Impact of the Connective Tissue Matrix in the Myocardium on the Restriction of Water Revealed with Diffusion Tensor MRI of a Decellularized Human Heart

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Target Audience: Scientists/clinicians interested in MRI of the myocardium.

Purpose: The myocardium contains a branching network of muscle fibers as well as a supporting network of connective tissue fibers. Both the myofiber and connective tissue networks are anisotropic but their relative contributions to the restriction of diffusion in the heart remain unknown. This has significant implications for the interpretation of diffusion tensor MRI (DTI) data in cardiac disease. To address this question we performed high-resolution DTI and two-photon microscopy of a decellularized human heart ex vivo. Diffusion in the decellularized heart was compared with diffusion in normal human hearts and in patients with recent myocardial infarction.

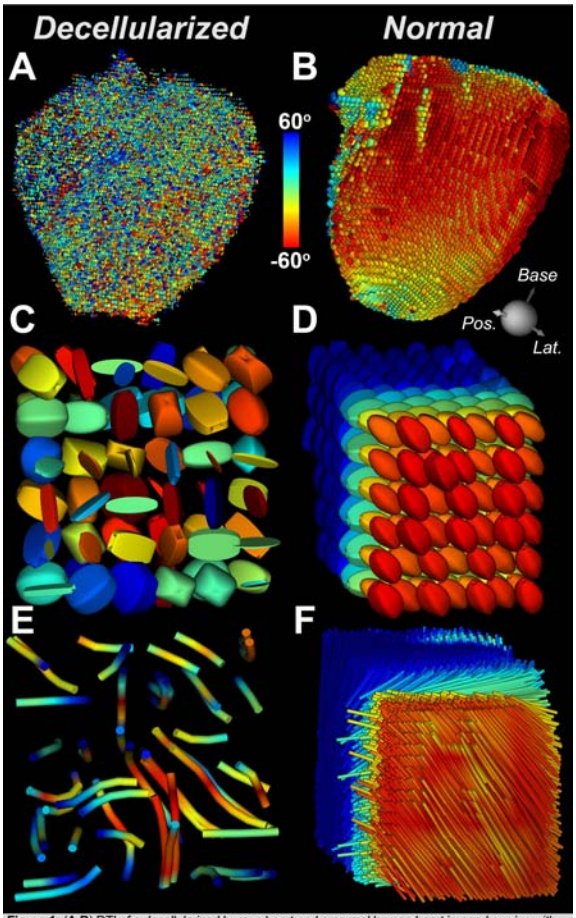

Figure 1: (**A,B**) DTI of a decellularized human heart and a normal human heart imaged ex vivo with the identical resolution. The eigensystem in the heart is represented by supertoroidal glyphs. The color and orientation of the glyphs is determined by their helix angle (HA) and their shape/volume by the diffusivity of water. (**C,D**) Magnified view of supertoroids in the anterior wall of the decellularized and normal hearts, respectively. In the decellularized hearts the glyphs are randomly oriented, consistent with isotropic diffusion. (**E-F**) Magnified view of fiber tracts in the decellularized and normal human hearts, respectively. Few coherent tracts can be resolved in the decellularized heart.

Methods: The human heart was obtained from the Institute for the Advancement of Medicine, fixed and processed as previously described.[1] The entire heart was immersed in a susceptibility-matching medium and imaged on a clinical 3T scanner with a diffusion-encoded spin echo echoplanar sequence and the following parameters: resolution of 2x2x2 mm^3, b-value of 1400 s/mm^2, and 6 diffusion-encoding directions. Following MRI, a plug of tissue was removed from the anterior wall of the left ventricle (LV) for high-resolution DTI at 9.4 Tesla (isotropic 100 μm resolution, b-value 507 s/mm^2, and 24 diffusion-encoding directions) and 2-photon microscopy. Excitation was performed at 920 nm, myofiber detection was at 525 nm, and collagen was detected by second harmonic imaging at 460 nm. Resolution was 0.9x0.9x2 μm^3 covering a depth of 300 μm. 5 normal human hearts were fixed in PFA and imaged ex vivo at 3T with the same parameters as the decellularized heart.[2] Three patients with recent myocardial infarction (MI) were imaged on a 1.5T clinical scanner with the following parameters: resolution 1.75x1.75x8 mm^3, b-value of 500 s/mm^2, 6 diffusion encoding directions, and 8 averages. Imaging was performed in the diastolic sweet spot of the cardiac cycle to mitigate the effect of strain. Late gadolinium enhancement was performed in these patients and used to define the infarct zone. Mean diffusivity (MD) and fractional anisotropy (FA) values in a region of interest in the lateral wall of the decellularized heart were computed at 16 short axis planes and compared with values in the normal human hearts and in the infarct zone of the patients with recent MI. The diffusion tensor was further modeled using supertoroid glyphs, and fiber tracts were constructed by integrating the primary eigenvector field into streamlines using a 5th order Runge-Kutta approach.

