiogenic status of the myocardium. First, a strong negative contrast effect was observed in the infarct region/border zone with cNGR-labeled pQDs that was mostly absent in sham-operated mice or in MI mice injected with unlabeled pQDs. Second, a lower contrast-to-noise ratio was found in the remote myocardium of both MI groups compared with the sham-operated mice. This may be explained by the development of myocardial hypertrophy (Table 1) because an increase in muscle tissue is normally accompanied by expansion of the vascular network and hence angiogenic activity. This is supported by TPLSM in which QDs were indeed found in the remote myocardium, although to a much lesser extent than in the infarct and border zones. Previous studies showed that significant differences resulting from hypertrophy could only be detected starting at 8 weeks after MI in rats. This may explain the absence of statistical significance in the present molecular MRI data acquired at 1 week after MI. Third, administration of cNGR-pQDs resulted in a larger hypointense area in the infarct/border zone than unlabeled pQDs, indicating specific accumulation of cNGR-pQDs in this area. This corresponds with intravital microscopy results, showing that injection of a cNGR-labeled fluorophore resulted in a significantly higher signal in the infarct/border zone compared with unlabeled fluorophore. Fourth, TPLSM investigation revealed a remarkably different vascular morphology in the infarct and border zone compared with remote myocardium, indicating vascular adaptation and remodeling in response to myocardial ischemia. This concurs with results of Grass et al, who described a transition into vascular...
remodeling with relatively large-caliber vessels crossing the infarct area at 7 days after MI. Fifth, TPLSM showed that both cNGR-pQDs and unlabeled pQDs were present in the infarction and border zone. cNGR-pQDs were found to colocalize with vascular endothelial cells, whereas unlabeled pQDs were found mostly in the extravascular space. This finding further supports the specificity of cNGR-pQDs for angiogenic vessels in the heart. Taken together, these results demonstrate that cNGR-pQDs are suitable for selectively detecting post-MI angiogenesis in the heart using in vivo molecular MRI and ex vivo TPLSM.

Contrast Agent

QDs were applied as contrast agent scaffold to allow non-invasive molecular MRI of the infarcted murine heart, validated by postmortem TPLSM. Although cNGR-labeled pQDs could selectively detect myocardial angiogenesis, the method cannot be translated directly into clinical trials. QDs have a cadmium-selenium core and accumulate mainly in the liver and spleen, and their long-term toxicity is still unknown. Nevertheless, the present data demonstrate that cNGR selectively targets the contrast agent to angiogenic neovessels in the heart, which corresponds with previous studies. Furthermore, validation of the MRI results with a subcellular resolution technique such as TPLSM is a necessary step in the development and evaluation of novel contrast agents. Once the targeting efficacy is fully established, cNGR could be coupled to more clinically applicable particles such as ultrasmall superparamagnetic iron oxide particles. These particles generate sufficient changes in image contrast, are cleared from the body, and were proven to be safe, even in patients with impaired renal function. In light of the advantageous short plasma half-life of pQDs (vide infra), using larger and fast clearing SPIO particles may also be suitable. The applicability of cNGR-labeled (ultra)small superparamagnetic iron oxide particles for cardiac imaging is a topic of future research.

Although pQDs were designed as a positive contrast agent by labeling QDs with multiple Gd-DTPA moieties, as observed in previous tumor angiogenesis studies, a strong negative contrast was observed in the myocardium. We suggest the following explanation. First, for proper T1 weight in gradient-echo imaging and consequent positive contrast, T2* relaxation effects can be minimized by setting the echo time much lower than tissue T2*. With echo times of 2.9 ms (shortest possible) and 6.0 ms and a tissue T2* of 10 ms, this was clearly not the case. Second, a hypothesised relaxation mechanism leading to negative contrast is presented in the Methods section in the online-only Data Supplement.

