Bone Submicron Mineralization in Osteogenesis Imperfecta: A Novel Application of Optical Photothermal Infrared (O-PTIR) Spectroscopy and Imaging

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INTRODUCTION: To elucidate the origins of bone health impairments, high-resolution compositional analysis is indispensable to assess the submicron-scale building blocks of bone quality and integrity [1]. Recently, a new modality of infrared spectroscopy, optical photothermal infrared (O-PTIR) spectroscopy, has been developed to allow data collection of thick samples with submicron spatial resolution. O-PTIR spectroscopy is a novel approach that overcomes resolution limitations of conventional Fourier transform infrared (FTIR) spectroscopy; however, validation of this technique is necessary to ensure it can be applied to evaluate bone tissue mineralization. In this study, our goal was to establish the novel application of O-PTIR spectroscopy and imaging to assess bone submicron composition in thick embedded samples.
METHODS: Our approach to validate this method was to assess cortical bone tissue submicron composition in samples from control tissues and tissues with a known bone mineralization defect (osteogenesis imperfecta, OI). Wildtype (WT, n=6) and *oim/oim* (OIM, n=7) female mouse tibias embedded in blocks of poly(methyl methacrylate) (PMMA) were obtained from an IACUC-approved research study at the Hospital for Special Surgery (New York, NY). The specimens underwent minimal sample preparation, primarily consisting of cross sectioning the blocks into 2-4 mm thick segments at the mid-diaphysis. No further sample treatment was required. Scanning electron microscopy (SEM) was utilized to visualize bone tissue structural features. O-PTIR spectral data were acquired using the Photothermal Spectroscopy Corp. mIRage infrared microscope. O-PTIR single-wavenumber imaging was used to map the distribution of sample components at 500 nm pixel size. Spectral line scans were acquired from the endosteum to the periosteum with a resolution of 500 nm and divided into three regions of cortical bone (endosteal, middle and periosteal). Quantification of tissue mineral content and crystallinity was done based on standard spectral peak ratios [2]. The heterogeneity of tissue composition was assessed based on the full width at half maximum (FWHM) of the distribution curve of the peak ratio values. Data from different regions of the cortical bone were compared between WT and OIM tissues using a t-test with statistical significance defined at $p < 0.05$. Machine learning analysis was carried out using the second derivatives of the raw spectral data from WT and OIM samples, grouped by bone regions (endosteal, middle, periosteal). Support vector machine (SVM) trained on principal component analysis (PCA)-reduced data was used to identify WT and OIM phenotypes based on spectral data.
RESULTS SECTION: O-PTIR spectra of PMMA-embedded cortical bone showed typical peaks of mineral and collagen, with little to no influence of PMMA. O-PTIR spectral imaging showed the different distributions of PMMA and bone mineral and protein components, and allowed a clear identification of tissue microporosity and osteocyte lacuna (Fig 1A). Quantification of peak ratios from the spectral line scans (Fig 1B) showed that the mineral content, indicative of the relative amount of mineral normalized to the amount of protein, was significantly greater in OIM than WT samples, in all bone regions. Additionally, mineral crystallinity, indicative of the maturity and structural order of the mineral, presented generally lower values in OIM samples compared to WT, but was only significant in the periosteal region. Quantification of the FWHM for the peak ratios demonstrated a significantly greater heterogeneity in the mineral content of OIM samples than WT. There was no significant differences in the heterogeneity of mineral crystallinity between WT and OIM. Finally, preliminary machine learning analysis could distinguish O-PTIR spectra from WT and OIM bones across the different cortical regions, with correct predictions ranging from 75-89%, demonstrating the potential of this approach to identify bones with different compositional properties.
DISCUSSION: Our study demonstrates the novel application of O-PTIR spectroscopy and imaging to assess bone tissue composition in thick embedded samples, addressing the high demand for this ability. Bone compositional data obtained using O-PTIR spectroscopy aligns with previous findings using standard FTIR spectroscopic methods to assess tissue mineralization in WT and OIM bones [3]. However, previous studies have been limited to analysis of bulk samples or to imaging at microscale resolution, which cannot properly assess the submicron features of bone. Here, using this novel approach with 500 nm spatial resolution revealed previously undocumented variations in matrix mineralization between WT and OIM across different cortical bone regions (endosteal, middle, periosteal), shedding new insights into how bone tissue compositional properties are affected in OI .
SIGNIFICANCE/CLINICAL RELEVANCE: O-PTIR spectroscopy and imaging can be readily implemented for the analysis of clinical samples aiming to elucidate the role of bone submicron compositional properties underlying skeletal diseases.
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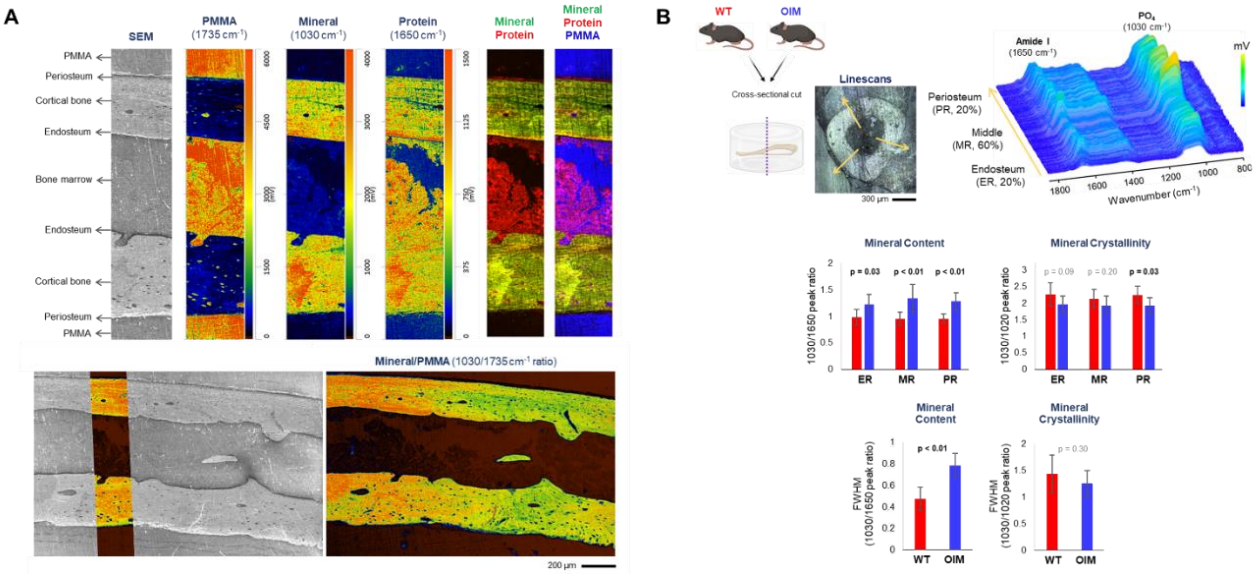

Figure 1. (A) O-PTIR spectral imaging of bone at submicron resolution. (B) Quantitative assessment of bone submicron composition in WT and OIM mice.