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Review

Bone quality characteristics obtained by Fourier transform infrared and Raman spectroscopic imaging

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ABSTRACT

Background: Bone strength, which is an indicator of the risk of fracture, is determined by bone mass (bone mineral density, 70%) and bone quality (30%). Bone quality results from a combination of various material and structural properties, making it difficult to determine a suitable method for the evaluation of bone quality based on clinical measurements. Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy are powerful techniques for the assessment of bone quality and reveal similar information on molecular structures; however, this molecular information is based on different physical phenomena. Therefore, a comparison of FTIR and Raman spectra is required for an accurate assessment of bone quality.

Highlight: We previously assessed the bone quality of femurs from rats with chronic kidney disease (CKD) using FTIR imaging, and found the carbonate-to-phosphate ratio in the hydroxyapatite was significantly reduced compared to control rats; however, there was no difference in crystallinity. Therefore, we focused on the crystallinity of the femoral cortical bone in rats with CKD, and compared the PO_4^{3-} bands in FTIR spectra in detail with those in the Raman spectra.

Conclusion: The PO_4^{3-} bands in the FTIR spectra were affected by changes in calcium phosphate composition rather than by changes in crystal size. Thus, FTIR is more suitable for the evaluation of mineral maturity than crystallinity; Raman spectroscopy is more sensitive to crystallinity than FTIR.

Contents

1. Introduction

Bone strength, an indicator of the risk of fractures associated with osteoporosis and other bone diseases, is determined 70% from bone mass (bone mineral content or bone mineral density; BMD) and 30% from bone quality. However, the clinical measurement of

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Abbreviations: BMD, bone mineral density; CKD, chronic kidney disease; FTIR, Fourier transform infrared spectroscopy; FWHM, full width at half maximum; MCT, mercury-cadmium-telluride; NIR, near-infrared; PMMA, polymethyl methacrylate

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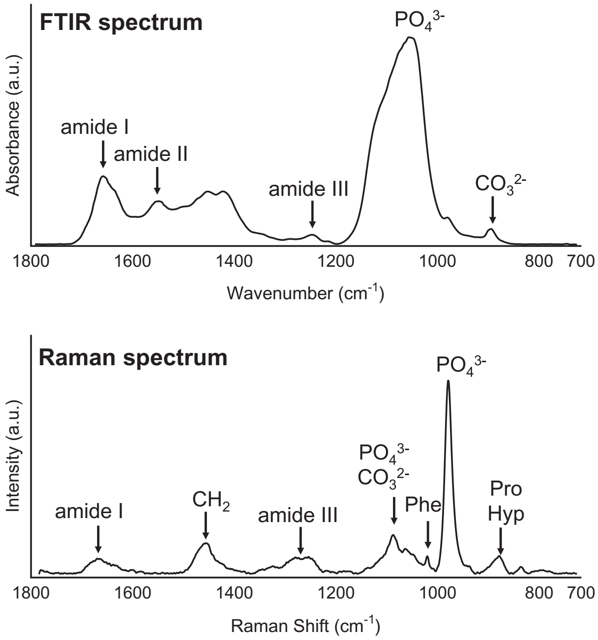

Fig. 1. Typical FTIR and Raman spectra of bone. In the FTIR spectrum (upper panel), PO_4^{3-}, CO_3^{2-}, amide I, amide II, and amide III bands can be observed, while PO_4^{3-}, CO_3^{2-}, amide I, amide III, CH_2, Phe, Pro, and Hyp bands can be observed in the Raman spectrum (lower panel). Both PO_4^{3-} and CO_3^{2-} were derived from bone minerals, primarily hydroxyapatite; the amides, CH_2, Phe, Pro, and Hyp were derived from proteins, primarily from type I collagen.

BMD is used as an indirect indicator of fracture risk. Bone quality results from a combination of various material and structural properties, including rate of turnover, architecture/geometry of trabecular and cortical bone, mineral/collagen matrix properties, and microdamage accumulations [1]. It is, therefore, difficult to determine a suitable method for the evaluation of bone quality based on clinical measurements.