Postmortem relaxation time quantification (Methods section in the online-only Data Supplement) revealed higher T1 and T2 values in infarcted myocardium compared with remote areas. This corresponds with previous studies and can be explained by edema in the infarction. Therefore, any small contrast agent-induced reduction in T1 or T2 in the infarct/border zones may have been canceled by these intrinsic relaxation time differences. In contrast, the postmortem T2* was found to be lower in infarcted myocardium compared with the remote area, which is likely related to increased capillary recruitment. Combined with the T2*-shortening effect of the contrast agent, this may have resulted in an enhanced contrast that exceeded the detection limit of MRI. In addition, any T1 contrast may have averaged out as a result of partial volume effects. In contrast, T2* is considerably less affected by partial volume effects. Taken together, the intrinsic differences in relaxation times between infarcted and healthy myocardium and the different dependence of T1 and T2* contrast on partial volume effects may explain the absence of any detectable T1 contrast and why T2* contrast was more dominant in detecting the presence of cNGR-pQDs in the heart.

Molecular Imaging

In the present MRI protocol, only postcontrast imaging was performed to reduce the total examination (ie, preparation and imaging) time. This was especially important for MI mice, which had a considerably reduced maximum tolerated imaging time compared with healthy mice because of their impaired cardiac function. The 2 control groups (MI mice injected with unlabeled pQDs and sham-operated mice injected with cNGR-pQDs) provided sufficient control evidence to justify the absence of precontrast images. In addition, comparison between infarct/border zone and remote myocardium could be performed in the same animal because both myocardial areas were present within the imaging slice. In future clinical applications, only the difference between infarct and remote myocardium would likely be evaluated.

Besides imaging angiogenic activity via endothelial cell targeting, other processes can be exploited for cardiac molecular imaging, including matrix metalloproteinase activity, blood coagulation factor XIII related to infarct healing and ventricular remodeling, cardiomycyte apoptosis, and fibrosis. A major advantage of endothelial cell targets is their easy accessibility from the bloodstream, resulting in relatively quick binding of the intravascular contrast agent to the target and high local concentrations. This is especially important in light of the short plasma circulation time of the currently applied contrast agent. From a clinical viewpoint, fast target binding and plasma clearance are also advantageous because they would allow the complete examination to be finished within a clinically acceptable timeframe. In contrast, imaging of an intracellular target would be performed at least 24 hours after contrast administration to allow sufficient contrast agent uptake, as is seen, for instance, in lymph node imaging.

An important issue regarding molecular imaging of the human heart is the spatial resolution of clinical MRI (~1 mm) versus nuclear imaging (5 to 10 mm). This implies that clinical MRI, in contrast to nuclear methods, can spatially resolve pathologically different regions/features in the radial direction of the myocardial wall, detect infarct size and transmural extent, and the adjacent area at risk, and localize the expression of angiogenic biomarkers with respect to morphological details of the infarcted myocardium. Because the area at risk is the part of the myocardium that is potentially salvageable upon revascularization, accurate detection of this area could have implications for the therapeutic strategy.
Practical Applications

Several future (pre)clinical applications can be envisioned for molecular MRI of myocardial angiogenesis. First, it provides information on global and local cardiac function (eg, EF and endocardial radial shortening) and the level of angiogenic activity within a single imaging session. It may therefore be applied for the early in vivo evaluation of the response to angiogenic treatments in both preclinical and clinical studies. In addition, molecular MRI may provide a surrogate marker of therapeutic efficacy and can serve as a timely end point in clinical trials. Second, future improvements in myocardial perfusion and function may be predicted from the early angiogenic response. Third, measurement of baseline angiogenic activity may allow risk stratification, development of individualized therapy, and patient selection for clinical trials. One may argue that patients with a limited level of angiogenesis have the largest need for proangiogenic therapy and may therefore benefit the most. It has also been suggested that these patients apparently have a failed natural angiogenic response and will therefore be more resistant to exogenous angiogenic stimuli. Fourth, conjugation of an imaging label to proangiogenic drugs may allow monitoring of drug delivery. Fifth, the presented method to detect angiogenesis in the mouse heart opens the way for studies in transgenic/knockout ery. Fifth, the presented method to detect angiogenesis in the mouse heart opens the way for studies in transgenic/knockout ery. Sixth, the presented method to detect angiogenesis in the mouse heart opens the way for studies in transgenic/knockout ery.

