A New Approach to Assess Tissue-Level Composition in Bone Biopsies: Towards Unveiling Osteoporosis

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INTRODUCTION: Osteoporosis is a degenerative bone disease that affects millions of people worldwide. Bone fragility due to osteoporosis is determined by a combination of factors, including bone mineral density (BMD), bone geometry and microarchitecture, bone turnover rates, and bone tissue composition [1]. However, conventional analysis of bone biopsies to improve the diagnosis of osteoporosis and subsequent fracture risk assessment does not take into account properties of bone mineral and collagen at the submicron scale—the building blocks of bone structural integrity [2]. This represents a critical gap in understanding how tissue-level compositional properties of bone underlie the poor bone quality associated with osteoporosis, which may be instrumental in informing the development of better therapies and treatment plans. Here, our goal is to establish the application of the recently developed technique optical photothermal infrared (O-PTIR) spectroscopy to assess the submicron-scale composition of human bone biopsies from subjects with osteoporosis, aiming to establish a new approach to gain insights into disease development and progression in clinical samples.

METHODS: Ten human iliac crest biopsies embedded in standard blocks of polymethyl methacrylate (PMMA) were acquired from the Hospital for Special Surgery (New York, NY). Each block was approximately 22 mm thick and had an exposed bone surface in one face. Standard assessment of bone tissue structure was carried out by histology (von Kossa staining), micro computed tomography (microCT), and scanning electron microscopy (SEM). 3D modeling of a sample holder for the O-PTIR microscope was done using SolidWorks. O-PTIR data from cortical and trabecular tissues were collected at 500 nm spatial resolution using the mIRage Sub-Micron IR Spectrometer (Photothermal Spectroscopy Corp), both as point spectra and single-wavenumber images at specific peaks of interest, including the phosphate (mineral), amide I (collagen), and PMMA peaks. The images were analyzed using ratio mapping and RGB overlays using PTIR Studio. Spectra were analyzed using The Unscrambler X to obtain second derivatives of the spectra and quantify peak ratios that reflect bone tissue compositional properties, such as averages and heterogeneity of mineral content (1030/1660 cm^{-1} ratio), mineral crystallinity (1030/1020 cm^{-1} ratio), carbonate content (880/1030 cm^{-1} ratio), and collagen integrity (1235/1450 cm^{-1} ratio) [3].

RESULTS SECTION: Histology (Fig 1a), microCT (Fig 1b) and SEM (Fig 1c) images of the bone biopsies show a range of tissue structural properties across subjects, with varying bone area and volume, trabecular architecture, and cortical thickness and porosity. For O-PTIR analysis, the first outcome to be achieved was the development of a custom sample holder to fit the thick bone biopsy blocks into the O-PTIR microscope stage, which was carried out by 3D modeling and printing (Fig 1c-d). Inspection of the bone surface using the visible mode of the microscope allowed clear identification of cortical and trabecular bone to select the regions of interest for O-PTIR spectral data collection. The spectra were typical, comprising bands of mineral (phosphate, carbonate) and collagen (amides, CH), with bands of PMMA (acrylate) seen only on the edges of the tissue and within tissue porosity (Fig 1e). Thousands of point spectra could be collected at cortical and trabecular bones, with quantitative analysis of peak ratios revealing overall trends in tissue-level compositional properties across subjects, including in mineral content, mineral crystallinity, carbonate content, and collagen integrity. Moreover, O-PTIR imaging of osteons and trabecula at 500 nm spatial resolution (Fig 1f) enabled the visualization of bone tissue-level composition in individual lamella and around osteocyte lacunae and Haversian canals.

DISCUSSION: We show that O-PTIR spectroscopy and imaging is an effective method to analyze the submicron tissue composition of human bone biopsies embedded in standard thick PMMA blocks. This approach brings a significant advantage to the field when compared to conventional Fourier transform infrared (FTIR) spectroscopy [3], as it can inform on submicron tissue-level composition without the need for cumbersome thin-sectioning of calcified tissue. Additionally, the method is non-destructive, preserving the clinical samples for further analysis. Future directions will involve implementing a machine learning approach to delve into the relationship between O-PTIR-derived tissue compositional properties and structural properties of bone obtained by microCT and SEM, aiming to provide new insights into compositional factors underlying poor tissue integrity and health.

SIGNIFICANCE/CLINICAL RELEVANCE: This study shows for the first time the application of O-PTIR spectroscopy to assess tissue-level composition in clinical bone tissues from subjects with osteoporosis, offering a new approach that may break new ground towards elucidating how the tissue-level composition of bone can inform on osteoporosis progression and therapeutic targets.

REFERENCES: [1] Curtis et al. Bone. 2017. [2] Reznikov et al. Acta Biomaterialia. 2014. [3] Querido et al. Molecules. 2021.
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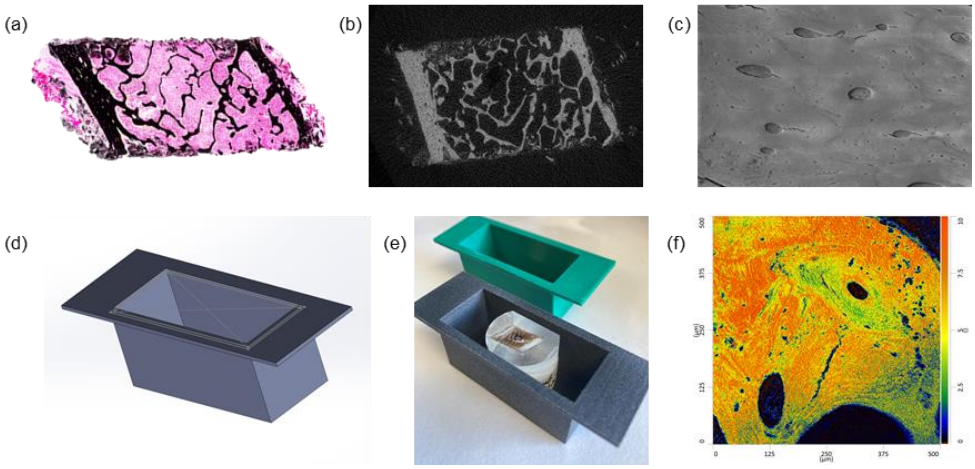

Figure 1. Analysis of bone biopsy. (a) Histology (von Kossa staining), (b) microCT, (c) SEM of cortical bone, (d-e) 3D modeling and printing custom sample holder to fit biopsy block into O-PTIR microscope, (f) O-PTIR imaging bone composition at 500 nm spatial resolution