Journal of Oral Biosciences 61 (2019) 115–119

Contents lists available at ScienceDirect

Journal of Oral Biosciences

journal homepage: www.elsevier.com/locate/job

Original Article

Degree of orientations of collagen fibers and bone apatite crystals in rat femora by infrared dichroism imaging

Teppei Ito , Hiromi Kimura-Suda *

Graduate School of Photonics Science, Chitose Institute of Science and Technology, 758-65 Bibi, Chitose, Hokkaido, 066-8655, Japan

ARTICLE INFO

Article history:
Received 15 February 2019
Received in revised form
23 March 2019
Accepted 29 March 2019
Available online 15 May 2019

Keywords:
Bone
FTIR
Collagen
Hydroxyapatite
Orientation

ABSTRACT

Objectives: The degree of orientations of collagen fibers and bone apatite crystals affects bone strength. We demonstrated that collagen fibers were aligned along the long axis of bone and that the degree of collagen fiber orientation changed with aging using infrared (IR) dichroism imaging. In this study, we developed a technique for evaluating bone apatite crystal orientation using IR dichroism imaging to investigate the relationships between collagen fiber and bone apatite crystal orientations.
Methods: Femora were harvested from male Sprague Dawley rats of different ages (6, 12, and 33 weeks); they were then embedded in poly (methyl methacrylate) and sectioned with a microtome into 3-μm longitudinal sections. The angle-dependent Fourier transform infrared (FTIR) spectra for sections were collected using FTIR imaging, and collagen fiber and bone apatite crystal orientations in the sections were assessed using IR dichroism imaging.
Results: Collagen fibers and poorly crystalline apatite in the femoral cortical bone were longitudinally aligned; however, the stoichiometric hydroxyapatite crystal and all of the bone apatite were not aligned. The degree of poorly crystalline apatite orientation was higher in 33-week-old rats than in 6-week-old rats.
Conclusions: Poorly crystalline apatite in the rat femoral cortical bone was aligned along the collagen fibers. The degree of poorly crystalline apatite orientation and collagen fiber orientation in the femoral cortical bone increased until at least 33 weeks; meanwhile, on aging, the stoichiometric hydroxyapatite crystal was not longitudinally aligned.

1. Introduction

Carbonated apatite and collagen are the main components of bone, and type I collagen fibers are mainly mineralized with nanocrystalline carbonated apatite. The material and structural properties of carbonated apatite crystals and collagen fibers in bone, bone quality including the rate of turnover, mineral/collagen matrix properties, microdamage accumulation, and architecture/geometry of trabecular and cortical bone contribute to bone strength with bone mineral density [1]. Bone quality has been assessed using various analytical imaging techniques such as vibrational spectroscopic imaging for material properties, quantitative computed tomography (CT), micro-CT, and high-resolution magnetic resonance imaging, and scanning electron microscopy for structural properties. The obvious common advantage in using these analytical imaging techniques is that they allow the distributions of structural/material properties in the bone to be visualized.

Fourier transform infrared (FTIR) imaging is a vibrational spectroscopic technique for determining the distribution of material properties. Therefore, it has attracted a good deal of attention for assessing bone quality. Conventional parameters and infrared (IR) band assignments for the assessment of bone quality are summarized in Table 1 [2–5].

Changes in the mineral-to-matrix ratio, carbonate-to-phosphate ratio, crystallinity, and mineral maturity affect bone strength [4]. The degree of orientations of collagen fibers and bone apatite crystals also affects bone strength [6]. We previously developed a new technique for evaluating collagen fiber orientation in bone using FTIR imaging with a polarizer, IR dichroism imaging [7–10]. IR dichroism imaging is particularly well suited for assessing bone

Abbreviations: CT, computed tomography; IR, infrared; FTIR, Fourier transform infrared; MCT, mercury-cadmium-telluride; PMMA, poly (methyl methacrylate).

* Corresponding author. Fax: +81 123 27 6008.
E-mail addresses: itotetu1019@gmail.com (T. Ito), kimurasu@photon.chitose.ac.jp (H. Kimura-Suda).

https://doi.org/10.1016/j.job.2019.03.004
1349-0079/

Table 1
Conventional bone quality parameters and IR band assignments.

