

Stem Cell Therapy in Acute Myocardial Infraction
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Introduction

The incidence of congestive heart failure (HF) is increasing with epidemic proportions, occurring most frequently in patients with structural heart disease resulting from myocardial infarction (MI). The process of cardiac remodeling that provides the substrate for HF results from fibrotic scar formation that replaces regions of myocyte necrosis. In addition, surviving myocytes undergo hypertrophy, which may be initially beneficial but subsequently transitions to a maladaptive process in which myocytes become vulnerable to apoptosis. The heart dilates due to infarct expansion, with wall thinning and dilatation of the ventricle, hyperplasia of fibroblasts, and scar formation, which results in changes to its geometry and loss of contractile function.¹

Recently, mitotic cardiomyocytes and endogenous cardiac stem cells have been identified within the mammalian heart²⁻⁴ suggesting that the heart is not a terminally differentiated organ. However, it is evident that these endogenous repair mechanisms are insufficient to allow a complete structural and functional recovery of the heart from a MI. Cellular regenerative approaches hold great promise to prevent remodeling and progression to HF in ischemically damaged hearts which has resulted in two closely related avenues of research. One approach has focused on the biology of endogenous stem or precursor cells that reside in the adult heart^{5;6}. Other researchers have used a variety of exogenous progenitor cells and stem cells as transplants into regions of ischemic damaged myocardium. In experimental models the cell types which have been investigated include fetal and neonatal cardiomyocytes, embryonic stem cell derived myocytes, tissue engineered contractile grafts, skeletal myoblasts, several cell types derived from adult bone marrow, and cardiac stem cells^{3;7-15}. This approach termed, cellular cardiomyoplasty^{16;17}, is currently under active investigation in large animal models, and has already prompted clinical trials^{16;18-21}.

Role of Non-Invasive MRI for Assessment of Cell Therapy

Imaging is playing an important role in assessing the myocardial response to stem cell therapy. The correlation of tissue specimen, allowing cellular and molecular characterization, with noninvasive imaging of myocardial function, viability, perfusion and cell tracking is the foundation to elucidate the effects of any novel cell therapy. However, it is critical to assess the efficacy regenerative therapy by having the ability to image tissues within the heart at various time-points.

It is essential to determine if regenerated tissue is indeed functional i.e. does the new tissue contract with the rest of the heart or is non-contractile and mechanically uncoupled from the rest of the heart, in which case it may actually be detrimental to cardiac function.

In experimental animal studies implantation of cells such as bone marrow derived mesenchymal stem cells (MSC) appears to restore the function of the damaged heart and decrease necrotic tissue. Whether they do so by generating actively and electromechanically coupled myocardium or by passive effects on the composition of the evolving scar remains unknown. Some groups advocate that these cells are not contractile, and indeed there is a possibility that new viable tissue functions to decrease myocardial wall tension at the infarcted region, thereby improving diastolic function and enhancing global cardiac function. MRI technology has successfully been used to address these important issues in a clinical relevant swine models.²²

Moreover, MRI has been applied in most clinical trials to determine the effect of cell therapy. The evaluation global contractility and endsystolic volume have been commonly used as study endpoints^{14;20;21}.

MRI is an ideal modality to assess regenerative cell therapy as it offers a number of quantitative measurements that are considered to be gold standard metrics a detailed below:

Global Left Ventricular Function

The ability of MRI to provide global function assessment in a number of pathophysiologic states has been well documented. Global function quantities, such as ventricular mass, ventricular volume, and ejection fraction can be obtained by image segmentation and definition of contours used along with Simpson's rule to calculate the LV volume at various cardiac phases. The volume of the cavity at end-systole and end-diastole (ESV and EDV) is determined based on the largest and smallest volumes. By measuring the percentage change in volume at end-systole relative to end-diastole, the ejection fraction of the heart is then measured ($EF=100*(EDV-ESV)/EDV$ %).

Regional Function - MR Myocardial Tagging

MRI tagging is a technique that places *non-physical* markers non-invasively inside the tissue by manipulating the magnetization of the tissue using special encoding pulses^{23;24}. These markers, called *tags*, appear in the acquired images as dark lines. These tags are generated by modulating the magnetization of the heart and deform with the motion of the myocardium during the cardiac cycle. In addition to simplifying the qualitative assessment of cardiac deformation, these tags can be used to quantify dynamic strain during the cardiac cycle. Significant local strains include circumferential shortening, radial thickening, and longitudinal compression.

Harmonic Phase (HARP) Analysis of Myocardial Tagging Data: While detailed 3D strain analysis of a cell treated myocardium segment is an excellent method to monitor improvements in regional function over time, it involves extensive post-processing time and thereby limits the application MRI tagging. A new method to analyze tagged data called harmonic phase (*HARP*) imaging. *HARP* measures the motion from tagged MR images by filtering certain regions in the k-space of the images called the harmonic peaks. The resulting image is a complex image which

can be decomposed into magnitude and phase, called *harmonic magnitude* and *harmonic phase*, respectively. The harmonic magnitude image is related to the underlying anatomy of the heart, which can be used for segmentation by simple thresholding, while the phase HARP image is directly related to the motion and deformation of the tags and thus the motion of the myocardium. Additionally, the gradients of the HARP images are related to the local strain which is used to produce strain maps. HARP measures local strain of tissue by measuring the tag line frequency changes that occur with contraction or stretching of the heart. Since the phase of any point in tissue does not change over time, it is possible to track the motion of a point simply by determining the position in the images at different cardiac phases that satisfy this condition. It is also possible to track multiple points that segment the heart using the HARP tracking method. A mesh that segments the LV into a number of segments can be built at one timeframe—preferably close to end-systole. Each of the points that constitute the mesh is tracked through the other timeframes. The mesh, therefore, tracks with the motion of the heart and allows measurements of *strain* and strain rate. By tracking the motion of different regions of the heart using a mesh, the regional strains are measured on trajectory of motion.