Conclusions

cNGR-labeled pQDs allowed specific detection of post-MI myocardial angiogenesis, as shown by the strong contrast observed in the infarcted mouse heart on molecular MR images and by the colocalization of cNGR-pQDs with vascular endothelial cells as detected by TPLSM. In addition, TPLSM provided unique, detailed information on microvascular structure and remodeling in different regions of the heart.

Sources of Funding

This research was funded by the SenterNovem Besluit Subsidies Investeringen Kennisinfrastructure program entitled Molecular Imaging of Ischemic Heart Disease (grant BSIK03033) and by the Smart Mix program entitled Translational Excellence in Regenerative Medicine (SMM06004). The TPLSM setup was financed by the Dutch Scientific Organization (NWO 902-16-276).

Disclosures

None.

References

Angiogenesis is a natural relief mechanism to restore perfusion to ischemic myocardium after acute myocardial infarction. Therapeutic angiogenesis is being explored as a novel treatment option for myocardial infarction patients; however, sensitive, noninvasive in vivo measures of therapeutic efficacy are lacking and need to be developed. Here, a molecular magnetic resonance imaging method is presented to noninvasively image angiogenic activity in vivo in a murine model of myocardial infarction.

**CLINICAL PERSPECTIVE**

Angiogenesis is a natural relief mechanism to restore perfusion to ischemic myocardium after acute myocardial infarction. Therapeutic angiogenesis is being explored as a novel treatment option for myocardial infarction patients; however, sensitive, noninvasive in vivo measures of therapeutic efficacy are lacking and need to be developed. Here, a molecular magnetic resonance imaging method is presented to noninvasively image angiogenic activity in vivo in a murine model of myocardial infarction with a targeted, bimodal (ie, paramagnetic and fluorescent) contrast agent. Intravenous injection of the contrast agent resulted in a strong increase in image contrast in the infarcted myocardium. Significantly less change in image contrast was found in myocardial infarction mice injected with a control contrast agent or in sham-operated mice injected with the targeted agent. The magnetic resonance imaging results were confirmed by ex vivo fluorescence microscopy, which also demonstrated significant microvascular remodeling in the infarction and border zones of the heart in response to myocardial infarction. Future clinical applications of the presented molecular magnetic resonance imaging method include early noninvasive evaluation of proangiogenic therapy, prediction of improvements in myocardial perfusion and function based on the early angiogenic response, risk stratification, development of individualized therapy, and monitoring of drug delivery.

**References**

Molecular Magnetic Resonance Imaging of Myocardial Angiogenesis After Acute Myocardial Infarction

Marlies Oostendorp, Kim Douma, Allard Wagenaar, Jos M.G.M. Slenter, Tilman M. Hackeng, Marc A.M.J. van Zandvoort, Mark J. Post and Walter H. Backes

_Circulation_. 2010;121:775-783; originally published online February 1, 2010;
doi: 10.1161/CIRCULATIONAHA.109.889451

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/121/6/775

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2010/01/26/CIRCULATIONAHA.109.889451.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/
SUPPLEMENTAL MATERIAL

*Molecular MRI of myocardial angiogenesis after acute myocardial infarction*

Supplemental Methods and Results

Contrast agent susceptibility

A thin cylindrical tube was filled with a sample of pQDs and suspended in water to reduce susceptibility artifacts due to air/sample interfaces. The sample was positioned perpendicular to the main magnetic field $B_0$. Next, coronal gradient echo images were recorded with TR 1500 ms, TE 3.2 or 7.0 ms, flip angle 40°, 4 averages, field-of-view 4×4 cm$^2$, matrix 256×256, 1 slice of 0.5 mm thickness.