Results: The decellularized heart exhibited a macroscopic appearance similar to chronic infarct scar/aneurysm. Scattered foci of myocytes, however, were seen on the epicardial surface. Diffusion in the decellularized human heart was largely unrestricted and isotropic in nature. MD in the decellularized heart averaged $1.20x10^{-3} \pm 0.08$ mm^2/s versus $0.81x10^{-3} \pm 0.11$ mm^2/s in the intact human hearts ($p<0.05$). FA in the decellularized heart averaged 0.38 ± 0.02 versus 0.58 ± 0.01 in the intact human hearts ($p<0.05$). MD and FA in the infarct zone of the 3 patients imaged were $1.07x10^{-3} \pm 0.42$ mm^2/s and 0.45 ± 0.07, respectively. The supertoroid glyphs confirmed the presence of near isotropic diffusion in the decellularized heart (Fig 1A-D). Coherent fiber tracts could not be detected in most regions of the decellularized heart (Fig 1E-F). High-resolution imaging of the tissue plug from the anterior LV wall revealed differences in microstructure between the epicardial portion of the plug, which contained visible foci of myocytes, and the bulk of the plug which did not. Anisotropic diffusion and coherent tracts could be resolved on the surface of the plug but not at the deeper levels (Fig 2A-B). Two-photon microscopy confirmed the high-resolution DTI findings (Fig. 2C-D). Coherently oriented cells (red) were seen near the surface but not at the deeper levels. The bulk of the plug contained only a network of collagen fibers (green).

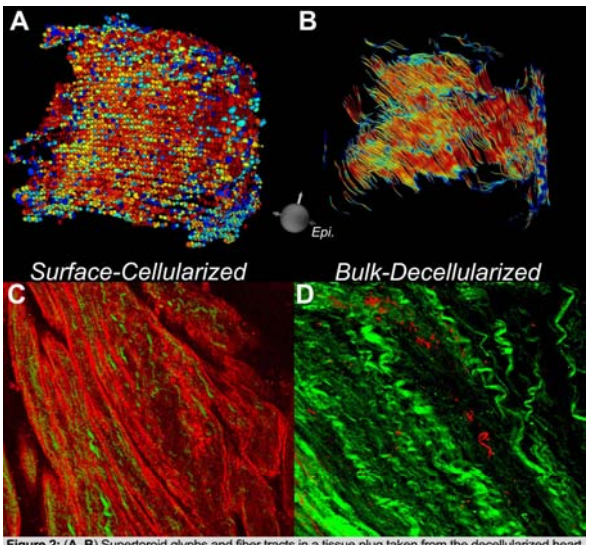

Figure 2: (**A, B**) Supertoroid glyphs and fiber tracts in a tissue plug taken from the decellularized heart and imaged at high resolution. Foci of coherent tracts were resolved near the epicardial surface of the plug but not in its bulk/interior. (**C, D**) Two-photon microscopy shows myofibers (red) at a plane just beneath the epicardial surface (**Fig. 2C**, red), corresponding to the plane of resolvable tracts. However, the bulk of the plug contains no myofibers but only a parallel network of collagen fibers (**Fig. 2D**, green). These collagen fibers do not restrict diffusion sufficiently to create resolvable tracts.

Discussion: Diffusion in the decellularized heart was minimally restricted despite the presence of a fairly dense, ordered, and anisotropic collagen network. This suggests that at the b-values commonly used for DTI of the myocardium (400-2000 s/mm^2), the myocardial connective tissue network has little impact on the diffusion eigensystem. Diseases characterized by diffuse collagen deposition may thus not be detectable with DTI unless changes in myofibroblast content, myofiber size, and myofiber orientation occur as well. Diffusion in the healing infarcts imaged here was moderately restricted, suggesting that myofiber debris, myofibroblasts, and inflammatory cells were present.

Conclusion: The restriction of diffusion in the myocardium predominantly reflects its cellular components and is minimally affected by its connective tissue network.

References: 1) Ott HC *et al*. Nature Med 2008. 2) Mekkaoui C *et al*. JCMR 2012.