Assessment of Heart Microstructure
From Mouse to Man

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tem cell therapy has undergone a rapid translation from bench research to clinical trials as a promising approach for the regeneration of the injured myocardium. Magnetic resonance imaging (MRI) plays a pivotal role in the assessment of stem cell therapy efficacy and elucidation of the mechanisms behind therapeutic effects. One important aspect of stem cell therapy, however, remains missing: There are currently no noninvasive methods to evaluate the restoration of myocardial tissue microstructure. A study by Sosnovik et al 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 described histologically 40 years ago in landmark studies by Streeter and Hanna. The myoarchitecture of a healthy heart is made up of 3 layers of crossing spiral myofibers. The subendocardium fiber orientation is a right-handed helix, the subepicardium is a left-handed cross spiral myofibers. The subendocardium fiber orientation from epicardium to endocardium in healthy myocardium demonstrates the feasibility of evaluating the integrity and spatial organization of myofibers after cell therapy. Diffusion tensor imaging (DTI), the first MRI method capable of visualizing cardiac microstructure, was developed in the mid 1990s. 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 magnetic resonance 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. 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, and axial and radial diffusivities) and reconstruction of fiber trajectories in tissues where diffusion has a preferred direction along fibers.

The latter approach, DTI tractography, has been used widely in brain imaging to study spatial organization of white matter fiber tracts over the past 2 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 magnetic resonance sequences. Whereas motion problems can be partially mitigated by cardiac gating and special motion-compensated pulse sequences, 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 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 provides the first demonstration of the feasibility of cardiac DTI tractography in live mice. Using previous developments in pulse sequence design and ultra-high-strength gradient insert, the authors were able to overcome these technical obstacles and obtain high-resolution 3-dimensional reconstructions of myofibrillar tracts in the murine heart in vivo.

Previous ex vivo cardiac DTI studies in animal models 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. 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. Ex vivo DTI was used to quantify infarct healing after ischemic injury in animal models.

The work by Sosnovik et al is unique in several aspects. First, it reveals the feasibility of 3-dimensional DTI tractography of the entire mouse heart in vivo with isotropic spatial resolution. Second, it provides rigorous validation of this technique with both histology and ex vivo diffusion spectrum imaging. It should be noted that diffusion spectrum imaging can be considered a gold standard diffusion imaging method that is free of certain DTI simplifications and is especially useful in resolving complex patterns of intersecting fibers although at the expense of extremely long acquisition times, making this technique inapplicable for in vivo studies. Third, Sosnovik et al provide the first example of serial DTI tractography as a monitoring tool in an animal model of heart disease. Fourth, the study establishes DTI as a new approach for assessment of the effect of stem cell therapy in a preclinical setting, in which 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 $\approx 1$ billion cardiomyocytes after acute infarction, leading to a rapid initial functional loss followed by a slower decline as the ventricle undergoes adaptive structural remodeling. Stem cells have an ability to differentiate to any cell type, including beating cardiomyocytes, and they therefore have 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 the complex 3-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. 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 because only 1 of 6 animals in the treatment arm of interest (bone marrow mononuclear cells from the same animal phenotype) showed improved healing on the basis of DTI data. Although this observation should be reproduced in larger-scale studies, it may explain the mixed results of human clinical trials utilizing the same cell type and emphasizes the critical importance of identifying optimal therapeutic regimens.

Some limitations of the study should be noted. Particularly, it was not investigated whether a postischemic defect of myofibrillar tracts is yet to be developed. Finally, the feasibility of the translation of the methodology outlined by Sosnovik et al. into clinical trials should be noted. Whereas the strength of magnetic field gradients is particularly important for DTI, the authors showed successful results of cardiac DTI tractography in a healthy volunteer on a 3T clinical scanner. This study also demonstrated an improvement in resolution ($2 \times 2 \times 4$ mm$^3$) of human cardiac DTI compared with earlier publications. Collectively, the present 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 on the basis of a direct comparison study. The choice should be made between spin-echo and stimulated-echo techniques for diffusion sensitization. Although the first approach offers a better signal-to-noise ratio, the second may be less sensitive to motion. Another question is the optimal readout for the magnetic resonance sequence. Although 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-echo and spin-echo techniques, have not been systematically evaluated in cardiac DTI. Another unresolved question involves the optimal angular sampling scheme 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. Although this question has been studied extensively in brain DTI, 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 will accelerate progress in this area and ultimately lead to new imaging tools for assessing the efficacy of stem cell therapy for cardiac regeneration.

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**Disclosures**

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**References**


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