INTRODUCTION:
Silver or gold chloride staining with light microscopy have been the most conventional methods used to date to study nerve endings associated with joints. The Freeman and Wyke (F and W) classification scheme, which has become the standard for categorizing articular nerve endings, is based on observations using these techniques (1). However, there have been concerns about limited resolution and restrictions to 2-dimensional analysis. In an attempt to improve resolution and gain a 3-dimensional appreciation of articular nerve endings, we developed a technique using fluorescent immunohistochemistry and confocal scanning laser microscopy and have applied this systematically to a comprehensive study of wrist ligaments.

METHODS:
Twenty dorsal radiocarpal (DRC), long radiolunate (LRL) and short radiolunate (SRL) ligaments and eighteen radioscaphocapitate (RSC) ligaments were harvested from ten paired fresh cadavers (5 males, 5 females, median age 74.5 yrs)(2). The ligaments were fixed, cryostat sectioned at 50µm, serially collected and processed for fluorescence immunohistochemistry using PGP9.5 and a secondary antibody conjugated to a fluorescent tag (Alexa Fluor 488). The sections were evaluated with a confocal scanning laser microscope (3). Scanned images of labeled nerve endings were 3-dimensionally reconstructed, measured and categorized.

RESULTS:
The high degree of specificity and resolution of imaging of the neural tissues with the current protocol was confirmed (Figure 1). The reconstructed images enable us to identify and characterize the nerve endings accurately. Of the total of 309 nerve endings identified in the radiocarpal ligaments, many were noted to have similar morphologic characteristics: 46.9% were oval shaped, 23.9% fusiform, 8.4% spherical, 5.2% cylindrical, 3.6% mushroom, 1.9% rectangular 1.6% crescent, 0.6% conic and 7.8% unclassifiable. The average size of each shape was reported in Figure 2. There was wide range in size in each shape group. The shape of spherical and oval receptors resembled the Type I category receptors described by F and W. The shape of fusiform, cylindrical and rectangular resembled the Type III category receptors. However, there is a large discrepancy in the sizes relative to the F and W classification.

DISCUSSION:
The high resolution images produced using the current protocol have provided enhanced precision and detailed descriptions of nerve endings associated with human wrist joint ligaments. The capability to virtually stack, reconstruct and rotate images has enabled observations on the entire 3-dimensional shape of the receptors. From this has emerged discrepancies with the original Freeman and Wyke classification scheme and an entirely new category based on shape and size.

CONCLUSION:
High resolution images resulting from the current protocol have provided more precise and detailed information of nerve endings associated with joint capsules. The 3-dimensional size and shape of the nerve endings in selected human wrist ligaments have been reported. It is anticipated that these new findings of 3-dimensional image characteristics for the nerve endings will contribute to the understanding of the functional role of the nerve endings in joint function.

REFERENCES:

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