Molecular Signaling Pathway Intervention of Fibrotic Alterations of Myo-architecture of Rabbit External Anal Sphincter Muscle following Surgical Myotomy – a DTI Fiber Tracking Study.

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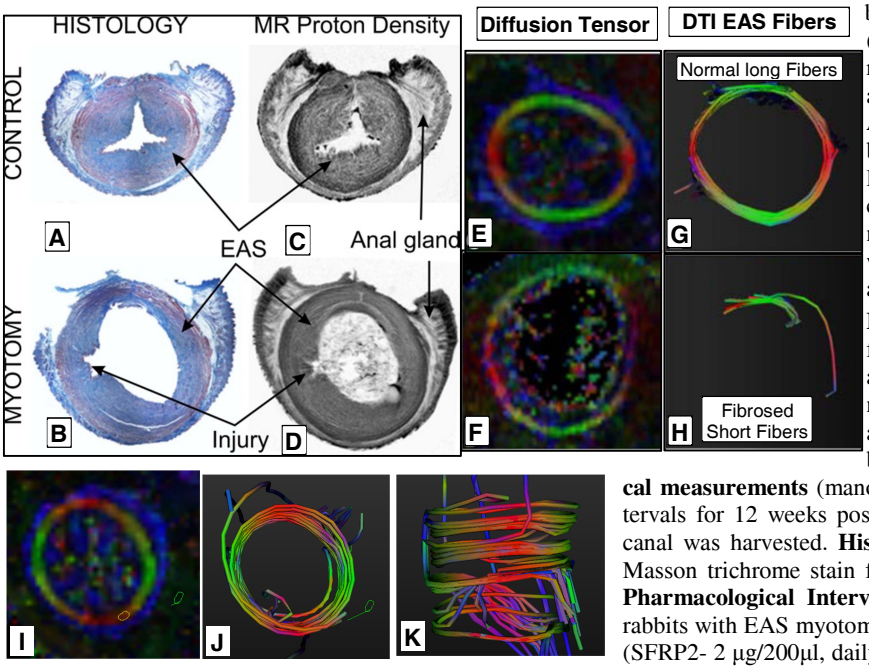
Target Audience: Gastroenterology clinicians, MR physicists, Pharmacologists working in Muscle Fibrosis.

PURPOSE: Childbirth related injury/trauma with anatomical disruptions to the external anal sphincter (EAS) muscle is quite common, resulting in impaired sphincter muscle function and anal incontinence. Our 1st hypothesis was that Diffusion Tensor Imaging (DTI) and Fiber Tracking (FT) could disambiguate well-ordered anal sphincter muscle architecture in healthy EAS muscle from disordered, fibrotic state in muscle recovering from injury. Our 2nd hypothesis was that alterations of molecular signaling pathways involved in muscle fibrosis, e.g. inhibition of Wnt signaling activity by augmentation of secreted frizzled-related protein-2 (SFRP2, a Wnt inhibitor) may mitigate fibrosis of muscles recovering from injury by attenuating collagen deposition and improving myo-architecture and muscle function. **AIM**: To examine the EAS muscle in an animal model before and after an experimental surgical injury, using DTI, FT, and histology, (also manometric measurement of anal canal function not emphasized here), at various stages from normal through the regenerative process, examined both without and with pharmacological intervention of Wnt antagonist, SFRP-2.