Bone quality parameters	IR band assignments (cm^{-1})
Mineral-to-matrix ratio (PO_4^{3-}/Amide I)	1200-900/1720-1585
Carbonate-to-phosphate ratio (CO_3^{2-}/PO_4^{3-})	890-850/1200-900
Crystallinity	1030/1020
Mineral maturity	1030/1110

quality because other bone quality parameters, which are obtained by FTIR imaging, can be simultaneously evaluated. We previously demonstrated that the collagen fiber was oriented approximately parallel to the long axis of the rat femoral cortical bone and that the degree of collagen fiber orientation was higher in 33-week-old rats than in 6-week-old rats; however, the mineral-to-matrix ratio also tended to be higher, and the crystallinity was nearly unchanged [10]. The relationship between collagen fiber orientation and bone apatite crystal orientation is still unclear.

In this study, we developed a technique for evaluating bone apatite crystal orientation using IR dichroism imaging to investigate the relationship between collagen fiber and bone apatite crystal orientations.

2. Materials and methods

2.1. Bone

Femora were harvested from male Sprague Dawley rats of different ages (6, 12, and 33 weeks); they were then washed with phosphate buffered saline (PBS), fixed with 70% ethanol, embedded in poly (methyl methacrylate) (PMMA), and then sectioned with a microtome into 3-μm longitudinal sections for IR dichroism imaging. The sections were mounted on BaF_2 windows.

2.2. Angle-dependent FTIR spectra for the femoral cortical bone

FTIR images of the femoral section of 12-week-old rats (12W) on the BaF_2 window were determined using the FTIR imaging system (Spotlight 400 system, PerkinElmer, Inc., MA, USA) with a mercury-cadmium-telluride (MCT) linear array detector and a wire grid polarizer (ST Japan Inc., Tokyo, Japan) to obtain angle-dependent FTIR spectra. The analytical condition for the instrument was in transmittance mode with a frequency region from 4000 cm^{-1} to 680 cm^{-1}, a resolution of 4 cm^{-1}, and a pixel size of 6.25 μm × 6.25 μm. The polarizer was rotated in the range from 0° to 180° in increments of 10°. The background spectrum was obtained through a BaF_2 window. A series of FTIR polarization spectra, each based on an average of 256 spectra for an area of 100 μm × 100 μm, was extracted from the femoral cortical bone in the FTIR images, and baseline collection and PMMA spectral subtraction were performed using Spectrum 10 software (PerkinElmer, Inc.).

2.3. Assessment using IR dichroism imaging

For IR dichroism imaging, FTIR images of the femoral sections were collected using the FTIR imaging system with the MCT linear array detector and wire grid polarizer in transmittance mode with a frequency region from 4000 cm^{-1} to 680 cm^{-1}, a resolution of 8 cm^{-1}, and a pixel size of 6.25 μm × 6.25 μm. The background spectrum was obtained through the BaF_2 window. The IR dichroism images of the rat femoral cortical bone were obtained to show orientations of collagen fibers, poorly crystalline apatite, stoichiometric hydroxyapatite crystals, and all of the bone apatite (including poorly crystalline apatite and stoichiometric hydroxyapatite crystals) using IR bands summarized in Table 2. Those bands were collected by two polarized IR beams (0° and 90°).

IR dichroism image is defined as $R = (A_{0°}-A_{90°})/(A_{0°}+A_{90°})$, where A stands for each integrated area of the bands.

The orientations of collagen fibers and bone apatite crystals in differently aged rats were compared with each other.

3. Results

3.1. Angle-dependent FTIR spectrum of the femoral cortical bone

A series of FTIR polarization spectra for the femoral cortical bone in 12W was obtained with a frequency region from 1800 cm^{-1} to 750 cm^{-1}. In the FTIR spectrum, both PO_4^{3-} and CO_3^{2-} were derived from bone minerals, primarily hydroxyapatite; the amides were derived from proteins, primarily from type I collagen. The amide I band and PO_4^{3-} sub-band near 1110 cm^{-1} became obviously smaller from 0°/180° (polarized parallel) to 90° (polarized perpendicular) (Fig. 1).

