Abstracts

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A NOVEL STUDY BY CONFOCAL RAMAN MICRO-SPECTROSCOPY IN THE RABBIT BONE-TENDON JUNCTION OF THE PATELLA-PATELLAR TENDON

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Objective: With confocal Raman micro-spectroscopy, we tried to distinguish different structures of rabbit patellar-patellar tendon junction (PPT) and explore the new method for further study to evaluate bone tendon junction healing.

Methods: The PPT samples were harvested carefully from four healthy bone matured male New Zealand rabbits cadavers and were sectioned crossing the median sagittal plane. All samples underwent no chemical treatment for Raman analysis. After all the Raman spectra were acquired, baseline correction for each individual spectrum was performed. The relative peak intensity of 960 cm⁻¹ for mineral and 2940 cm⁻¹ for collagen as well as mineral-to-collagen ratio (960 cm⁻¹/2940 cm⁻¹) was used as indicators to identify which structure the scanning spot belongs to. Meanwhile, through X-axis coordinate of each individual position, the thickness of the junction in rabbit PPT can be calculated. After Raman spectroscopy scanning, the PPT samples were observed optically in histological sections (HE staining).

Results: According to Raman analysis, three different yet continuous structures of PPT could be well identified: bone, fibrocartilage, and tendon (Fig 1A). The thickness of fibrocartilage was about 1800 μm. This result was highly consistent with histological study. The collagen content (the peak intensity strength of 2940 cm⁻¹) in bone was higher than tendon (Fig 1A). We also found that the mineral-to-collagen ratio gradually increased across the junction from the tendon to bone. The 960 cm⁻¹ band across the junction became narrow in the same mineral zone. Interestingly, we found that combined with histological observation, some scanning spots around the tidemark showed higher mineral-to-collagen ratio than bone (Fig 1B). This might be the result of the process of continuous and dynamic mineralization existing around the boundary (i.e., tidemark) region. Furthermore, it could detect the distribution and the degree of mineralization as well as the content of the organic tissue (almost collagen) across the tendon—bone junction.

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Fig 1. A: The classical spectra of sample #2. Spectrum a represented for tendon, b for fibrocartilage and c for bone. The position of spectrum c was around the tidemark. Mineralization increased from tendon to bone. The collagen content in bone was higher than tendon.

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CHARACTERIZATION OF ZINC AND CALCIUM SPATIAL DISTRIBUTION AT THE FIBROCARTILAGE OF RABBIT PATELLA-PATELLAR TENDON COMPLEX: A SYNCHROTRON RADIATION MICRO X-RAY FLUORESCENCE STUDY

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Objective: Zinc (Zn) and calcium (Ca) play important roles in the normal growth, remodeling and mineralization of fibrocartilage zone of patella-patellar tendon complex (PPTC). Synchrotron radiation micro X-ray fluorescence (SR-μXRF) allows in situ mapping of Zn and Ca at nanometer level with high sensitivity. Therefore, the main purpose of this study was to characterize the distribution of Zn and Ca at the fibrocartilage zone of PPTC.

Methods: (1) Sample Preparation: Four PPTC of rabbits were embedded with polymethylmethacrylate and cut sagittally with a thickness of 100 μm. (2) Backscattered electron imaging (BEI): An electron-probe microanalyzer (EPMA) was utilized to acquire BEI images. The region of interest and tidemark (TM) of PPTC were determined based on the BEI images (Fig 1.A). (3) SR-μXRF: the distribution of Zn and Ca at the fibrocartilage zone of PPTC were examined at BL15U1 (Shanghai Synchrotron Radiation Facility, China) and analyzed with Igor pro program (Version 6.1, WaveMetrics, Inc, USA).

Results: (1) The distribution of Zn and Ca at the fibrocartilage zone of PPTC was successfully visualized by using SR-μXRF. The spatial resolution of elemental mapping was as small as 3 μm (Fig. 1.B). (2) The distribution of Zn and Ca at the fibrocartilage zone of PPTC was inhomogeneous. The content of Zn in the TM zone was 2.8 times higher than that of patellar tendon (Fig 1.D) (Fig 2.D). The position of highest Ca content (21.9 times of that of patellar tendon) was located in the calcified fibrocartilage zone and is about 100 μm far from where the highest Zn content is. Most importantly, Ca was distributed in a gradually decreasing manner from bone to tendon.