Collagen Fiber Orientation in the Femur of Rats with Chronic Kidney Disease∗

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(Received 15 February 2015; Accepted 1 April 2015; Published 23 May 2015)

Collagen fiber orientation in the femur of chronic kidney disease (CKD) rats was determined using Fourier transform infrared (FTIR) imaging with a wire grid polarizer in order to characterize bone quality in CKD. Eleven-week-old male rats underwent 5/6 nephrectomy (Nx) to replicate CDK. These CDK rats, as well as sham-operated rats (sham), were kept for 16 weeks. After sacrifice, femurs in the sham and CKD rats were removed and embedded in PMMA. Longitudinal sections (~3 µm) of the femurs were prepared for FTIR imaging. IR dichroism images were calculated from the FTIR images obtained using two polarized IR beams (0° and 90°). The IR dichroism images of the collagen fibers in the CKD rat femurs indicated that the collagen fibers in the cortical bone were aligned longitudinally, and the degree of collagen fiber orientation was decreased in the metaphysis compared to that in the diaphysis. The carbonate-to-phosphate ratio in the hydroxypatite was reduced in both the cortical bone and trabecular bone of the CKD rat femurs. In the cortical bone, reductions in the carbonate-to-phosphate ratio and collagen fiber orientation were particularly evident in the metaphysis in the CKD rats.

[DOI: 10.1380/ejsnt.2015.244]

Keywords: Infrared absorption spectroscopy; Bioimaging and engineering; Vibrations of adsorbed molecules; Biological molecules-proteins; Biological aspects of nano-structures

I. INTRODUCTION

The kidneys play an important role in calcium and phosphate homeostasis, maintaining acid-base balance, extracellular fluid and blood pressure. CKD is characterized by a progressive loss of kidney function over a period of months or years, leading to mineral and bone disorder (CKD-MBD) [1]. Bone consists of a matrix of cross-linked type I collagen fibers mineralized with carbonated apatite [2]. Bone strength, which is a parameter of bone health, primarily reflects the integration of two features: bone quantity (bone mineral density) and bone quality [2, 3]. FTIR imaging is a powerful tool for the characterization of bone quality, including parameters such as the mineral-to-collagen matrix ratio, carbonate-to-phosphate ratio in the hydroxypatite, crystallinity, and collagen maturity [1, 4–7]. In our previous work [1], we used FTIR imaging to characterize the bone quality of the femur in a rat model of CKD induced by 5/6 nephrectomy (Nx). We observed excess bone resorption compared to bone formation, and bone mineral loss was observed in the endosteum rather than in the periosteum. We also found that the trabecular bone and epiphysis had a rapid turnover rate compared to that of the cortical bone. The mineral and bone disorder found in patients with CKD leads to a decrease in bone strength and an increase in the risk of fracture. On the other hand, collagen maturity [7, 8] and collagen fiber orientation [9, 10] also affect bone strength. In this work, collagen fiber orientation was characterized using FTIR imaging with a wire grid polarizer in order to characterize bone quality in CKD.

II. EXPERIMENTAL

Eleven-week-old male rats underwent 5/6 Nx to replicate CDK. These CDK rats, as well as sham-operated rats (sham), were kept for 16 weeks. The rats were sacrificed at 27 weeks of age and, after sacrifice, the femurs were removed and embedded in PMMA. Longitudinal sections (3 µm) were prepared using a microtome and then mounted on BaF₂ disks for FTIR imaging.

FTIR images of the longitudinal sections were collected using an FTIR imaging system with a mercury-cadmium-telluride (MCT) linear array detector (Spotlight 400 system, PerkinElmer, MA, USA) in transmittance mode with a frequency region from 4000 to 680 cm⁻¹, a resolution of 8 cm⁻¹, and a pixel size of 25 µm × 25 µm. FTIR spectra extracted form the trabecular bone and cortical bone in the images were then used to evaluate the mineral-to-collagen matrix ratio, carbonate-to-phosphate ratio, crystallinity, and collagen maturity in the bone. FTIR images for the calculation of IR dichroism images were also collected using FTIR imaging system with a wire grid polarizer in transmittance mode with a frequency region from 4000 to 680 cm⁻¹, a resolution of 8 cm⁻¹, and a pixel size of 6.25 µm × 6.25 µm.