3.2. Collagen fiber and bone apatite crystal orientations in the femoral cortical bone

Both collagen fiber and bone apatite crystal orientations in rat femora were evaluated from IR dichroism images. Fig. 2 shows IR dichroism images showing (a) collagen fiber orientation, (b) poorly crystalline apatite orientation, (c) stoichiometric hydroxyapatite crystal orientation, and (d) the orientation of all of the bone apatite in 12W. The collagen fibers and poorly crystalline apatite in the rat femoral cortical bone were longitudinally aligned; however, the other apatite bands, which were derived from the stoichiometric hydroxyapatite crystal and all of the bone apatite, were not aligned. The degree of poorly crystalline apatite orientation in the femoral cortical bone increased until at least 33 weeks (Fig. 3)

4. Discussion

The degree of orientations of collagen fibers and bone apatite crystals affects bone strength [4] as well as other bone quality parameters [6]. We previously developed a new technique for evaluating the orientation of the collagen fiber in the bone using IR dichroism imaging and demonstrated that the collagen fiber was oriented approximately parallel to the long axis of rat femoral cortical bone and that the degree of collagen fiber orientation was higher in 33-week-old rats than in 6-week-old rats; however, the mineral-to-matrix ratio also tended to be higher, and the crystallinity was nearly unchanged [10]. The relationship between collagen fiber and bone apatite crystal orientations was still unclear. In the present study, we developed a technique for evaluating bone apatite crystal orientation using IR dichroism imaging and investigated the relationship between collagen fiber and bone apatite crystal orientations in rat femoral cortical bone.

A series of FTIR polarization spectra for femoral cortical bone in 12W indicated that a height of PO_4^{3-} sub-band near 1110 cm^{-1}

Table 2
IR band assignments to show collagen fiber and bone apatite crystal orientation.

Orientation	IR band assignments (cm^{-1})
Collagen fiber	amide I band (1712-1609)
Poorly crystalline apatite	PO_4^{3-} sub-band near 1110 cm^{-1} (1118-1102)
Stoichiometric hydroxyapatite crystal	PO_4^{3-} sub-band near 1030 cm^{-1} (1038-1022)
All of the bone apatite	PO_4^{3-} band (1185-900)

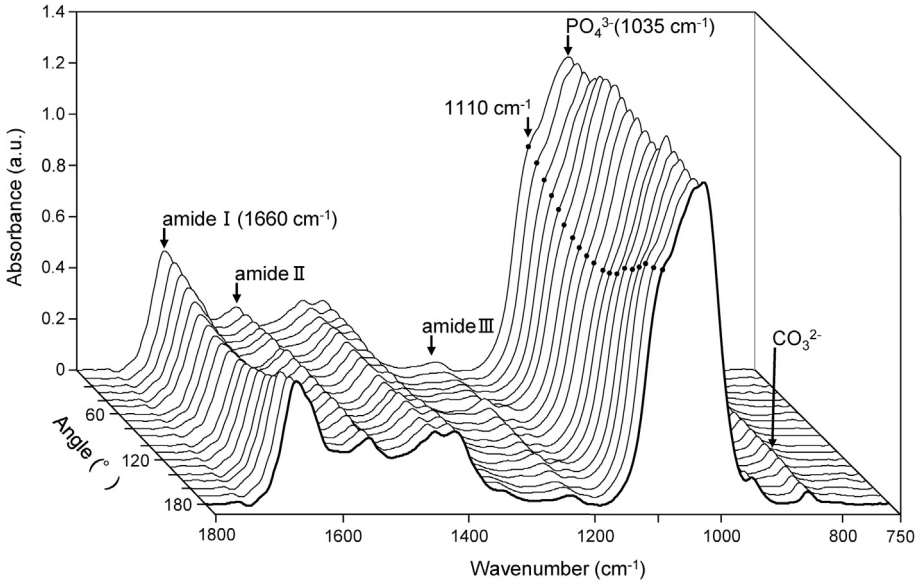

Fig. 1. A series of Fourier transform infrared (FTIR) polarization spectra for the femoral cortical bone in 12-week-old rats. Each spectrum was based on an average of 256 spectra for an area of 100 μm × 100 μm and recorded as the polarizer angle varied from 0° to 180° in increments of 10°.

