American Heart Association | American Stroke Association

Seeing What We Build—The Need for New Imaging Techniques in Myocardial Regeneration

David E. Sosnovik, MD

The largest experience with stem cell therapy in the heart has been with bone marrow mononuclear cells (BMMCs). Several single-center studies have shown modest, and in some cases transient, increases in ejection fraction (EF) following BMMC injection in patients with reperfused myocardial infarction.[1] While this experience clearly demonstrated the safety of BMMC injection, the imaging readouts used in these phase 1 to 2 studies did not provide a clear "stop or go" signal on which to base further decisions. Several multicenter phase 3 studies of BMMC injection were thus conducted and have been negative, showing no significant changes in EF.[2,3] A clear need, therefore, exists for more refined imaging tools to guide the development of regenerative therapies in the heart.

Successful regeneration in the heart requires the injected cell to be delivered to the correct zone of the myocardium, survive in the host microenvironment, exert beneficial paracrine effects, differentiate, and integrate with the host myocardium. Advanced imaging techniques to characterize many of these processes have been developed (Figure) and several are poised for use in humans. In the current issue of *JAHA*, Dash and colleagues add to this armamentarium with an elegant approach involving dual-contrast magnetic resonance imaging (MRI) and positron emission tomography (PET) of a reporter probe.[4] The value of this and other advanced imaging approaches is discussed below.

Characterization of the Host Myocardium

Acute necrosis elicits an intense inflammatory response in the myocardium.[5,6] Highly degradative macrophages infiltrate the infarct and release cytotoxic and proteolytic enzymes including cathepsins, myeloperoxidase, and matrix metalloproteinases.[7] Any cell that is injected, or migrates, into the infarct zone must be able to survive in this highly hostile inflammatory environment. MRI of iron-oxide nanoparticles and PET imaging of ^{18}fluorodeoxyglucose can be used to image the degree of macrophage infiltration in infarcted myocardium.[6] In addition, preclinical probes to myeloperoxidase and matrix metalloproteinases have been developed for MRI and nuclear imaging, respectively.[7] Infarcted myocardium has a poor vascular supply, limiting the availability of nutrients and oxygen to any injected cells. The degree of angiogenesis in healing infarcts has been imaged using a PET tracer to the $\alpha_V\beta_3$ integrin.[8] Imaging tools are thus available to characterize the receptiveness of the host myocardium to cell therapy and to personalize the timing and location of cell injection.

The alternative to this image-guided approach is an empiric strategy in which cells are injected at predefined time points. This formed the basis of the LateTIME trial, where cell injection was performed 2 to 3 weeks after infarction.[2] However, no advantage was seen in the delayed injection strategy used in LateTIME. While this may reflect the inherent limitations of BMMCs, the absence of an imaging readout to characterize inflammation and angiogenesis in the myocardium prior to cell injection may also have contributed to the negative result.

Confirmation of Local Cell Delivery

The delivery of cells to the myocardium must be confirmed for the analysis of the subsequent response to be interpreted in its true context. A large body of preclinical experience exists with MRI of the ferumoxides nanoparticle,[9] which unfortunately is no longer available. However, ferumoxytol is Food and Drug Administration–approved and can be used for cell labeling as well. The advantage of this cell-labeling approach is that late gadolinium enhancement (LGE) of the infarct can

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

From the Martinos Center for Biomedical Imaging, Department of Radiology and Cardiovascular Research Center, Cardiology Division, Massachusetts General Hospital, Harvard Medical School, Boston, MA.

Correspondence to: David E. Sosnovik, MD, Martinos Center for Biomedical Imaging, 149 13th St, Charlestown, MA 02129. E-mail: sosnovik@nmr.mgh.harvard.edu

J Am Heart Assoc. 2015;4:e002306 doi: 10.1161/JAHA.115.002306.

