Effects of Shear Injury on the Subsynovial Connective Tissue and Median Nerve within the Rabbit Carpal Tunnel: A Potential Animal Model of Carpal Tunnel Syndrome

INTRODUCTION
The synovial tissues in the carpal tunnel are unique in containing a substantial, multilayered subsynovial connective tissue (SSCT) and normally, the SSCT loosely connects the flexor tendons and median nerve. We tested the basic premise of our hypothesis, i.e. SSCT injury leads to progressive SSCT fibrosis and subsequent median neuropathy, by investigating the effect of a surgically induced shear injury to the SSCT on SSCT fibrosis and median nerve function in a rabbit model.

METHODS
Surgical Procedure: After approval of our Institutional Animal Care and Use Committee, forty two female New Zealand white rabbits were used for this study. Two volar incisions were made at proximal and distal of the carpal tunnel of one randomly selected forepaw of 30 rabbits, and the flexor digitorum superficialis (FDS) tendon of the middle digit was exposed and cut at the muscle-tendon junction. Two marks with a distance of 5 mm were made on the middle digit FDS tendon and tied together. In effect, the middle digit FDS tendon was distally shifted by 5 mm. For the sham group, two incisions were made and the third FDS tendon was just exposed. The rabbits were allowed 30 minutes of exercise outside their cage twice a week until sacrifice after wounds had healed. All rabbits were sacrificed 12 weeks after the surgery. Twelve rabbits were also used as a normal control group.

Evaluation of Subsynovial Connective Tissue
Mechanical Testing: The method has been described previously (Yamaguchi, et al., 2008). In brief, the middle digit FDS tendon was moved proximally until the tendon was fully pulled through the carpal tunnel. The energy absorption and the stiffness were calculated.

Light Microscopy: Twelve weeks after the surgery we observed hypercellularity, vascular proliferation, thicker collagen bundles and higher fibroblast density in the SSCT of the shear injury group. The fibroblast density of the shear injury group (9244.03 ± 1968.44/mm2) was significantly higher than both sham (5002.74 ± 1436.53/mm2, p<0.001) and normal control group (4020.47 ± 1631.25/mm2, p<0.001).

Transmission Electron Microscopy (TEM): The mean size of the collagen fibers of the shear injury group (58.91 ± 10.80 nm) was significantly higher than either the sham (54.13 ± 7.72 nm, p=0.0004) or normal control groups (55.55 ± 8.61 nm, p=0.0006).

Scanning Electron Microscopy (SEM): Twelve weeks after the surgery we observed thicker collagen bundles in the SSCT of the shear injury group. In contrast, the sham group histology appeared to be similar to the normal control group.

EVALUATION OF MEDIAN NERVE (n=25):
There was no significant difference in distal motor latency either between the shear and sham groups or comparing before and after treatment. The distal motor amplitude, however, showed a significant decrease at 12 weeks in the shear injury group compared to before surgery (p=0.0027), but not in the sham surgery group (p=0.43). There was no evidence of nerve demyelination in the specimens studied.

DISCUSSION
In our study, we observed higher fibroblast density, thicker mean size of the collagen fibers and thicker collagen bundles in the SSCT of the shear injury group; these results are similar to those reported in specimens from patients with carpal tunnel syndrome. While the distal motor latency did not change significantly in our study, the distal motor amplitude did show a significant reduction at 12 weeks in the shear injury group (p=0.0018). These findings are similar to those seen in mild cases of CTS, where a drop in amplitude has been shown to be an earlier finding than a change in latency, both clinically and experimentally. We did not see any evidence of demyelination, but demyelination is associated with decreased nerve conduction velocity, increased temporal dispersion or even conduction block, whereas a reduction in amplitude is more consistent with axonal loss. Longer follow up is maybe necessary to detect delayed distal motor latency and nerve demyelination.

CONCLUSION
The shear injury procedure induced thickening of SSCT collagen fibers, increased fibroblast density, changed SSCT mechanical properties and changed the median nerve amplitude. This suggests that the shear injury procedure may be the basis of a new animal model to study the etiology of carpal tunnel syndrome.

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