showing poorly crystalline apatite was changed with a height of amide I band derived from the collagen fiber. The collagen fiber was aligned approximately parallel to the long axis of rat femoral cortical bone [10]; therefore, this result suggested that poorly crystalline apatite was aligned along the long axis of the bone. Orientations of collagen fibers, poorly crystalline apatite, stoichiometric hydroxyapatite crystals, and all of the bone apatite (including poorly crystalline apatite and stoichiometric

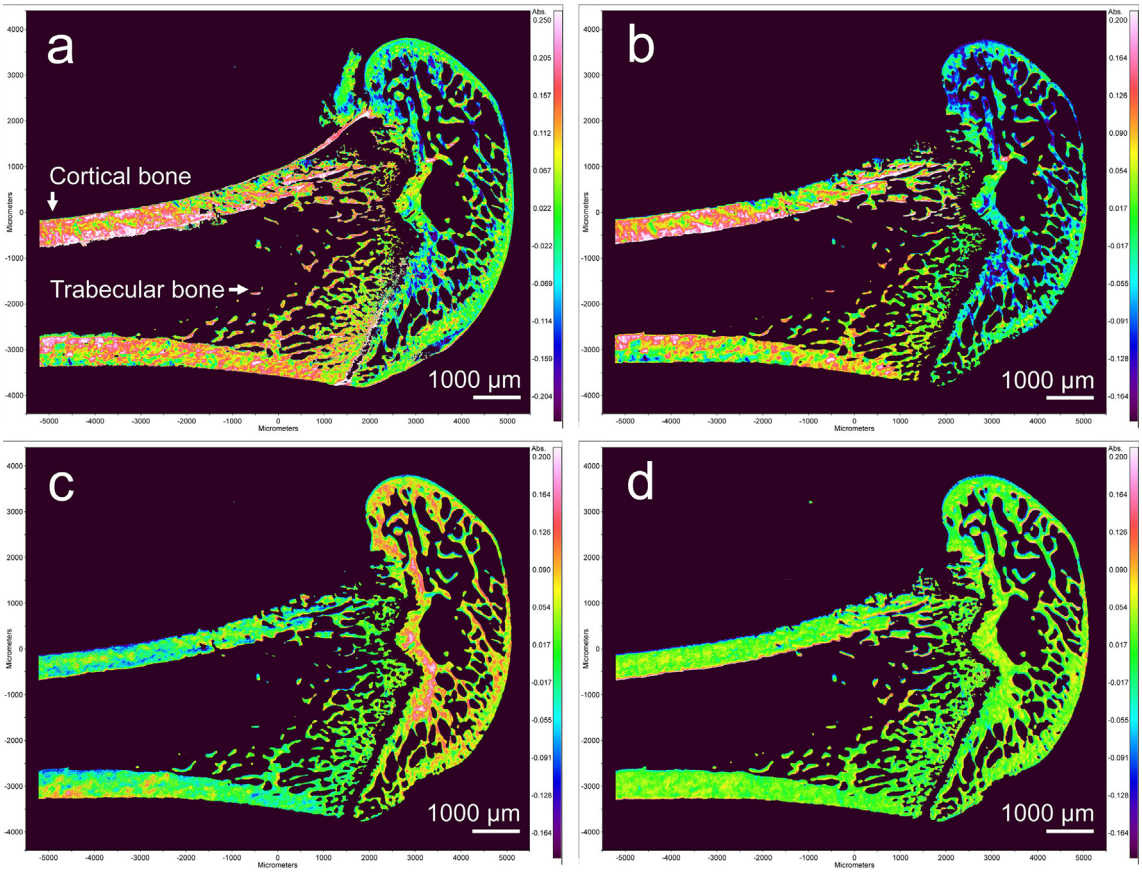

Fig. 2. Infrared (IR) dichroism images showing both collagen fiber and bone apatite orientation in 12-week-old rats. (a) Collagen fiber orientation, (b) poorly crystalline apatite orientation, (c) stoichiometric hydroxyapatite crystal orientation, and (d) orientation of all of the bone apatite. White and red show that the orientation is parallel to the long axis of the cortical bone, and blue shows that orientation is perpendicular to the long axis of the cortical bone. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