HARP analysis can be used to determine peak circumferential negative strain ($E_{cc_{max}}$) which is used as a primary metric of regional function since this parameter most directly reflects maximal myocardial shortening in a selected layer of myocardial tissue. Thus, values of increasing strain (towards positive values) reflect relatively dyskinetic or non-contracting myocardial segments. Tracking of $E_{cc_{max}}$ over time provides valuable quantitative information regarding the restoration of contractile function in the treated myocardial region.

Myocardial Viability Imaging

Delayed contrast enhanced (DCE) MRI for assessment of myocardial viability has been well validated over the past several years and is performed routinely by several cardiac MRI centers. Several studies have demonstrated that MRI accurately represents the spatial extent of myocardial cell death and is routinely used to perform delayed enhancement MRI in animal models and humans following acute and chronic myocardial infarction²⁵.

The mechanism of myocardial hyper-enhancement in the setting of infarction is based on the action of gadolinium-DTPA as an extra-cellular contrast agent. In normal myocardium myocytes are densely packed and thus intracellular space of the myocytes represents a majority of the total myocardial volume. In the setting of acute infarction, the myocyte membrane integrity is compromised and Gd-DTPA is able to diffuse into the intracellular space to greatly increase the volume of distribution and result in hyperenhancement. In the setting of chronic infarction, myocytes have been replaced with collagenous scar which increases interstitial space and leads to spatially increased gadolinium concentration and hyperenhancement.

The typical pattern of hyper enhancement occurs in patients with prior myocardial infarction and thus in those with ischemic HF can be explained by the

patho-physiology of ischemia. Following coronary occlusion, myocardial contractile function falls almost immediately throughout the region of ischemia. However, little or no cellular necrosis is found until approximately 15 minutes after occlusion. From this point on a 'wave front' of necrosis begins in the sub-endocardium and grows towards the epicardium over the next few hours. During this period the region of ischemia remains the same size, but the infarcted region within the ischemic zone increases continuously towards a transmural infarction.

DCE has been applied in animal model to evaluate MSC therapy demonstrating reduction infarct size²². Furthermore, DCE can determine prognosis in patients post-MI, which can be a useful tool to determine the effects of cell therapy

Perfusion

MRI is increasingly applied as an alternative to nuclear medicine assessing microcirculation. Myocardial perfusion imaging using contrast-enhanced first-pass MRI can obtain whole-heart coverage at a resolution double that of a PET scanner and 4 times the resolution of SPECT²⁶. MRI first-pass perfusion studies can be performed either at rest or under physical or pharmacological stress to evaluate the myocardial microcirculation.

Clinically, MR perfusion imaging is mainly applied to detect coronary artery disease using parameters like coronary flow reserve. Coronary flow reserve, defined as the ratio of coronary flow at maximal vasodilatation compared with rest is a guide in clinical applications to the significance of coronary narrowing. Changes in first-pass enhancement in a cardiac segment during pharmacologically induced vasodilation is directly proportional to the perfusion of that segment²⁷.

Both, rest and pharmacological stress perfusion is applied to evaluate stem cell therapy in animal models and clinical trials²⁸. Infusion of bone marrow derived cells showed increased myocardial blood flow in the relevant territories in treated patients. Despite the ongoing controversy which mechanisms underly cellular therapy. Two major functional benefits have emerged from exogenous cellular therapy. One is cardiac regeneration through cell fusion or cell differentiation to cardiac phenotypes, which can be evaluated by MRI tagging methods as discussed above. The other principal is improvement of neovascularization, which has attracted much attention lately.

Neovascularization and angiogenesis, occurs mostly on the level of myocardial microcirculation, which is amendable to MRI perfusion techniques²⁹ and offers the opportunity to study these biological processes. Indeed, we recently could demonstrate an effect of MSCs therapy on the microcirculation shortly after cell delivery using first pass perfusion MRI³⁰.

Cell Tracking

Cellular imaging based on MRI technology requires labeling of the target cells. Currently, most cellular MRI *in vivo* labeling strategies have applied iron oxide particles, so called ultra-small superparamagnetic iron oxide (SPIO) compounds³¹⁻³³.

The compound is internalized into the living cell, because surface labels may become detached and transferred to other cells. The great advantage of SPIO labeling is that picogram quantities of iron per cell can lead to large hypo-intense signals which can easily be visualized on T2* weight images. Another advantage of SPIO labeling is that when the cells are lysed the iron oxide is only taken up by phagocytic cells and ultimately recycled in the iron pool thereby preventing to damage tissue at the injection side. However, on the other hand, this is also the biggest problem of SPIO labeling because iron particles from degraded cells are incorporated by adjacent phagocytic cells. Thus, hypo-intense artifacts in images at later time points after cell delivery may represent cells other than the originally labeled ones. Therefore strict quantification of cell numbers based on the change in volume of the hypo-intense signal is difficult to determine.

SPIO have been used to verify cell delivery and follow up studies up to two months have been performed^{22;34}. Furthermore, it has been recently demonstrated that SPIO labeling does not impair the biological effect of injected stem cells³⁵, which might open the opportunity to use this MRI tracking method in clinical studies.

Conclusion

Cardiac MRI assessment of acute stem therapy provides valuable information regarding both the functional response to cell therapy and provides insight into mechanism of observed global and functional improvement. MRI combined with other high resolution imaging modalities such as MDCT will be important tools to provide answers for a number of unresolved issues surrounding cell therapy in acute myocardial infarction.

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