Vibrational spectroscopies, including Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy, are powerful techniques for the assessment of material properties. Moreover, spectrometers equipped with both a microscope and an imaging system, such as FTIR and Raman imaging systems, are suitable for the characterization of material and structural property distributions. Therefore, both FTIR and Raman imaging systems have attracted a good deal of attention as tools for the assessment of bone quality. Fig. 1 shows typical FTIR and Raman spectra of bone. PO_4^{3-}, CO_3^{2-}, amide I, amide II, and amide III bands can be observed in the FTIR spectrum (Fig. 1, upper panel), while PO_4^{3-}, CO_3^{2-}, amide I, amide III, CH_2, Phe, Pro, and Hyp bands can be observed in the Raman spectrum (Fig. 1, lower panel). Both PO_4^{3-} and CO_3^{2-} are derived from bone minerals (mainly hydroxyapatite and carbonated apatite), and amides I-III, CH_2, Phe, Pro, and Hyp are derived from proteins, primarily from type I collagen. The FTIR and Raman band assignments and parameters for the assessment of bone quality are summarized in Table 1.

FTIR and Raman spectra provide similar information regarding molecular structures; however, this molecular information is based on different physical phenomena (infrared absorption vs. Raman scattering) and, therefore, FTIR and Raman spectroscopy are generally used in a complementary manner. However, either FTIR or Raman spectroscopy alone is used for the characterization of bone quality. Although comparisons of FTIR and Raman spectroscopy have been conducted previously in various research fields to identify better analytical techniques, there have been few reports on the assessment of bone quality. An assessment of changes with aging in rabbit cortical bone clarified differences between FTIR and Raman microscopic analysis [2], with only the results of collagen cross-linking found to be correlated and Raman analysis found to be more sensitive than FTIR for the analysis of inorganic matrices. In our previous work [3], bone quality in the femur of rats with chronic kidney disease (CKD) was characterized using FTIR imaging, and no difference in crystallinity was observed between CKD and sham rats. However, we found that the hydroxyapatite carbonate-to-phosphate ratio was significantly reduced in the CKD rat femur. In this study, we focused on crystallinity in the femoral cortical bone in rats with CKD, and we undertook a detailed comparison of the PO_4^{3-} bands in the FTIR and Raman spectra.

Table 1
FTIR and Raman band assignments and parameters for bone quality.

Assignment & Bone quality parameter	FTIR Wavenumber (cm^{-1})	Raman Raman shift (cm^{-1})
B-type CO_3^{2-}	871	1065–1070
A-type CO_3^{2-}	878	
CO_3^{2-}	890–850	
Hydroxyproline (Hyp)		876
Proline (Pro)		855, 921
PO_4^{3-}	1200–900	945–964
Phenylalanine (Phe)		1002
Amide I	1720–1585	1720–1616
Amide II	1590–1510	
Amide III	1320–1210	1320–1243
Mineral-to-matrix ratio	amide I/PO_4^{3-}	amide I/PO_4^{3-}
Carbonate-to-phosphate ratio	CO_3^{2-}/PO_4^{3-}	CO_3^{2-}/PO_4^{3-}
Crystallinity	1030/1020	FWHM of PO_4^{3-}
Mineral maturity	1030/1110	

2. Materials and methods

2.1. Bone

Three eleven-week-old male Sprague Dawley® (Japan SLC, Inc., Shizuoka, Japan) rats underwent 5/6 nephrectomy to replicate CKD. The rats were sacrificed at 27 weeks of age, and the femurs were removed and embedded in polymethyl methacrylate (PMMA). Longitudinal Sections 3 μm thick were prepared using a microtome, and bone quality was assessed using both FTIR and Raman spectroscopy.

2.2. Assessment by 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, Inc., 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. The background spectrum was obtained through a BaF_2 window. Seven spectra, each based on the average of 16 spectra for an area of 100 μm × 100 μm, were extracted from both the metaphysis and diaphysis of the femoral cortical bone in the FTIR image, and baseline collection and PMMA spectral subtraction were performed using Spectrum 10 software (PerkinElmer, Inc.). Each PO_4^{3-} band in the frequency region from 1200 to 900 cm^{-1} was normalized against 1 absorbance to compare both the shape and height of the PO_4^{3-} band in the metaphysis to that in the diaphysis. The crystallinity was calculated by dividing the absorption of the PO_4^{3-} band at 1030 cm^{-1} by the absorption of the PO_4^{3-} band at 1020 cm^{-1} (1030 cm^{-1}/1020 cm^{-1}). The mineral maturity [4] was calculated by dividing the absorption of the PO_4^{3-} band at 1030 cm^{-1} by the absorption of the PO_4^{3-} band at 1110 cm^{-1} (1030 cm^{-1}/1110 cm^{-1}).

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