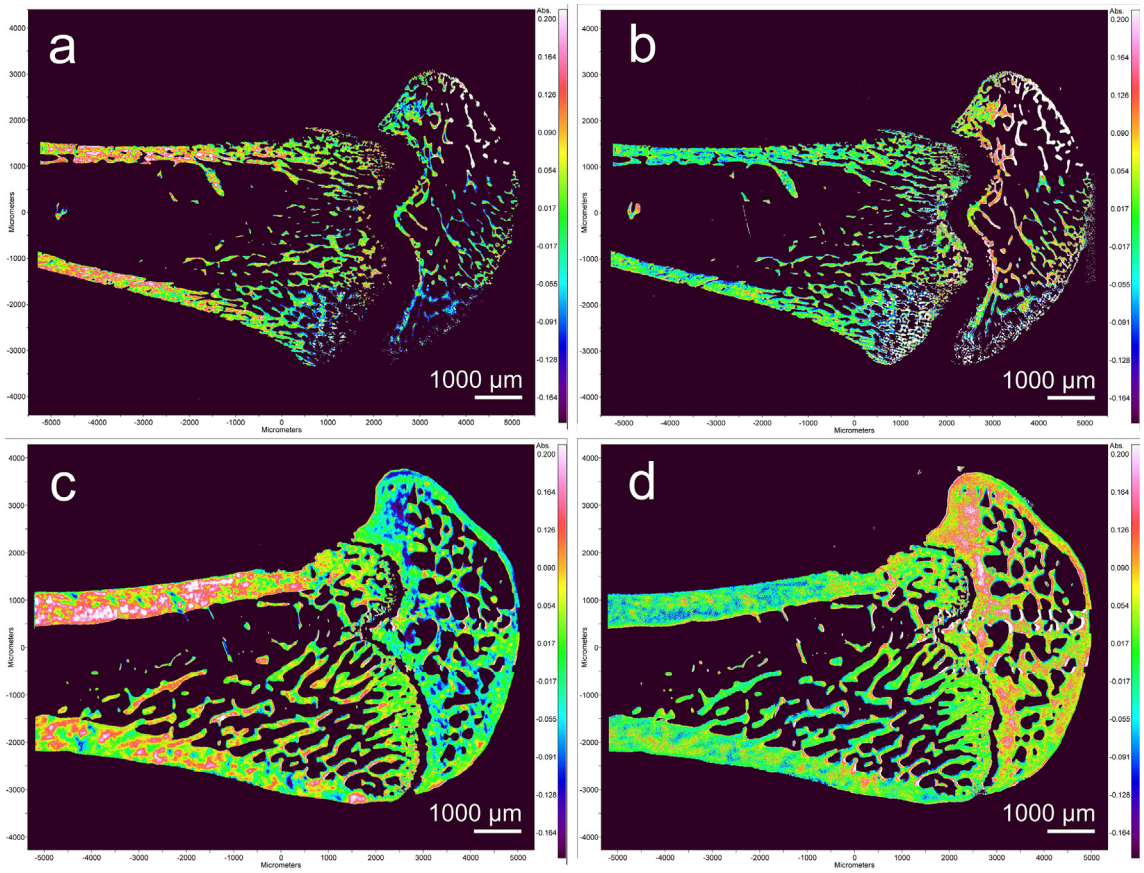

Fig. 3. Infrared (IR) dichroism images showing bone apatite orientation in 6- and 33-week-old rats. (a) Poorly crystalline apatite orientation in 6-week-old rats, (b) stoichiometric hydroxyapatite crystal orientation in 6-week-old rats, (c) poorly crystalline apatite orientation in 33-week-old rats, and (d) stoichiometric hydroxyapatite crystal orientation in 33-week-old rats. White and red show that the orientation is parallel to the long axis of the cortical bone, and blue shows that the orientation is perpendicular to the long axis of the cortical bone. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

hydroxyapatite) in the femoral cortical bone of 12W were assessed using IR dichroism imaging. The orientation of poorly crystalline apatite in the femoral cortical bone was coincident with that of collagen fibers; however, the other apatite bands, which were derived from the stoichiometric hydroxyapatite crystal and all of the bone apatite, were not aligned. More specifically, poorly crystalline apatite in the rat femoral cortical bone was aligned along the collagen fibers.

