Journal of Oral Biosciences 55 (2013) 61–65

Contents lists available at SciVerse ScienceDirect

Journal of Oral Biosciences

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

Review

Studies on bone metabolism by using isotope microscopy, FTIR imaging, and micro-Raman spectroscopy

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ARTICLE INFO

Article history:
Received 9 November 2012
Received in revised form
30 March 2013
Accepted 12 April 2013
Available online 21 May 2013

Keywords:
Bone quality
FTIR imaging
Raman
Isotope microscopy

ABSTRACT

We describe the comprehensive analysis of bone quality by using the isotope microscopy, Fourier transform infrared (FTIR) imaging, and Raman spectroscopy, which are non-destructive techniques. Isotope microscopy is a novel tool for visualizing microdomains within materials through the imaging of the 3-dimensional distribution of isotopes. This technique enabled us to observe calcium metabolism in the tibiae of mice. Thus, a stable calcium isotope fed to mice was observed in the trabecular and cortical bones. FTIR and Raman spectroscopies are powerful tools for characterizing the chemical compositions of materials and provide both qualitative and quantitative information on molecular structure. An FTIR imaging system, which is an accessory for FTIR spectroscopy, provides a distribution map of functional components in the sample. The crystallinity, secondary structure of collagen, carbonate-to-phosphate ratio, and mineral-to-matrix ratio of bone can be obtained from the IR spectra extracted from the selected area of an FTIR image. Raman spectroscopy complements FTIR spectroscopy; however, the Raman spectrum provides information about functional groups in a sample as well as its FTIR spectrum. The major advantage of Raman spectroscopy for bone analysis is the ability to obtain spectra with higher spatial resolution compared with those acquired using FTIR spectroscopy. Moreover, a wide range of samples, including aqueous solutions, fibers, powders, or frozen materials can be readily analyzed, without any special preparation. Raman spectroscopy generates data on crystallinity, carbonate-to-phosphate ratio, and mineral-to-matrix ratio in bone.

Contents

1. Introduction

Osteoporosis leads to an increase risk of fracture caused by reduced bone mineral density (BMD), deteriorates the bone microarchitecture, and alters collagen and other proteins. Primary

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http://dx.doi.org/10.1016/j.job.2013.04.006

Table 1
Comparison of FTIR and Raman spectroscopy.

	FTIR spectroscopy	Raman spectroscopy
Principle	The absorption of light by vibrating molecules is observed. The change in dipole moment during the vibration is detected.	The scattering of light by the vibrating molecules is observed. The change in polarizability is detected.
Light source	Mid-Infrared light and near-Infrared light are commonly used.	Lasers listed below are commonly used. Nd:YAG (1064), diode (785 nm), Helium-Neon (632 nm), doubled Nd:YAG (532 nm)
Detector	TGS or MCT	CCD detectors for visible lasers, InGaAs detector for NIR lasers
Spatial resolution	10 μm 3 μm (ATR imaging)	1 μm 0.3 μm (Global mapping)
Advantage	Many IR spectra libraries are provided.	Water can be used as a solvent. Sample in a glass bottle can be analyzed.
Disadvantage	Water cannot be used as a solvent.	There is a risk of sample damage.

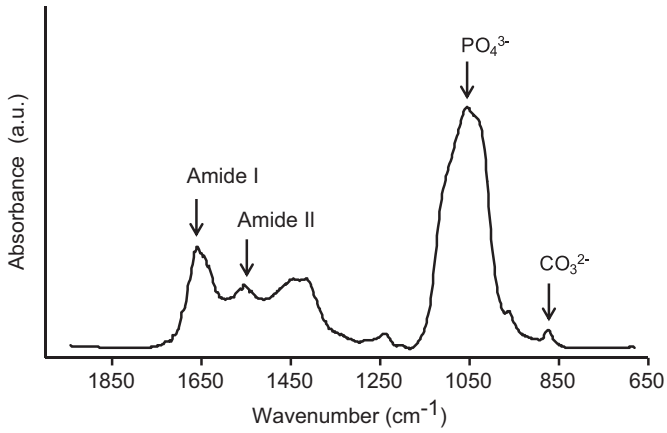

Fig. 1. Representative FTIR spectrum of bone. The PO_4^{3-} (1200–950 cm^{-1}) and CO_3^{2-} (950–850 cm^{-1}) bands in hydroxyapatite and the amide I (1750–1600 cm^{-1}) and amide II (1600–1520 cm^{-1}) bands in collagen are indicated.

osteoporosis is caused by age-related bone loss and occurs commonly in older women [1]. Secondary osteoporosis arises regardless of age or gender and is caused by an underlying specific disease or disorder, such as chronic kidney disease (CKD) or diabetes [2]. Osteopenia is a condition when your bone density is lower than normal, but not low enough to be considered osteoporosis. An estimated 11 million people in Japan and 52 million people in the United States suffer from osteoporosis, and it has been estimated that the number of people with osteopenia/osteoporosis in the US will increase to 61 million by the year 2020.