The mineral-to-collagen matrix ratio (PO₄⁢³⁻/amide I) was calculated by integrating the area of the phosphate band (PO₄³⁻, 1180-911 cm⁻¹) and dividing it by the area of the amide I band (1709-1606 cm⁻¹). The carbonate-to-phosphate ratio (CO₃²⁻/PO₄³⁻) was calculated by integrating the area of the carbonate band (CO₃²⁻, 890-856 cm⁻¹) and dividing it by the area of the PO₄³⁻ band. The crystallinity was calculated by dividing the height of the PO₄³⁻ band at 1030 cm⁻¹ by the height of the PO₄³⁻ band at 1020 cm⁻¹ (1030 cm⁻¹/1020 cm⁻¹). The collagen maturity was calculated by dividing the height of the amide I band at 1660 cm⁻¹ by the height of the amide I band at 1690 cm⁻¹ (1660 cm⁻¹/1690 cm⁻¹) [5]. The IR dichroism images showing the collagen fiber orientation

∗ This paper was presented at the 15th Chitose International Forum “Nanotechnology - From Synthesis to Devices,” Chitose Institute of Science and Technology, Chitose, Japan, October 2-3, 2014.
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FIG. 1. FTIR images of the chemical composition of the sham rat femur (a) and CKD rat femur (b) showing the distribution of phosphate (PO$_4^{3-}$). The images are comprised color pixels corresponding to the intensity of a selected PO$_4^{3-}$ band in the FTIR spectra.}

was obtained from the amide I bands (1709-1606 cm$^{-1}$), which were collected by two polarized IR beams (0° and 90°). The IR dichroism (R) is defined as

$$ R = \frac{A_{0^\circ} - A_{90^\circ}}{A_{0^\circ} + A_{90^\circ}} $$

where A stands for the integrated area of amine I band considered

FIG. 2. Bone quality of the metaphysis in sham and CKD rat femurs evaluated using FTIR spectra. (a) Mineral-to-collagen matrix ratio; (b) carbonate-to-phosphate ratio; (c) crystallinity; (d) collagen maturity.

FIG. 3. IR dichroism images of collagen fibers in the sham rat femur (a) and CKD rat femur (b). Collagen fibers in cortical bone were aligned longitudinally, and the degree of collagen fiber orientation was reduced in the metaphysis. In the cortical bone of the CKD rat femur, the reduction in the collagen fiber orientation was particularly apparent in the metaphysis.

FIG. 4. Carbonate-to-phosphate ratio in the metaphysis and diaphysis in the cortical bone of rat femurs evaluated using FTIR. The carbonate-to-phosphate ratio in the hydroxyapatite was significantly reduced in the metaphysis of the CKD rats. (a) Sham rat femur; (b) CKD rat femur.

III. RESULTS AND DISCUSSION

Bone quality of the femur in CKD rats was characterized using FTIR imaging. Figure 1 shows FTIR images of the chemical composition of the sham rat femur (a) and CKD rat femur (b) showing the distribution of phosphate (PO$_4^{3-}$). The image is comprised of color pixels corresponding to the intensity of a selected PO$_4^{3-}$ band in the FTIR spectrum. The FTIR images of the femurs demonstrate that the total phosphate in the trabecular bone and cortical bone was lower in CKD rats than in the sham rats. That means bone mass and density was lower in CKD rats than in the sham rats. Figure 2 shows the results for various parameters of bone quality in sham and CKD rat femurs evaluated using FTIR spectra extracted from the FTIR images: (a) mineral-to-collagen matrix ratio, (b) carbonate-to-phosphate ratio, (c) crystallinity,
and (d) collagen maturity. The carbonate-to-phosphate ratio in the hydroxyapatite was significantly reduced in the CKD rat femur; however, there was no significant difference in the mineral-to-collagen matrix ratio between the sham and CKD rats. These were not also significant differences in the crystallinity, or collagen maturity in femur between the CKD and sham rats.

Collagen fiber orientation in the femur was evaluated from IR dichroism images of the amide I band. Figure 3 shows IR dichroism images of the collagen fibers in the sham rat femur (a) and CKD rat femur (b) and indicates that the collagen fibers in cortical bone were aligned longitudinally, and the degree of collagen fiber orientation was reduced in the metaphysis. In the cortical bone of the CKD rat femurs, the reduction in the collagen fiber orientation was particularly apparent in the metaphysis. Figure 4 shows the carbonate-to-phosphate ratio in the metaphysis and diaphysis in the cortical bone of rat femurs evaluated using FTIR spectra. In the cortical bone, a reduction in the carbonate-to-phosphate ratio was observed in the metaphysis in the CKD rats (b); however, there was no significant difference in the carbonate-to-phosphate ratio between metaphysis and diaphysis in the sham rats (a).

IV. CONCLUSIONS

Bone quality in the femurs of CKD rats was characterized using FTIR and IR dichroism images. Collagen fibers in the cortical bone in the sham and CKD rats were aligned longitudinally, and the degree of collagen fiber orientation was lower in metaphysis than in the diaphysis. The carbonate-to-phosphate ratio in the hydroxyapatite was reduced in both the cortical bone and trabecular bone of the CKD rat femurs. In the cortical bone of the CKD rat femurs, the reductions in the carbonate-to-phosphate ratio and collagen fiber orientation were particularly apparent in the metaphysis.

ACKNOWLEDGMENTS

This study was supported by JSPS KAKENHI Grant Number 24591237.