Subsequently, the susceptibility shift $\Delta \chi$ was calculated from the phase difference $\Delta \phi$ between two gradient echo images recorded with two different echo times according to\(^1\):

$$
\Delta \chi = \frac{\Delta B}{B_0} = 2 \cdot \frac{\Delta \phi}{\gamma(TE_2 - TE_1)B_0}
$$

(1)

Here, $\Delta B$ is the change in magnetic field strength induced by the contrast agent, and $\gamma$ is the gyromagnetic ratio. The factor 2 corrects for the perpendicular orientation of the cylindrical sample with respect to $B_0$. The resulting susceptibility shift was $3.1 \times 10^{-2}$ ppm per mM Gd, which is comparable to previously published values for Gd-DTPA.\(^2\)

Animal model

0.1 mmol/kg burprenorphin s.c. (Temgesic®, Schering-Plough, Utrecht, The Netherlands) was given as pre-operative analgesia. Mice were anesthetized using 5% isoflurane (Abbott Laboratories Ltd, Queensborough, UK) in medical air, intubated and ventilated with 1-2% isoflurane at 150 respirations per minute using a microventilator (UNO Roestvaststaal BV, Zevenaar, The Netherlands). The heart was exposed via lateral thoracotomy and the left anterior descending coronary artery was ligated just below the branch-point of the anterior interventricular descending and diagonal arteries using a 6-0 polypropylene thread (Surgipro II, Syneture, Tyco Healthcare, Gosport, UK). This ligation site produced transmural, anterolateral, apical infarctions with a good post-operative survival rate of approximately 80%. The thorax was closed using 5-0 polypropylene and the skin was sutured using 5-0 silk. Animals recovered...
overnight at 30 °C. Sham-operated mice underwent the same surgery without tying the ligature (thread was only pulled through the myocardium).

Postmortem relaxation time measurements
To obtain more insight into the effects in image contrast induced by the quantum dots, postmortem $T_1$, $T_2$ and $T_2^*$ quantification was performed. All images were recorded with a field-of-view of 4×4 cm$^2$, a 256×256 matrix, and 5 slices of 1 mm thickness, resulting in a spatial resolution of 0.16×0.16×1.0 mm$^2$. $T_1$ was measured using a series of inversion recovery experiments with a repetition time TR of 3500 ms, an echo time TE of 8.2 ms, and inversion times TI of 200, 500, 750, 1000, 1500 and 2000 ms. $T_2$ was quantified using a multi-slice multi-echo spin echo sequence with TR 3500 ms, and TE ranging between 10 and 80 ms, with an echo interval of 10 ms. For $T_2^*$, a multi-slice multi-echo gradient echo sequence was applied with a TR of 2500 ms and TE ranging between 4 and 58 ms, with an echo interval of 6 ms, and a 30° flip angle.

Regions of interest were drawn manually. For MI-mice, the infarct/border zone was defined based on the thin myocardial wall which could be readily visualized postmortem. The thick myocardium was defined as remote area. No differentiation was made for sham-operated mice. Next, average relaxation times were calculated in Matlab via non-linear fitting of their respective signal intensity functions using the Levenberg-Marquardt optimization algorithm. The obtained results are summarized in Supplemental Table 1.

