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Infrared spectroscopic characterization of mineralized tissues

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Abstract

Vibrational spectroscopy (Infrared and Raman), and in particular micro-spectroscopy and micro-spectroscopic imaging has been used to characterize developmental changes in bone and other mineralized tissues, to monitor these changes in cell cultures, and to detect disease and drug-induced modifications. Examples of the use of infrared micro-spectroscopy and micro-spectroscopic imaging are discussed in this review.

Keywords: Vibrational spectroscopy; Cell culture; Infrared and Raman; Bone; Hydroxyapatite

1. Introduction

The mineralized tissues found in vertebrates can be subdivided into those which develop through normal physiologic processes (e.g., bones, teeth, calcified cartilage, etc.), and those that form through pathologic processes (e.g., atherosclerotic plaques, kidney and salivary stones, and other pathologic deposits). Table 1 lists examples of these two types of calcified tissues, and the mineral phase or phases they most frequently contain. As can be seen from the table, all the physiologic deposits contain an analogue of the naturally occurring mineral, hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$; (HA). With the exception of the enamel of teeth, all the other physiologically mineralized tissues are deposited upon a collagen matrix, while that of enamel is collagen-free. Because the spectra of these mineral components are quite distinct, vibrational spectroscopy (Raman and infrared) has been extensively used to study all of these tissues providing information on the nature of the mineral phases present, quantitative information on the changes in mineral and matrix composition as mineralization occurs, and the nature and amounts of substituents in the mineral (e.g., [1–7]). In accord with the theme of this issue, this review is focused on IR microscopy and microscopic imaging for characterization and diagnoses of normal and diseased mineralized tissues. Specifically, we shall examine developmental studies of bones and teeth using whole tissues and cell cultures, pathologic calcifications, and the study of bone disease.

In addition to the hyperspectral images (x–y position, z intensity or value of parameter in question) showing the distribution of the phosphate ν_1, ν_3, ν_2 carbonate or amide I peaks (Fig. 1), several calculated parameters have been validated for the HA-containing tissues. The ratio of the area of the ν_1, ν_3 phosphate vibration (900–1200 cm^{-1}) to that of the amide I vibration is directly related to the chemically determined mineral content (ash weight) [8,9]. Carbonate to amide I ratios or carbonate to phosphate ratios indicate the extent of carbonate incorporation in the hydroxyapatite lattice, and curve-fitting of the carbonate band reveals whether the carbonate has replaced hydroxide (A-type) or phosphate (B-type) in the apatite lattice [10]. The relative areas of sub-bands at 1060 cm^{-1} [11] or the ratio of the 1030 and 1020 cm^{-1} sub-bands [12] correlate linearly with the HA crystal size and perfection in the c-axis direction as determined by X-ray diffraction analyses. In IR imaging, this ratio is often expressed as a ratio of peak height intensities [13] because it is time consuming to curve fit the

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Table 1
Vertebrate calcified tissues

Physiologic			Dystrophic		
Tissue	Mineral	Matrix	Tissue/disease	Mineral	Notes
Bone	Hydroxyapatite	Collagen	Atherosclerotic plaques	Hydroxyapatite	Lipid involvement
Calcified cartilage	Hydroxyapatite	Collagen	Prosthetic heart valves	Hydroxyapatite	Collagen based
Tendon and ligament insertions	Hydroxyapatite	Collagen	Tumoral calcinosis	Hydroxyapatite	Juxta-articular space
Cementum	Hydroxyapatite	Collagen	Juvenile dermatomyositis	Hydroxyapatite	Muscle and fat deposits
Dentin	Hydroxyapatite	Collagen	Milk alkali disease	Hydroxyapatite	Vitamin D toxicity
Enamel	Hydroxyapatite	Amelogenin Enamelin	Kidney stones and salivary stones	Calcium oxalate, whitlokite, hydroxyapatite	
			Thalessemia	Iron oxides	Skin deposits related to transfusions
			Articular cartilage and intervertebral disk deposits	Calcium pyrophosphate dihydrate, monosodium urate, hydroxyapatite, calcium oxalate	

number of spectra in a single image, not to mention multiple images. The areas of sub-bands at 1660 and 1686 cm^{-1} (or their intensity ratios) is related to the amount of non-reducible as contrasted with reducible collagen-cross links [14,15]. Hyperspectral images enable visualization of each of these parameters in the systems under examination.

