Assessment of the Heart Microstructure: From Mouse to Man

Running title: Naumova et al.; Assessment of the Heart Microstructure

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Stem cell therapy has undergone a rapid translation from bench research to clinical trials as a promising approach for the regeneration of the injured myocardium\textsuperscript{1,2}. Magnetic resonance imaging (MRI) plays a pivotal role in the assessment of stem cell therapy efficacy and elucidation of the mechanisms behind therapeutic effects\textsuperscript{3}. One important aspect of stem cell therapy, however, remains missing: there are currently no non-invasive methods to evaluate the restoration of myocardial tissue microstructure. A study by Sosnovik et al.\textsuperscript{4} published in this issue of Circulation fills this gap and demonstrates the feasibility of evaluating the integrity and spatial organization of myofibers after cell therapy.

The microstructure of the heart was histologically described more than 40 years ago in landmark studies by Streeter et al\textsuperscript{5}. The myoarchitecture of a healthy heart is made up of three layers of crossing spiral myofibers. The subendocardium fiber orientation is a right-handed helix, while the subepicardium is a left-handed helix, and fibers in the mid-myocardium are circumferential\textsuperscript{5}. This structure allows for maximal contractile force to ensure effective blood pumping. Despite the discovery of the complex cardiac myoarchitecture and its role in heart function, opportunities to study this aspect of the cardiac anatomy non-invasively were not available for several decades.

Diffusion tensor imaging (DTI), the first MRI method capable of visualizing cardiac microstructure, was developed in the mid-1990s\textsuperscript{6}. DTI allows characterization of anisotropic diffusion of water molecules in tissues. Diffusion anisotropy arises from natural barriers, such as cell membranes, and is most prominent in tissues consisting of coherent fiber bundles, such as brain white matter or muscle. The MR signal can be sensitized to diffusion by applying sufficiently strong magnetic field gradients, which cause the loss of phase coherence of individual molecular magnetizations in the presence of diffusion\textsuperscript{6}. In DTI, a series of data
acquisitions is performed to probe diffusion in many directions along applied gradients. DTI data can be utilized in several ways, including voxel-based calculation of scalar indexes characterizing diffusion rate and anisotropy (such as mean diffusivity, fractional anisotropy, axial, and radial diffusivities) and reconstruction of fiber trajectories in tissues where diffusion has a preferred direction along fibers.

The latter approach, DTI tractography\(^7\), has been widely used in brain imaging to study spatial organization of white matter fiber tracts over the past two decades. In contrast to neuroimaging, cardiac applications of DTI are much more technically challenging. The main difficulty is associated with cardiac motion, which is especially problematic for diffusion-sensitized MR sequences. While motion problems can be partially mitigated by cardiac gating and special motion-compensated pulse sequences\(^8,9\), it is difficult to accommodate relatively long diffusion gradients within the quiescent time interval of the heart cycle. Another challenge is the low signal-to-noise ratio (SNR) caused by both diffusion signal attenuation and short T2 relaxation times of heart tissue, resulting in long acquisition times. These challenges have been prohibitive for in vivo cardiac DTI in small animals. The study by Sosnovik et al.\(^4\) provides the first demonstration of the feasibility of cardiac DTI tractography in live mice. Using previous developments in pulse sequence design\(^9\) and ultra-high-strength gradient insert, the authors\(^4\) were able to overcome these technical obstacles and obtain high-resolution 3D reconstructions of myofibrillar tracts in the murine heart in vivo.

Previous ex vivo cardiac DTI studies in animal models\(^10-13\) have demonstrated the capability of DTI to adequately depict spatial organization of cardiac myofibers. Heart myoarchitecture evaluated with DTI has demonstrated high concordance with histology\(^10\). DTI tractography has shown a smooth transition in fiber orientation from epicardium to endocardium.
in healthy myocardiums and severe disruption of myofiber architecture after infarction\(^{11}\). Ex vivo DTI was used to quantify infarct healing after ischemic injury in animal models\(^{12}\).

The work by Sosnovik et al\(^{4}\) is unique in several aspects. First, it reveals the feasibility of 3D DTI tractography of the entire mouse heart \textit{in vivo} with isotropic spatial resolution. Second, it provides rigorous validation of this technique with both histology and \textit{ex vivo} diffusion spectrum imaging (DSI). It should be noted that DSI\(^{13}\) can be considered a gold standard diffusion imaging method, which is free of certain DTI simplifications and is especially useful in resolving complex patterns of intersecting fibers, though at the expense of extremely long acquisition times, making this technique inapplicable for \textit{in vivo} studies. Third, Sosnovik et al\(^{4}\) provide the first example of serial DTI tractography as a monitoring tool in an animal model of heart disease. Fourth, the study\(^{4}\) establishes DTI as a new approach for assessment of the effect of stem cell therapy in a preclinical setting, where it could prove whether cardiomyocytes derived from stem cells are actually aligned with host myofibers to regenerate heart structure and function.