TPLSM protocol
In-depth TPLSM images were recorded with an Eclipse E600FN upright microscope (Nikon, Tokyo, Japan) and a Radiance 2100MP optical imaging system (Bio-Rad, Hemel Hempstead, UK). Fluorophores were excited by a mode-locked Tsunami Ti:Sapphire laser (Spectra-Physics, Mountain View, CA) with an 800 nm central wavelength and a 120 fs pulse width. Datasets were acquired using a 60× water-dipping objective lens (Nikon) with a 1.00 numerical aperture. FITC (520–560 nm) and QD (570–600 nm) fluorescence were detected by photo-multiplier tubes (Electron Tubes, Ruislip, UK) and color-coded in green and red, respectively. An in-plane resolution of 0.35×0.35 μm$^2$ was obtained using a 179×179 μm$^2$ field-of-view and a 512×512 matrix. The pixel dwell time was 11.8 μs, which together with a two-fold Kalman averaging
resulted in an acquisition rate of 0.16 Hz for each of the imaging planes in the 3D dataset. The interplanar distance was 1.05 μm or 0.6 μm.

**Hypothetical relaxation mechanism**

Figure S1 shows a hypothetical relaxation mechanism that might explain the negative contrast induced by bound cNGR-pQDs in the heart. Vascular endothelial cells in quiescent vessels (left panel) form a nicely aligned network and show no upregulation of specific receptors. The targeted contrast agent flows freely through the vasculature and the magnetic properties of pQDs have a relatively weak effect on the water proton resonance frequency. In angiogenic vessels (right panel), endothelial cells display upregulated receptors to which the targeted agent can bind with high affinity. This results in high local contrast agent concentrations, which in turn generates static field perturbations due to the magnetic properties of pQDs in combination with the dense gadolinium concentration on the QD surface. These field perturbations, which are aligned with the main magnetic field, likely result in an altered resonance frequency of nearby water protons and a consequently reduced signal intensity due to locally strong intravoxel dephasing effects of the water proton spins.

For unlabeled pQDs, there is no binding to upregulated receptors and contrast agent may extravasate through the permeable EC layer. Contrast agent distribution is relatively diffuse in the extravascular space and high local contrast agent concentrations are not reached. Consequently, negative contrast is not observed on the corresponding MR images.

Whether the negative contrast effect can be applied to generate positive contrast images using techniques like susceptibility gradient mapping or white marker imaging needs to be explored. Furthermore, these novel techniques need to be tailored to cardiac imaging.

**Estimation of resonance frequency shift**

To further investigate the negative contrast mechanism, we estimated the resonance frequency shift $\delta \omega$ using the method of Bauer et al., who showed that the $T_2^*$ in healthy cardiac muscle tissue can be described by:

$$T_2^* = \frac{\tau (1 + \varsigma)}{\sqrt{1 + \left(\frac{\varsigma \delta \omega}{\tau}\right)^2} - 1} + \varsigma \left[\frac{\sqrt{1 + \left(\frac{\varsigma \delta \omega}{\tau}\right)^2} - 1}{\sqrt{1 + \left(\frac{\varsigma \delta \omega}{\tau}\right)^2} - 1}\right] \quad (2)$$
Here, $\tau$ is the correlation time of the field fluctuations and $\varsigma$ is the relative intracapillary blood volume. The correlation time and the intracapillary blood volume can be calculated according to equations 3 and 4, respectively.

$$\tau = \left( \frac{R_c^2}{4D} \right) \ln \left( \frac{\varsigma}{\varsigma - 1} \right)$$

$$\varsigma = \frac{R^2}{R_s^2}$$

In equation 4, $R_c$ is the capillary radius and $R_s$ is the cylindrical supply region around a vessel.

Using the TPLSM images, $R_c$ and $R_s$ were estimated manually in the infarct and remote myocardium (Supplemental Table 2). To this extent, 4 images from each area were randomly selected from different mice and the vascular parameters were measured at 8 randomly chosen positions within each 3D imaging stack. Furthermore, the diffusion coefficient $D$ was obtained from Strijkers et al.\textsuperscript{7} For mice injected with cNGR-pQDs, the postmortem $T_2^*$ values in the infarct and remote myocardium were 8.1 and 9.5 ms, respectively (see Supplemental Table 1). Using these data and equations 2-4, the correlation time $\tau$ was calculated analytically, whereas the resonance frequency shift $\delta \omega$ was estimated numerically using Maple.