METHODS: **Animal Surgery:** Three New Zealand White female rabbits were used as controls. Another six were anesthetized and subjected to surgical myotomy by a cranio-caudal incision that extended along the entire length and thickness of the EAS muscle. The skin was sutured back and the animals were allowed to recover. **Physiological measurements** (manometric studies of anal canal) were recorded at weekly intervals for 12 weeks post-myotomy, after which animals were sacrificed and anal canal was harvested. **Histochemical Processing** (Fig. A, B) was carried out with Masson trichrome stain for muscle /connective tissue and Sirius red for collagen. **Pharmacological Intervention of Molecular Signaling Pathway:** Three of the rabbits with EAS myotomy received injection at the myotomy site of Wnt antagonist (SFRP2- 2 µg/200µl, daily x 5 days), starting immediately after surgery. **MR Imaging:** To determine the myo-architechtural changes of the EAS muscle, MR and DTI was performed on all 9 specimens, in a 7T Bruker Avance II system. ***High-Resolution MRI*** (Fig. C, D)***:*** A 2D RARE double-echo sequence with TE1/TE2/TR: ~17/52/~5,000 ms, slice thickness: 0.5 mm, contiguous; matrix: 256×256; FOV:3x3 cm; in a scan time of ~22 min. ***DTI of the sphincter muscle*** (Fig. E to J) was carried out with a fat saturated EPI SE sequence (TE/TR: 26/18750 ms) with a b value of 600 s/mm^2, 32 diffusion gradient directions and 5 baseline images. Geometry parameters include: 3×3 cm FOV, 256×256 Matrix, 0.5 mm thick/0.5 mm gap and ~70 slices to provide coverage of entire specimen and match the high resolution images. ***DTI and Fiber Tracking Analysis:*** DTIStudio [1] was used to calculate the diffusion tensor, which yielded the fractional anisotropy (FA), and the 3 eigenvectors for the control, without and with SFRP2 intervention specimens. These parameters were then exported to DTITools [2] for fiber tracking and visualization, using typically a FA threshold of 0.15, min/max of 4 and 40 mm fiber length and 50^o max turning angle/step.

RESULTS: Histological images shown in Fig. A and B reveal that EAS myofibers, appearing as red, form continuous circular trajectories bordering the mucosa in normal and SFRP2 treated muscle, while in untreated muscle show considerable fibrosis with a hetereogenous structure of the same fibers. Quantitatively, a 30% connective tissue (fibrosis marker) in controls increased significantly to 72% in untreated injured tissue. The histological images compared very well with the proton-density and T2 high resolution MR images (Fig. C, D). Diffusion Tensor λ_1 Images showed quite explicitly the very homogenous distribution in EAS in both Fig. E: Normal and I: treated injured muscle, which however degenerated to very heterogenous appearance in Fig. F: untreated injured muscle. DTI fiber tracking reinforced the presence of continuous intact fibers in Fig. G: normal and J: treated, injured EAS muscle which degenerated to disorganized, much shorter fibers with loss of continuity shown in Fig. H, untreated, injured muscles. Fig. K shows a side-view of the circular fibers of the EAS. Manometric measurements revealed that surgical myotomy of the EAS significantly impaired its function with minimal recovery during the 12 week.

DISCUSSION and CONCLUSION: We successfully demonstrated, using DTI and fiber tracking, validated against the gold standard of histology, the onset of fibrosis in EAS muscle subsequent to injury and, established the efficacy of a pharmacological intervention aimed at the down-regulation of the canonical Wnt signaling pathway in rabbit model. We found excellent correlation between histology and DTI and fiber tracking, with long, continuous myo-fibers detected in normal and SFRP2 treated injured muscle, in contrast to short disorganized fibers in untreated fibrosed muscle tissue recovering from injury. Loss of normal myo-architecture and collagen deposition in the EAS muscle, possibly due to an impaired regenerative capacity of satellite cells are most likely the cause of impaired function of the EAS muscle following surgical trauma. Sphincter muscle DTI and fiber tracking could evolve as a novel non-invasive diagnostic modality to monitor sphincter injury/ regeneration after childbirth related injury to anal sphincters in women. Such pharmacologic intervention might possibly inhibit other instances of fibrosis as in aging and sarcopenia. **References:** [1] *https://www.**dtistudio**.org/*; [2] www.bmia.bmt.nl/Software/DTITool; **Acknowledgement**: Drs. V. Bhargava and R. Mittal.