The orientations of collagen fiber and bone apatite crystal in differently aged rats were compared. The degree of orientation of poorly crystalline apatite in the femoral cortical bone and collagen fiber increased until at least 33 weeks [10]; meanwhile, the stoichiometric hydroxyapatite crystal was not aligned to the long axis of the bone with aging.

5. Conclusion

We demonstrated that the relationship between collagen fiber and bone apatite crystal orientation in rat femoral cortical bone using IR dichroism imaging. Collagen fibers and poorly crystalline apatite in the cortical bone were aligned along the long axis of the femur; however, the stoichiometric hydroxyapatite crystal and all of the bone apatite, were not aligned. The degree of poorly crystalline apatite orientation increased with aging until at least 33 weeks.

Ethical approval

All animal studies were approved by the Committee of Ethics on Animal Experiments at Kureha Corporation (Tokyo, Japan) and were conducted in accordance with the guidelines of Kureha Corporation.

Funding

This study was partially supported by JSPS KAKENHI Grant Number 24591237 and 16K10916.

Conflict of interest disclosure

All authors state that they have no conflicts of interest.

CRediT authorship contribution statement

Teppei Ito: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft. **Hiromi Kimura-Suda:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Writing - original draft, Writing - review & editing.

Acknowledgements

H.K.-S. would like to thank to Dr. Hiroyuki Sasabe, Professor Emeritus of the Chitose Institute of Science and Technology, for his useful comments and valuable advice.

References

[1] Burr DB, Akkus O. Bone morphology and organization. In: Burr DB, Allen MR, editors. Basic and applied bone biology. 1st ed. Cambridge: Academic Press; 2013. p. 3–25.
[2] Kimura-Suda H, Ito T. Bone quality characteristics obtained by Fourier transform infrared and Raman spectroscopic imaging. J Oral Biosci 2017;59:142–5.
[3] Kimura-Suda H, Kajiwara M, Sakamoto N, Kobayashi S, Ijiro K, Yurimoto H, et al. Studies on bone metabolism by using isotope microscopy, FTIR imaging, and micro-Raman spectroscopy. J Oral Biosci 2013;55:61–5.
[4] Paschalis EP, Mendelsohn R, Boskey AL. Infrared assessment of bone quality: a review. Clin Orthop Relat Res 2011;469:2170–8.
[5] Farlay D, Panczer G, Rey C, Delmas PD, Boivin G. Mineral maturity and crystallinity index are distinct characteristics of bone mineral. J Bone Mineral Metab 2010;28:433–45.
[6] Sekita A, Matsugaki A, Nakano T. Disruption of collagen/apatite alignment impairs bone mechanical function in osteoblastic metastasis induced by prostate cancer. Bone 2017;97:83–93.
[7] Kimura-Suda H, Ito T, Kanazawa K, Chaki N, Akiyama H. Collagen fiber orientation in the femur of rats with chronic kidney disease. e-JSSNT 2015;13:244–6.
[8] Shimizu T, Takahata M, Kimura-Suda H, Kameda Y, Endo K, Hamano H, et al. Autoimmune arthritis deteriorates bone quantity and quality of periarticular bone in a mouse model of rheumatoid arthritis. Osteoporos Int 2016;28:709–18.
[9] Ito T, Kimura-Suda H. Analysis of collagen fiber orientation in bone using infrared dichroism imaging in reflectance mode. Mol Cryst Liquid Cryst 2017;654:244–8.
[10] Ito T, Kanazawa K, Kimura-Suda H. Analysis of collagen fiber orientation in bone of different aged rats using FTIR imaging. Mol Cryst Liq Cryst 2015;622:114–9.