For the remote myocardial region, the obtained results match relatively well with the findings described by Bauer et al.\textsuperscript{6}, especially for the capillary radius, the intracapillary blood volume and the correlation time (supplemental Table 2). For the infarcted myocardium, an approximately 3-fold lower $\delta \omega$ was found compared with the remote myocardium, whereas it was expected to be larger due to the higher local concentration of cNGR-pQDs. However, it is important to note that the mathematical derivations described by Bauer et al. assume a regular, parallel architecture of muscle fibers and capillaries in the myocardium. Although this is approximation is valid for remote myocardium, it is not suited for infarcted myocardium, where the microvasculature lacks any distinct structure (Figure 5). Furthermore, in the infarct area there were multiple regions where no vessels could be detected with TPLSM, likely resulting in an underestimation of $R_s$ and a consequent overestimation of $\varsigma$. A thorough mathematical evaluation of $\delta \omega$ in the infarct area therefore requires an extension of the theory of Bauer et al. to take the abnormal vasculature into account, which is beyond the scope of this paper.
Supplemental Tables

**Table 1** Postmortem relaxation times in different cardiac regions for MI-mice injected with cNGR-pQDs, MI-mice injected with unlabeled pQDs, and sham-operated mice injected with cNGR-pQDs. Data are presented as mean ± standard error. NA: not applicable.

<table>
<thead>
<tr>
<th></th>
<th>$T_1$ (ms)</th>
<th>$T_2$ (ms)</th>
<th>$T_2^*$ (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infarct/border</td>
<td>Remote</td>
<td>Infarct/border</td>
</tr>
<tr>
<td>MI + cNGR</td>
<td>1645 ± 56</td>
<td>1529 ± 52</td>
<td>37 ± 3.0</td>
</tr>
<tr>
<td>MI + unlabeled</td>
<td>1711 ± 146</td>
<td>1589 ± 131</td>
<td>34 ± 1.4</td>
</tr>
<tr>
<td>Sham + cNGR</td>
<td>NA</td>
<td>1447 ± 70</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Table 2** Input parameters and estimated intracapillary blood volume $\varsigma$, correlation time $\tau$, and resonance frequency shift $\delta\omega$ according to the method of Bauer et al.\cite{6} $R_c$: tubular capillary radius, $R_s$: cylindrical supply region, $D$: apparent diffusion coefficient. Values for $R_c$ and $R_s$ are presented as mean ± standard error.