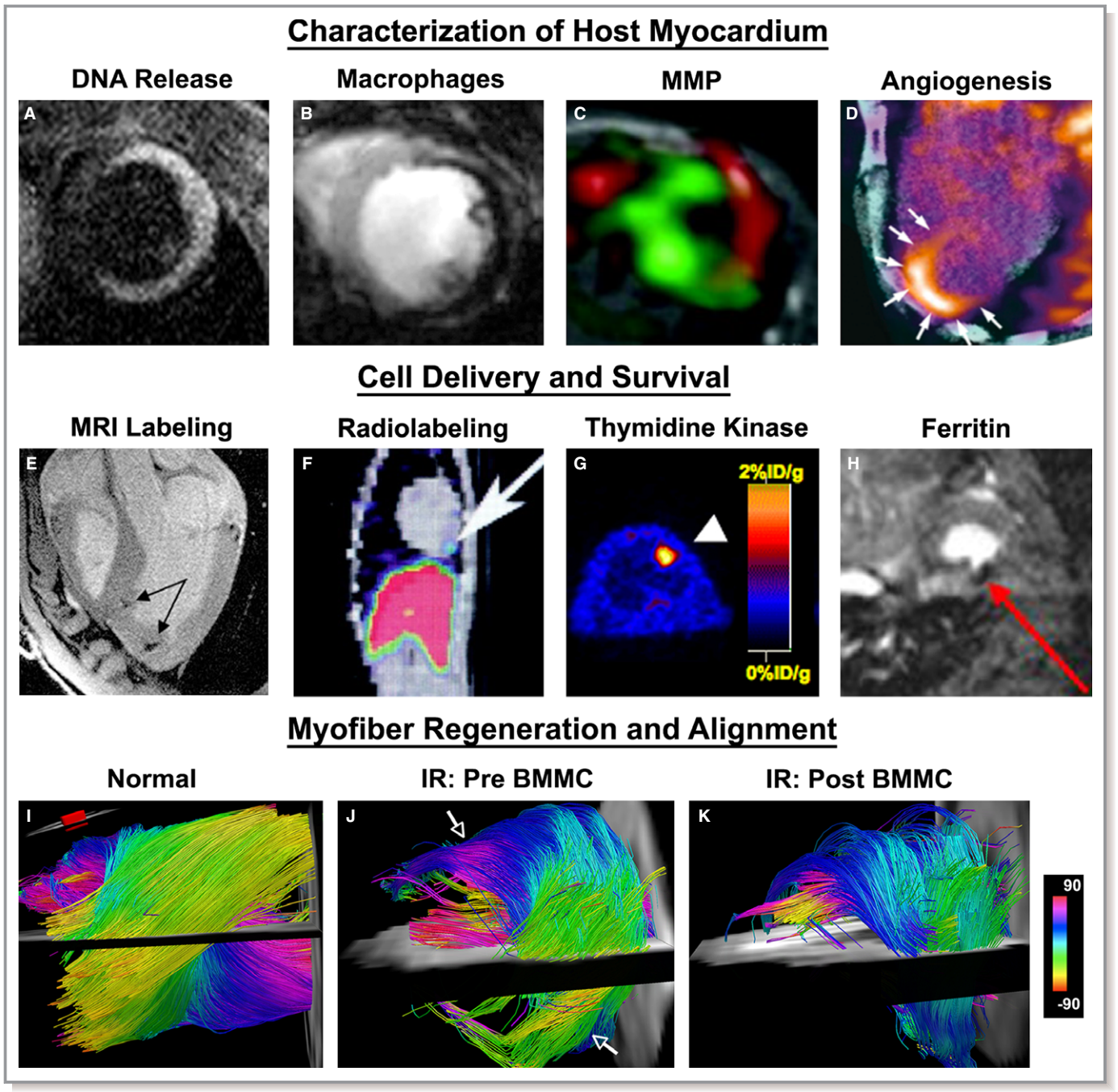

Figure. Advanced imaging techniques in myocardial regeneration. *Top row:* (A) DNA release from acute necrosis (hyperenhanced area), reproduced with permission from Huang et al[5]; (B) macrophage infiltration into the infarct (hypoenhanced area), reproduced with permission from Sosnovik et al[6]; (C) MMP production (red) in the perfusion defect defined by thallium (green), reproduced with permission from Su et al[7]; (D) PET imaging of angiogenesis (arrows) in the healing infarct, reproduced with permission from Makowski et al[8]; *Middle row:* (E) Intramyocardial injection of iron-oxide-labeled cells (arrows), reproduced with permission from Kraitchman et al[9]; (F) homing of radiolabeled cells to the infarct (arrow), reproduced with permission from Kraitchman et al[10]; (G) PET imaging of embryonic stem cells expressing the thymidine kinase reporter gene, reproduced with permission from Cao et al[11]; (H) MRI of the ferritin reporter gene producing signal hypoenhancement (arrow), reproduced with permission from Naumova et al[12]; *Bottom row*: (I through K) DTI-tractography of fibers in the lateral wall of a normal mouse and a mouse with IR injury, reproduced with permission from Sosnovik et al.[13] Fibers intersecting a standardized region-of-interest (inset) are shown and are color-coded by their helix angle. After IR, coherent myofiber tracts can no longer be visualized in the apical half of the ventricle. Serial in vivo imaging shows that fiber tracts that were present in the border zone preinjection (arrows) have been lost after BMMC injection.[13] BMMC indicates bone marrow mononuclear cell; DTI, diffusion tensor MRI; IR, ischemia–reperfusion; MMP, matrix metalloproteinases; MRI, magnetic resonance imaging; PET, positron emission tomography.

be simultaneously performed to further guide the location of cell injection.[9] Intravenous delivery of stem cells is more challenging. The vast majority of the cells accumulate in the liver, and radiolabeling is needed to detect cell delivery to the myocardium.[10] Concerns regarding the potential bioeffects of radiolabeling are legitimate but can be mitigated in part by labeling only a small fraction of the cells.

Assessment of Cell Survival and Differentiation