The heart is one of the least regenerative organs in the body. A common clinical scenario in heart failure is characterized by a loss of roughly one billion cardiomyocytes after acute infarction, leading to a rapid initial functional loss followed by a slower decline as the ventricle undergoes adverse structural remodeling\(^{1}\). Stem cells have an ability to differentiate to any cell types, including beating cardiomyocytes, therefore the potential to rejuvenate injured human tissues after severe cell loss. Stem cell therapy can be considered effective if transplanted cells not only survive in the infarcted environment, but integrate structurally and functionally with host tissue. The real challenge is to achieve adequate alignment of the transplanted cardiomyocytes with host myocardium to restore complex three-dimensional microstructure of the heart. This is critical for efficient contractile and conductive functions of the renewed...
myocardium.

The key finding of the study by Sosnovik et al.\textsuperscript{4} is the persisting chronic abnormalities of myofiber alignment and coherence after ischemic injury in the ischemia-reperfusion model. These abnormalities remain detectable by DTI tractography and are consistent with histology during a relatively long period after injury despite restoration of both signal abnormalities on conventional MRI and quantitative scalar indexes derived from DTI data (mean diffusivity and fractional anisotropy). These observations suggest that DTI tractography may provide more sensitive and specific biomarkers of myocardial injury and regeneration than conventional MRI techniques. At the same time, the results of stem cell therapy in mice reported by Sosnovik et al. are not particularly encouraging, as only one animal out of six in the treatment arm of interest (bone marrow mononuclear cells from the same animal phenotype) showed improved healing based on DTI data. While this observation should be reproduced in larger-scale studies, it may explain the mixed results of human clinical trials utilizing the same cell type\textsuperscript{2,14} and emphasizes the critical importance of identifying optimal therapeutic regimens.

Some limitations of the study\textsuperscript{4} should be noted. Particularly, it was not investigated whether a post-ischemic defect of myoarchitecture (with or without stem cell treatment) is associated with impaired heart function, contractility, and perfusion. Establishment of such associations in animal and human studies is of particular importance for understanding the clinical relevance of cardiac DTI tractography. To date, only three studies investigated associations between quantitative indexes derived from DTI, heart morphology, wall motion, and viability in human myocardial infarction\textsuperscript{5,16} and hypertrophic cardiomyopathy\textsuperscript{17}. It is particularly encouraging that all of the above aspects of cardiac pathophysiology can be assessed using currently available CMR techniques.
A word of caution should be added regarding interpretation of the results of DTI reconstruction as an anatomical depiction of actual myofibers. In fact, visual representation of tractographic reconstructions does not correspond to the density, length, and diameter of the fibers. Likewise, the loss of fiber coherence on DTI tractograms does not necessarily indicate the physical loss of fiber tracts, but could rather be caused by edema, proliferation of connective tissue, or imaging artifacts. More rigorous quantitative approaches to characterize integrity of myofibrillar tracts are yet to be developed.

Finally, the feasibility of the translation of the methodology outlined by Sosnovnik et al.\textsuperscript{4} into clinical trials should be pointed out. While the strength of magnetic field gradients is particularly important for DTI, the authors\textsuperscript{4} showed successful results of cardiac DTI tractography in a healthy volunteer on a 3T clinical scanner. This study\textsuperscript{4} also demonstrated an improvement in resolution (2x2x4 mm\textsuperscript{3}) of human cardiac DTI compared to earlier publications\textsuperscript{8,9,15-19}. Collectively, the current and earlier studies have established the feasibility of DTI tractography in the human heart in vivo. However, a significant amount of work remains to translate this challenging technology to serial applications in clinical trial settings. Particularly, an optimal pulse sequence for acquisition of source data needs to be chosen based on a direct comparison study. The choice should be made between spin-echo\textsuperscript{9} and stimulated-echo\textsuperscript{8} techniques for diffusion sensitization. While the first approach offers a better SNR, the second may be less sensitive to motion. Another question is the optimal readout for the MR sequence. While the current practice is based on a single-shot echo-planar sequence, potential benefits or disadvantages of other single-shot readout techniques, such as fast spin-echo or combined gradient- and spin-echo, have not been systematically evaluated in cardiac DTI. Another unresolved question is what the optimal angular sampling scheme is for DTI acquisition. Such a
scheme should enable robust reconstruction of myofibrillar tracts, but avoid long acquisition times so as to be applicable in clinical settings. While this question has been extensively studied in brain DTI\textsuperscript{20}, it remains unclear whether DTI sampling approaches derived for brain imaging will be optimal for cardiac applications. Additional work is also needed for the standardization of the tract reconstruction algorithms and development of quantitative measures adequately characterizing damage and repair of myofibers.

In summary, DTI provides a unique imaging approach for evaluation of structural restoration of myofiber architecture. We believe that the inspirational study by Sosnovik et al.\textsuperscript{4} will accelerate progress in this area and ultimately lead to new imaging tools in the assessing efficacy of stem cell therapy for cardiac regeneration.

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\textbf{References:}


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