

FT-IR Spectroscopy As A Tool in orthopedics To Study Cells And Tissue Diseases

G. Graziani¹, F. Pegreff², G. D'Atri³, E. Gabusi⁴, L. Saldaña⁵, E. García-Rey⁵, G. Lisignoli⁴

¹University Rome San Raffaele, Rome, IT; ² University Kore of Enna, Enna, IT; ³Politecnico di Milano, Milan, IT; ⁴IRCCS Rizzoli Orthopaedic Institute, Bologna, IT; ⁵Hospital Universitario La Paz-IdiPAZ, Madrid, SP
Gabriela.graziani@gmail.com

Disclosures: Nothing to disclose.

INTRODUCTION: Fourier transform infrared spectroscopy (FT-IR) is largely used for the study of inorganic and organic biomaterials. Thanks to its specific advantages, which include high sensitivity, need for small volume of samples, fast acquisition time and possibility to analyze a broad range of compounds, in the past years, FT-IR has emerged as a powerful technique for the study of cells and tissues, and as a potential diagnostic tool to investigate physiopathology *in vivo*, *ex vivo* and *in vitro*.

In orthopedics, FT-IR is increasingly being proposed for the study of different tissues and pathologies, including cancer, osteomyelitis and osteoarthritis. However, the use of this technique for tissues is at its early stages, and applications to cells and extracellular vesicles is scarcely investigated.

Here, we show the applications of FT-IR in orthopedic field, for the study of tissue (bone), cells and extracellular vesicles, to show potential applications in bone disease. Indeed, for these application, FT-IR allows to obtain a fingerprint of the analyzed material, providing information on the inorganic and organic phases and on biological molecules that are contained (i.e. proteins, lipids, nucleic acids), in terms of quantity and organization, providing important insights on pathophysiology, disease and efficacy of therapy.

METHODS: Trabecular bone explants were obtained from patients with osteoarthritis during total hip replacement and were directly analysed after deproteinization in sodium hypochlorite. For the acquisition, the following parameters were used: resolution 4 cm⁻¹, 16 scans, can step size 1 cm⁻¹.

Cells were analysed after culturing for the relevant timepoints. For the acquisition, either micromass culture, or cultures on Thermanox coverslips (5000 cell/cm²) can be used.

Extracellular vesicles spectra were acquired starting from 5 µL drops containing about 10 µg total protein.

All samples were acquired in triplicate and in three different experiments (n=9).

For cells and Evs, the following parameters are used: 4000-400 cm⁻¹, resolution 4 cm⁻¹, acquisition: 128 scans, step size: 0.5 cm⁻¹.

Data processing was carried out by Matlab and statistical analysis was performed using the Graph Pad Prism 7.04 software.

RESULTS SECTION: Cells, extracellular vesicles and tissues can be characterized by FT-IR in a fast and highly reproducible way.

For trabecular bone, FT-IR permits to identify changes in the composition of the inorganic phase (i.e. different carbonation in hydroxyapatite and/or presence of metastable phases), which correlate with bone fragility.

For healthy cells (chondrocytes and mesenchymal stem cells (MSC)) and tumor cells (MG-63), lipids, GAGs, collagen and aggrecan bands are present in different areas of the spectrum and can be analysed and quantified. Mineralization can be also evaluated; FT-IR helps distinguish the specific calcium phosphate phase secreted by MSC and its abundance, by varying the microenvironment conditions and/or applying therapeutics.

A “biomolecular fingerprinting” can also be defined for extracellular vesicles, secreted by different cells, including MSCs and tumor cells, identifying nucleic acids, lipids, proteins, and carbohydrates. We have also show that extracellular vesicles from doxorubicine-resistant cells present specific markers (bands) in the IR spectrum, which could in the future be used as a marker of sensitivity/resistance to drugs.

DISCUSSION:

FT-IR is a powerful tool with unique advantages, which include high sensitivity, small volume of samples, fast acquisition time, and broad range of analyzable compounds. It allows to obtain a fingerprint of cells, tissues and extracellular vesicles, providing information on the biological molecules that are contained (i.e., proteins, lipids, nucleic acids, etc.), in terms of quantity and organization, permitting to differentiate between healthy and pathological conditions and thus providing information about tissue pathophysiology, disease progression, and therapeutic evaluation.

SIGNIFICANCE/CLINICAL RELEVANCE: FT-IR appears as a powerful tool in orthopaedics, that can be used as an indicator of pathology and a method to investigate treatment efficacy, with applications *in vitro*, *in vivo*, *ex vivo* and clinical studies. Future perspectives include its use to analyze synovial fluid, as a biomarker of pathology. The technology is fast and inexpensive, permitting potential clinical translation.