An important caveat of all cell-labeling techniques is that the presence of the label in the myocardium does not imply survival of the cell. The label can persist in free form, or in inflammatory cells, well after the death of the injected cell. Dedicated approaches are needed to assess cell survival and differentiation. Reporter genes encoding for bioluminescent, fluorescent, MR-detectable, and PET-detectable probes have all been used to assess cell survival preclinically.[11,12] Translation of the MR- and PET-based approaches is feasible, but the injection of genetically manipulated cells is complex and will require extensive testing. Ultimately, however, this information will be crucial to understand the mechanism of benefit or mode of failure of any injected cell.

Generation and Alignment of New Myofibers

The regeneration of infarcted myocardium requires new myofibers to be generated within the infarct. Moreover, these myofibers must be correctly aligned and integrated with the surrounding myocardium. Diffusion Tensor MRI-tractography allows myofiber architecture to be imaged noninvasively by tracking the diffusion of water along myofibers.[13] Serial in vivo imaging with the technique can be used to determine whether new myofibers are being regenerated and whether they are spiraling around the left ventricle with the correct helix angle.[13] The difference in helix angle ($\approx$120°) between the subendocardial and subepicardial fibers plays a key role in the mechanical and electrical function of the heart and is vital to replicate during regeneration.

Diffusion tensor MRI provides a direct and fundamental measure of myofiber regeneration that is likely to be highly predictive of downstream clinical response. Serial in vivo diffusion tensor MRI-tractography in infarcted mice injected with BMMCs revealed a neutral response,[13] consistent with the results of the TIME, LateTIME, and Swiss-AMI trials.[2,3] In addition, in occasional cases, the response to BMMC injection was negative.[13] Diffusion tensor MRI-tractography can be performed in humans and could play a valuable role in early clinical trials. One limitation of the technique, however, is its inability to distinguish new myofibers generated from endogenous repair from those generated directly from the injected cells. Reporter imaging approaches, capable of making this distinction, will thus need to be developed.

Molecular and Metabolic Imaging of the Myocardium

Molecular imaging techniques to follow many of the processes involved in infarct healing and remodeling have been developed (Figure). Metabolic imaging with PET and MR spectroscopy can also provide important insights into the efficiency of myocardial contraction after cell therapy. The use of ^{31}P to measure high-energy phosphates in the myocardium is well established, and could potentially be combined with hyperpolarized ^{13}C MRI and ^{11}C PET in a multiplexed approach.