2. Characterization of the development of physiologically mineralized tissues

The formation of the mineralized tissues starts with the patterning of the skeletal elements [16] and proceeds through the differentiating and proliferation of the cells that

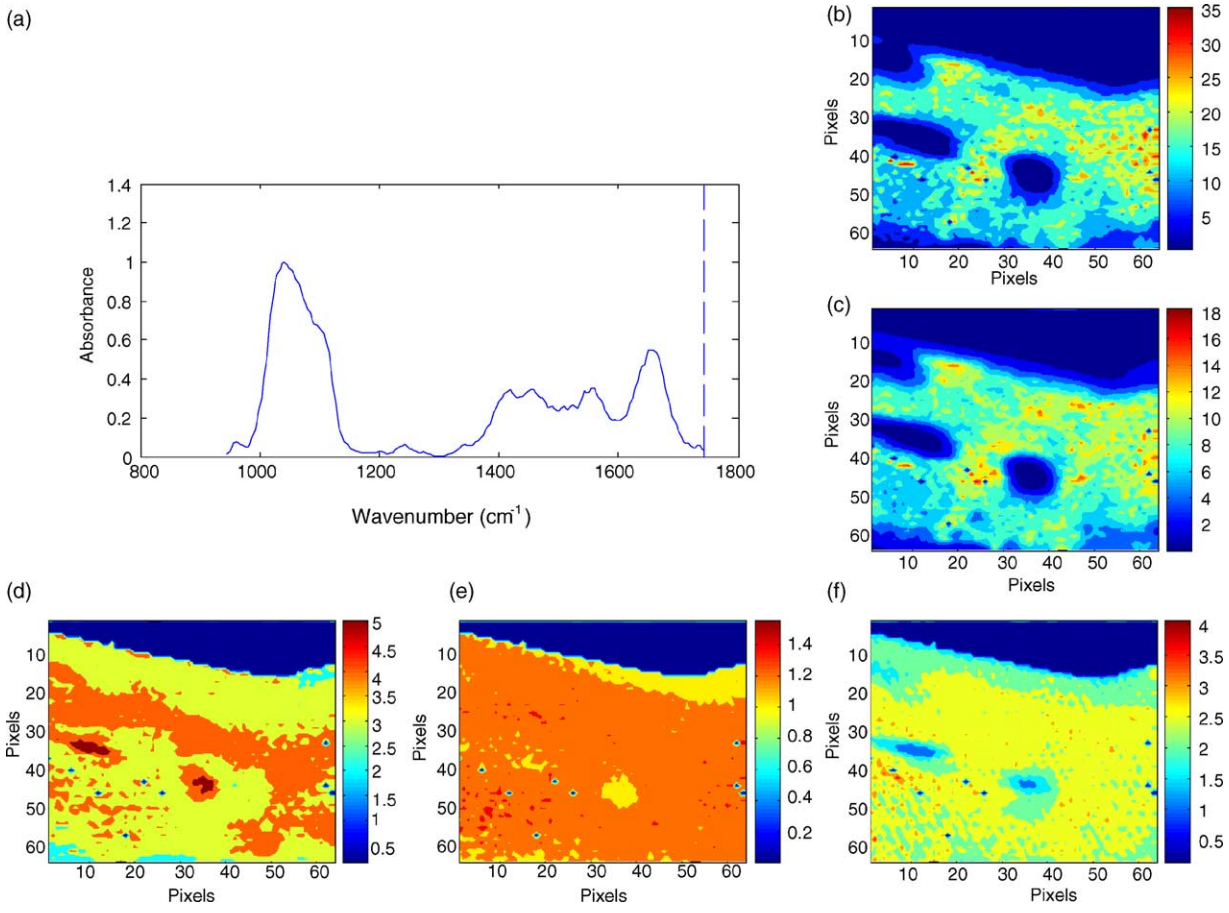

Fig. 1. Hyperspectral images of bone mineral properties: in normal human cortical bone (a) typical spectrum from a single image pixel, (b) image of the mineral distribution in the biopsy, (c) image of the matrix distribution in the biopsy, (d) image of carbonate distribution, (e) image of mineral:matrix ratio, (f) image of crystallinity and (g) image of collagen cross link ratio. *Note*: all images are corrected for the presence of the embedding media, PMMA.