<table>
<thead>
<tr>
<th></th>
<th>$R_c$ (µm)</th>
<th>$R_s$ (µm)</th>
<th>$D$ ($\times 10^{-3}$ mm$^2$/s)</th>
<th>$\varsigma$</th>
<th>$\tau$ (ms)</th>
<th>$\delta\omega$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infarct</td>
<td>4.6 ± 0.3</td>
<td>9.1 ± 0.7</td>
<td>0.56</td>
<td>0.26</td>
<td>17.3</td>
<td>350</td>
</tr>
<tr>
<td>Remote</td>
<td>2.1 ± 0.2</td>
<td>6.5 ± 0.4</td>
<td>0.66</td>
<td>0.14</td>
<td>4.2</td>
<td>1018</td>
</tr>
</tbody>
</table>
Table 3  Semi-quantitative analysis of TPLSM data. Data were scored with respect to microvascular morphology, QD presence, and QD localization. Data are presented as mean ± standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Number of analyzed images</th>
<th>Number of vessels (0 = little, 3 = many)</th>
<th>Vessel size (0 = small, 2 = large)</th>
<th>Vessel structure (0 = chaotic, 2 = parallel)</th>
<th>Number of pQDs (0 = absent, 3 = many)</th>
<th>Intravascular pQDs (0 = no, l = yes)</th>
<th>Colocalized pQDs (0 = no, l = yes)</th>
<th>Extravasated pQDs (0 = no, l = yes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI + cNGR-pQDs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remote</td>
<td>24</td>
<td>2.4 ± 0.6</td>
<td>0.6 ± 0.5</td>
<td>1.6 ± 0.6</td>
<td>1.1 ± 1.3</td>
<td>0.4 ± 0.5</td>
<td>0.5 ± 0.5</td>
<td>0.6 ± 0.5</td>
</tr>
<tr>
<td>Border</td>
<td>39</td>
<td>2.5 ± 0.6</td>
<td>1.2 ± 0.7</td>
<td>0.7 ± 0.7</td>
<td>1.2 ± 1.1</td>
<td>0.3 ± 0.5</td>
<td>0.6 ± 0.5</td>
<td>0.5 ± 0.5</td>
</tr>
<tr>
<td>Infarct</td>
<td>11</td>
<td>1.5 ± 0.8</td>
<td>1.4 ± 0.4</td>
<td>0.3 ± 0.4</td>
<td>1.3 ± 0.9</td>
<td>0.3 ± 0.5</td>
<td>0.8 ± 0.5</td>
<td>0.3 ± 0.5</td>
</tr>
<tr>
<td>MI + unlabeled pQDs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remote</td>
<td>22</td>
<td>2.4 ± 0.5</td>
<td>0.3 ± 0.5</td>
<td>1.7 ± 0.5</td>
<td>0.5 ± 0.7</td>
<td>0.2 ± 0.4</td>
<td>0.2 ± 0.4</td>
<td>0.3 ± 0.5</td>
</tr>
<tr>
<td>Border</td>
<td>33</td>
<td>1.8 ± 0.8</td>
<td>1.3 ± 0.6</td>
<td>0.8 ± 0.4</td>
<td>1.5 ± 0.8</td>
<td>0.3 ± 0.5</td>
<td>0.2 ± 0.4</td>
<td>0.6 ± 0.5</td>
</tr>
<tr>
<td>Infarct</td>
<td>14</td>
<td>1.2 ± 0.8</td>
<td>1.0 ± 0.7</td>
<td>0.4 ± 0.5</td>
<td>1.1 ± 1.0</td>
<td>0.2 ± 0.4</td>
<td>0.2 ± 0.4</td>
<td>0.4 ± 0.5</td>
</tr>
<tr>
<td>Sham + cNGR-pQDs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole heart</td>
<td>27</td>
<td>1.4 ± 0.9</td>
<td>0.7 ± 0.7</td>
<td>1.5 ± 0.7</td>
<td>0.9 ± 0.9</td>
<td>0.4 ± 0.5</td>
<td>0.1 ± 0.3</td>
<td>0.4 ± 0.5</td>
</tr>
</tbody>
</table>
Supplemental Figure

**Figure 1** Hypothetical negative contrast mechanism for cNGR-pQDs. In quiescent vessels (left panel) the contrast agent flows freely through the vasculature and therefore hardly influences the water proton resonance frequency. However, in angiogenic vessels (right panel), cNGR-pQDs bind to the vessel wall, generating a high local contrast agent concentration with static field inhomogeneities, which likely results in an altered resonance frequency of nearby water protons and a consequently reduced signal intensity (see text for more details).
Movie Legends

**Movie 1** Horizontal long axis cine image of mouse with myocardial infarction. A clearly dilated left ventricle is observed. The infarction is identified as an akinetic apical region with a thin myocardial wall.

**Movie 2** Horizontal long axis cine image of a sham-operated mouse, showing normal contractility and wall thickness of the left ventricle.

**Movie 3** In depth 3D TPLSM imaging stack recorded in the infarct area of an MI-mouse injected with cNGR-pQDs, showing abundant colocalization of cNGR-pQDs (red) with vascular endothelial cells (green).

Supplemental References