Myocardial Viability and Infarct Size

LGE is being increasingly used to detect a reduction in infarct size following cell therapy.[14] It is critical to understand, however, what exactly is being imaged with LGE. Clinically used gadolinium chelates cannot cross cell membranes and accumulate nonspecifically in the extracellular space. Any process that expands the extracellular space with thus lead to an accumulation of gadolinium. In chronic infarction, the correlation between LGE and infarct size is excellent. However, in acute infarction the extent of LGE can overestimate infarct size, particularly in the border zones. Manganese (Mn)-based contrast agents are transported through calcium channels into viable cells and provide a specific signature of cell viability. A loss of viability is characterized by the absence of Mn uptake and defines an area that is frequently smaller than that defined by LGE.

In their elegant study, Dash and colleagues use the difference between the areas defined by LGE and the absence of Mn uptake to define the peri-infarct zone (PIR).[4] A reduction in the size of the PIR through the generation of new myofibers is one of the central aims of cell therapy following acute infarction. The response to intramyocardial injection of human amniotic mesenchymal stem cells was examined in their study. Cell injection decreased the size of the infarct core and the PIR, and resulted in lower end-diastolic volumes and higher EF.[4] A strong correlation was seen between increased viability in the PIR and cell survival via PET imaging of the thymidine kinase reporter gene. However, consistent with prior studies of mesenchymal stem cells, no evidence of cardiomyocyte differentiation was seen.[4]

T1 mapping, both with and without gadolinium, is being increasingly used to detect changes in the properties of the myocardium, and the detection of myocardial edema with T2-weighted imaging is widely used to detect the area-at-risk.

How then do we interpret the dual-contrast technique described by Dash and colleagues in the context of these advances? While the combination of T2, T1, and gray zone imaging with LGE could all conceivably detect changes in the size of the PIR, they lack the specific signature of viability provided by Mn uptake. One could thus potentially envisage a scenario where Mn-based viability imaging, rather than LGE, is used in conjunction with native T1 and T2 mapping to characterize changes in the infarct zone and PIR after cell therapy.

Conclusions

The early trials of stem cell therapy in the heart were based largely on the measurement of EF by echocardiography, while in later trials MRI was used. Interestingly, a meta-analysis of BMMC trials revealed positive results by echo-derived EF and negative results using MRI-derived EF.[1] It would be a mistake, however, to assume that the use of MRI, including LGE, provides us with all the tools we need. The complexity of the myocardium and of the regenerative process will require advanced imaging techniques to be developed for optimal results. As shown in the excellent article by Dash and colleagues, the imaging community is rising to meet this challenge.

Sources of Funding

Supported by R01HL093038 and R01HL112831 to Sosnovik.

Disclosures

None.

