INTRODUCTION:
Idiopathic carpal tunnel syndrome (CTS) is known to be associated with non-inflammatory fibrosis and thickening of the subsynovial connective tissue (SSCT). However, there have been no animal models to study the process of this disease. In a previous study, we reported the injection of 10% dextrose induced mechanical property changes and SSCT fibrosis in a rabbit model, and showed the possibility to create a CTS animal model with dextrose injection [1]. In this study we investigated the effects of different concentration and number of hypertonic dextrose injections on the tissues in the rabbit carpal tunnel.

METHODS:
Surgical Procedure
The experimental protocol was approved by the Institutional Animal Care and Use Committee. Sixty-six New Zealand white rabbits weighing 4.0-4.5 kg were used. Following the induction of anesthesia, one fore paw was injected with 0.1 ml of hypertonic dextrose solution (Hospira inc., IL) into the SSCT at the carpal tunnel level. The contralateral forepaw was injected a similar amount of saline. We made five different types of injection groups, namely, saline-single (n=48), saline-double (n=20), 10% dextrose-single (n=28), 20% dextrose-single (n=18), 10% dextrose-double (n=20) injections. For the double injection group, the second injection was made 1 week after the first injection. After 12 weeks of the initial injection, the rabbits were sacrificed for the following evaluations.

1. Evaluations of Subsynovial Connective Tissue
Mechanical Property Testing
After sacrificing the animals, the forepaws were harvested with the carpal tunnel intact. The specimens were mounted on a custom-made microtensioner, which was composed of a linear servo motor (MX 80 Daedal, Irwin, PA) and a load cell (MDB-5, Transducer Techniques, Temecula CA). The middle digit FDS tendon was exposed and the proximal end was connected with a suture to the load cell. The middle digit FDS tendon was then sharply cut 5 mm distal to the distal edge of the carpal tunnel. Under displacement control, the middle finger FDS tendon was moved through the carpal tunnel at a rate of 0.5 mm/s. The ultimate tensile load, the energy absorption, and stiffness, which was defined by tilt of the shear force curve, were measured to estimate the shear force within the SSCT.

Histological Analysis
Tissues were formalin fixed and paraffin embedded. Five μm sections were made and stained with standard Hematoxylin and Eosin. Specimens were evaluated for cellularity, neovascularization, fibrosis, and inflammation. In addition, the distance between middle finger FDS tendon and FDP tendon, and the average of the distance between index-middle and middle-ring finger FDS tendons were measured with imageJ software (National Institute of Mental Health, Bethesda, MD, USA).

2. Evaluations of Median Nerve
Electrophysiological Analysis
Twelve weeks after the initial injection, electrophysiology (EP) testing was performed on each paw, with recording from the thenar muscle with stimulation of the median nerve 3.0cm proximal to the recording point. The distal latency and amplitude were evaluated.

(Statistical Analysis)
Nerve tissue was fixed in a solution of 10% glutaraldehyde and 10% paraformaldehyde. The tissues were embedded in plastic resin. 0.6 μm cross sections of the median nerve were made at the carpal tunnel level and stained with toluidine blue. Fascicular demyelination and subperineurial edema were assessed. In addition, the short and long diameter ratio of median nerve bundles was measured.

REFERENCES:
1. Yoshii Y, et al. The effects of hypertonic dextrose injection on the subsynovial connective tissue in the carpal tunnel; a novel animal model of carpal tunnel syndrome. 54th ORS, Proceeding.

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