INTRODUCTION
The subsynovial connective tissue in the carpal tunnel is highly specialized for tendon gliding and tendon nutrition. Commonly encountered pathologic findings in the subsynovial connective tissue of carpal tunnel syndrome are proliferation with thickening of tenosynovial walls, intimal hyperplasia, vascular proliferation, and thrombosis. These pathological changes are caused by interactions of synoviocytes and quantitative changes of extracellular matrix components such as collagen, elastin, and proteoglycans. In general, fibrotic changes closely correlate with overproduction and structural changes of collagen. However, the ultrastructural changes of the collagen fibrils in the subsynovial connective tissue have not been studied. In this study, the morphological changes of the collagen fibrils in the subsynovial connective tissue of patients with idiopathic carpal tunnel syndrome was analyzed using transmission electron microscope.

METHODS
We used three specimens of SSCT from idiopathic CTS patients during surgery and three from fresh frozen cadaver donors, who had no antemortem history of CTS. Through chart review, we eliminated patients and cadaver donors with endocrine disease, inflammatory disease, degenerative joint disease, flexor tendinitis, hemodialysis, BMI>30, sarcoidosis, amyloidosis, peripheral nerve disease or traumatic injuries to the ipsilateral arm.

Biopsies were fixed overnight in 0.1% glutaraldehyde and 4% formaldehyde in 0.1 M phosphate buffer at pH 7.2. Thin sections were mounted on copper grids for evaluation with a transmission electron microscope (JEOL 1200, JEOL USA, Peabody, MA). For the morphovolumetric study of the collagen fibrils, eight randomly selected regions of sections showing transversely cut collagen fibrils were photographed at a magnification of 50,000 for each specimen grid. One of the eight images per specimen was selected for analysis by a person not otherwise associated with this study. Collagen fibril diameter was measured using Photoshop 6.0. The photo was placed in a Photoshop window and overlapped by a dotted grid composed of 10 columns and 10 rows to randomly select collagen fibrils to be measured. In addition, three portions from the randomly selected photo were randomly cropped. Numbers of collagen fibrils per unit area were then calculated from the resulting images. The same procedure was repeated 3 times and the three results per specimen were averaged for statistical analysis.

RESULTS
A total of 30 randomly selected photos in each group was analyzed. A total of 2029 collagen fibrils in the control group and 2180 collagen fibrils in the patient group were measured in diameter. The average diameter of collagen fibrils was 45.47±7.96 nm in the control group and 54.79±15.20 nm in the patient group. The diameter of collagen in the patient significantly larger than the control (P<0.05). Although the mode of distribution in both groups was 45–50 nm, the distribution graph of the patient group skewed to a larger mean diameter compared with that of control group (Figure 1).

Ninety randomly cropped photos in each group were analyzed. The mean number of collagen fibrils per unit area (density) was 201.38±48.88 in the control group and 157.08±54.38 in the patient group. The collagen density of the patient was significantly lower than the control (P<0.05). The mode in the control group was 250–260 collagen fibrils per area. However, in the patient group, the mode was bi-modal, at 100–110 and 120–130 collagen fibrils per area (Figure 2).

DISCUSSION
There are significant changes in the extracellular matrix and cell components in CTS SSCT compared with normal SSCT, including enlarged diameter of the collagen fibril, deformation of collagen fibrils, decreased collagen density, destruction of elastin, increase in cellularity, and phagocytosis of aberrant collagen fibrils (Figure 3). This suggests that the dynamic equilibrium of the extracellular matrix in the SSCT of CTS patients is shifted to a pattern of fibrosis, which could affect the mechanical properties of the SSCT in CTS patients. Future studies are necessary to determine the relationship between the morphological and mechanical changes, and also the possibility of altering collagen type.

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MORPHOLOGICAL CHANGES OF COLLAGEN FIBRILS IN THE SUBSYNOVIAL CONNECTIVE TISSUE IN PATIENTS WITH CARPAL TUNNEL SYNDROME

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