References

1. Jeevanantham V, Butler M, Saad A, Abdel-Latif A, Zuba-Surma EK, Dawn B. Adult bone marrow cell therapy improves survival and induces long-term improvement in cardiac parameters: a systematic review and meta-analysis. *Circulation*. 2012;126:551–568.
2. Simari RD, Pepine CJ, Traverse JH, Henry TD, Bolli R, Spoon DB, Yeh E, Hare JM, Schulman IH, Anderson RD, Lambert C, Sayre SL, Taylor DA, Ebert RF, Moye LA. Bone marrow mononuclear cell therapy for acute myocardial infarction: a perspective from the cardiovascular cell therapy research network. *Circ Res*. 2014;114:1564–1568.
3. Surder D, Manka R, Lo Cicero V, Moccetti T, Rufibach K, Soncin S, Turchetto L, Radrizzani M, Astori G, Schwitter J, Erne P, Zuber M, Auf der Maur C, Jamshidi P, Gaemperli O, Windecker S, Moschovitis A, Wahl A, Buhler I, Wyss C, Kozerke S, Landmesser U, Luscher TF, Corti R. Intracoronary injection of bone marrow-derived mononuclear cells early or late after acute myocardial infarction: effects on global left ventricular function. *Circulation*. 2013;127:1968–1979.
4. Dash R, Kim PJ, Matsuura Y, Ikeno F, Metzler S, Huang NF, Lyons JK, Nguyen PK, Ge X, Po Foo CW, McConnell MV, Wu JC, Yeung AC, Harnish P, Yang PC. Manganese-enhanced MRI enables in vivo confirmation of peri-infarct restoration following stem cell therapy in porcine ischemia-reperfusion model. *J Am Heart Assoc*. 2015;4:e002044 doi: 10.1161/JAHA.114.002044.
5. Huang S, Chen HH, Yuan H, Dai G, Schuhle DT, Mekkaoui C, Ngoy S, Liao R, Caravan P, Josephson L, Sosnovik DE. Molecular MRI of acute necrosis with a novel DNA-binding gadolinium chelate: kinetics of cell death and clearance in infarcted myocardium. *Circ Cardiovasc Imaging*. 2011;4:729–737.
6. Sosnovik DE, Nahrendorf M, Deliolanis N, Novikov M, Aikawa E, Josephson L, Rosenzweig A, Weissleder R, Ntziachristos V. Fluorescence tomography and magnetic resonance imaging of myocardial macrophage infiltration in infarcted myocardium in vivo. *Circulation*. 2007;115:1384–1391.
7. Su H, Spinale FG, Dobrucki LW, Song J, Hua J, Sweterlitsch S, Dione DP, Cavaliere P, Chow C, Bourke BN, Hu XY, Azure M, Yalamanchili P, Liu R, Cheesman EH, Robinson S, Edwards DS, Sinusas AJ. Noninvasive targeted imaging of matrix metalloproteinase activation in a murine model of postinfarction remodeling. *Circulation*. 2005;112:3157–3167.
8. Makowski MR, Ebersberger U, Nekolla S, Schwaiger M. In vivo molecular imaging of angiogenesis, targeting alphavbeta3 integrin expression, in a patient after acute myocardial infarction. *Eur Heart J*. 2008;29:2201.
9. Kraitchman DL, Heldman AW, Atalar E, Amado LC, Martin BJ, Pittenger MF, Hare JM, Bulte JW. In vivo magnetic resonance imaging of mesenchymal stem cells in myocardial infarction. *Circulation*. 2003;107:2290–2293.
10. Kraitchman DL, Tatsumi M, Gilson WD, Ishimori T, Kedziorek D, Walczak P, Segars WP, Chen HH, Fritzges D, Izbudak I, Young RG, Marcelino M, Pittenger MF, Solaiyappan M, Boston RC, Tsui BM, Wahl RL, Bulte JW. Dynamic imaging of allogeneic mesenchymal stem cells trafficking to myocardial infarction. *Circulation*. 2005;112:1451–1461.
11. Cao F, Lin S, Xie X, Ray P, Patel M, Zhang X, Drukker M, Dylla SJ, Connolly AJ, Chen X, Weissman IL, Gambhir SS, Wu JC. In vivo visualization of embryonic stem cell survival, proliferation, and migration after cardiac delivery. *Circulation*. 2006;113:1005–1014.
12. Naumova AV, Reinecke H, Yarnykh V, Deem J, Yuan C, Murry CE. Ferritin overexpression for noninvasive magnetic resonance imaging-based tracking of stem cells transplanted into the heart. *Mol Imaging*. 2010;9:201–210.
13. Sosnovik DE, Mekkaoui C, Huang S, Chen HH, Dai G, Stoeck CT, Ngoy S, Guan J, Wang R, Kostis WJ, Jackowski MP, Wedeen VJ, Kozerke S, Liao R. Microstructural impact of ischemia and bone marrow-derived cell therapy revealed with diffusion tensor magnetic resonance imaging tractography of the heart in vivo. *Circulation*. 2014;129:1731–1741.
14. Malliaras K, Smith RR, Kanazawa H, Yee K, Seinfeld J, Tseliou E, Dawkins JF, Kreke M, Cheng K, Luthringer D, Ho CS, Blusztajn A, Valle I, Chowdhury S, Makkar RR, Dharmakumar R, Li D, Marban L, Marban E. Validation of contrast-enhanced magnetic resonance imaging to monitor regenerative efficacy after cell therapy in a porcine model of convalescent myocardial infarction. *Circulation*. 2013;128:2764–2775.

Key Words: Editorials • imaging • magnetic resonance imaging • myocardium • regeneration • stem cell