Bone is a composite material consisting of about 60 wt% mineral formed mostly from carbonate-containing hydroxylapatite, 8–10 wt% water, and organic materials consisting primarily of type I collagen and smaller amounts of noncollagenous proteins and lipids. By volume, these proportions are approximately 40%, 25%, and 35% [3]. Bone is normally evaluated by its strength, which reflects the integration of 70% BMD and 30% bone quality. Bone quality is defined by at least 4 factors as follows: (1) the rate of bone turnover, (2) the properties of the mineral/collagen matrix, (3) accumulation of microdamage, and (4) the architectures of trabecula and cortical bone [4]. Bone is continuously replaced through modeling or remodeling generated by the coordinated actions of osteoblast and osteoclasts. The rate of bone turnover is reflected by mineral balance in the body and is commonly determined using markers such as osteocalcin, bone-specific alkaline phosphatase, pyridinoline, and deoxypyridinoline. However, measurements of bone turnover by using these markers are time consuming. Therefore, establishing analytical methods without such markers will enhance the determination of bone turnover. Recently, natural stable calcium isotopes, such as ^{40}Ca, ^{42}Ca, and ^{44}Ca have been employed to measure bone mineral balance [5]. Isotopes are useful tracers in cosmo- and geochemistry to determine the origin and circulation of elements in nature [6].

Vibrational spectroscopic techniques, including infrared (IR) and Raman spectroscopy are powerful tools for characterizing the chemical compositions of materials, because they can provide both qualitative and quantitative information on molecular structure. Research in the laboratories of Boskey [7–14] and Morris [15–20] has succeeded in determining bone quality using noninvasive techniques, such as FTIR microspectroscopy, FTIR imaging, and Raman spectroscopy. Boskey and collaborators focused on collagen cross-links in bone and characterized the nonreducible:reducible collagen cross-link ratio in diseased bone matrix using FTIR microspectroscopy and FTIR imaging [13], and Morris and colleagues characterized microcracks in bone mineral using a newly developed Raman system [16]. However, further detailed studies on the various bones are required to better understand bone quality. In this review, we describe recent advances in the analysis of bone metabolism using isotope microscopy, FTIR imaging, and micro-Raman spectroscopy.

2. Overview of vibrational spectroscopy

Vibrational spectroscopies using FTIR and Raman techniques have been used frequently to identify molecular structures and specific molecular functional groups within them [21]. Molecules constantly vibrate and can absorb energy received from photons to increase vibrational frequency, which is detected by these techniques. In FTIR analysis, molecular targets absorb photons in the mid-infrared range (2.5–25 μm) causing their vibrations to increase. This is associated with symmetrical stretching, antisymmetrical stretching, scissoring, rocking, wagging, and twisting. To be detected, the vibrational mode in a molecule that interacts with IR is associated with changes in its polarity. The specific frequencies of absorbed light, the energies of which are typically defined as wavenumber (cm^{-1}), depend on the nature of the molecular bonds in the sample [21]. A full range of FTIR accessories, including FTIR imaging, are available for use in a variety of sampling techniques. The FTIR image comprises pixels, whose color corresponds to the absorption of infrared light by the sample, and each pixel contains the IR spectrum of the sample. If a specific functional group is selected in the spectrum, the FTIR image provides a distribution map of the functional group.

Raman spectroscopy is complementary to FTIR spectroscopy. In Raman measurements, the samples are excited with a source of monochromatic laser incident radiation in the ultraviolet (UV), visible (Vis), or near-infrared (NIR) regions, and the radiation scattered from the molecules is detected. A small fraction of incident photons returns not to the lowest vibrational ground state, but to an excited vibrational state, resulting in a shift in the energy of the incident and scattered photons. The shift represents the energy of a vibrational transition within the sample [22]. The