Investigating the properties of silk formation in Bombyx mori silkworms using T1 and T2 image maps.

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Introduction: *Bombyx mori*, are a domesticated species of silkworm that have been used for centuries in the commercial production of silk. Silk fibres are formed from a proteinaceous mixture, also referred to as silk dope. The process of silk spinning occurs in two large silk glands; see Figure 1. The posterior section of the gland is responsible for secreting the protein components (Fibroins) that constitute the silk dope, which is then stored in the middle section before being spun through a specially tapered silk duct which initiates silk fibre formation via flow induced protein denaturation before exiting the body through the animal's spinneret. Previous rheological studies on excised silk dope from the silk gland have shown that silk is stored as a viscous gel before undergoing various chemical and mechanical changes during spinning[1]. In this study we determine the T1 and T2 per voxel in contiguous slices of live silkworms as an indicator of the rheological properties of the silk dope as it passes through the gland and duct. Better knowledge of the silk spinning process would help in future development of novel fibre spinning techniques. Furthermore, the study of fibroin aggregation is a potential model for amyloidogenesis[2].

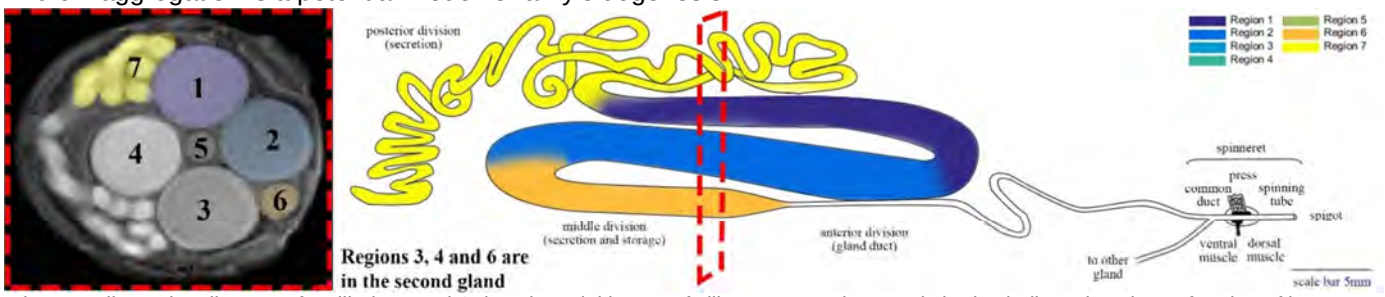

Figure 1: Illustrative diagram of a silk duct; and Spin echo axial image of silk worm. Numbers and shades indicate locations of region of interest used in T1 and T2 maps.

Method: 5th Instar *Bombyx mori* were tightly wrapped up in Clingfilm to restrict their movement, additionally cold nitrogen gas was passed through the coil to induce a state of dormancy. MRI images were obtained using a 9.4T MRI scanner with a 10mm ^{1}H volume coil. A RAREVTR sequence was used to estimate the T1 and T2 values from each image (FOV=15x15mm, Matrix 64x64, 25 1mm contiguous axial slices, TR= 1397.83, 1703.35, 2094.91, 2640.75, 3548.71, 6989.13 ms, TE = 12.56, 25.11, 37.67, 50.23 ms).Images were reconstructed using Bruker Paravision 5.1 software. A custom MatLab program was used to estimate the T1 and T2 relaxation time for each voxel per slice to create a T1 and T2 map for each image. A region of interest was manually drawn around the major silk ducts and the mean±S.D. determined for each region and slice. Slices 1-3 was removed from the analysis due to noisy images.

Results: Regions 1-4 were located in the major silk glands within the midsection and regions 5 and 6 were narrower section of the duct towards the posterior and region 7 was collected from the entangled posterior section (Figure 1). The T1 and T2 were observed to vary smoothly traveling along the gland. Region 5 showed a significant difference in mean T1 compared to all regions except for region 2 (1-way ANOVA). No significant differences in T2 were observed between the regions. The results show that changes in the T1 and T2 properties of the silk dope could be used to track changes in the rheology. Further work is ongoing to track the silk duct in the images to construct a single continuous gland.

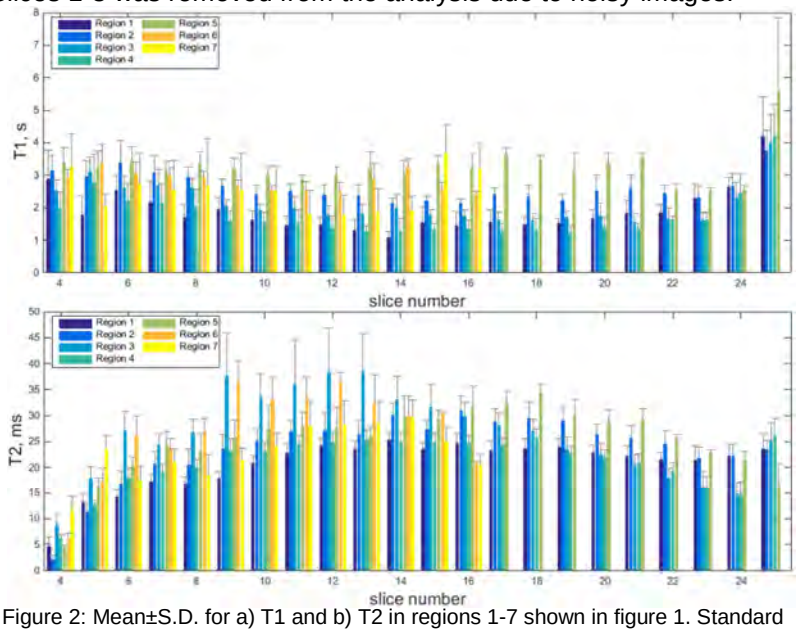

Figure 2: Mean±S.D. for a) T1 and b) T2 in regions 1-7 shown in figure 1. Standard deviation represents the variation in T1/T2 across the region of interest in each

References

1. C. Holland, D. Porter, F. Vollrath. Comparing the Rheology of Mulberry and "Wild" Silkworm Comparing the Rheology Spinning Dopes. Biopolymers. 2012, 97:362-7.
2. Functional Amyloid Aggregation, S. Rigacci and M. Bucciantini eds., 2010.