

Tendon Collagen Fibril Identification via Phase Contrast Microscopy

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ABSTRACT

Tendons play a key role in the musculoskeletal system as their main functions are to connect muscles to bones, provide shock absorption, and increase range of motion and the efficiency of locomotion. Due to the tendons' hierarchical structure, the ability to image subfibrillar units plays a key role in visualizing how changes at the microscopic level affect the macroscopic structure and function. **PURPOSE:** To provide an accessible method for confirming the separation of tendon tissue to the collagen fibril level. **METHODS:** Previously frozen (-80°C) 657BL/6J mouse tendons were treated with a chemical and mechanical extraction technique which led to the separation of the whole tendon to tendon subunits. The tendon complex was then imaged with phase contrast microscopy (PCM), which is an illumination technique under an optical microscope. Collagen fibrils were then confirmed with high-resolution imaging by atomic force microscopy (AFM). **RESULTS:** Quantitative analysis of the AFM images (n=38 fibril sections) revealed fibrils of 4 distinct sizes with an average D-banding period of 66 nm ± 2.1 and an average fibril height of 63 nm ± 23.5. **CONCLUSION:** The smallest subunits of the processed tendon complex visualized with PCM were confirmed to be collagen fibrils under high resolution imaging with the AFM. Quantitative analysis of AFM images revealed the 66 nm D-banding pattern in collagen fibrils of different sizes as described in previous literature. These findings provide a new method of confirming the presence of collagen fibrils utilizing an accessible mode of imaging and is applicable to tissue that has been previously frozen.