

Discrimination of different human cell lines by using FT-IR spectra spectroscopy

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Introduction

The Fourier Transform Infrared (FT-IR) spectroscopy is a powerful analytical tool used to study the molecular composition of various biological samples, including cell lines. Several studies have indicated the potential use of this technique for distinguishing between healthy and cancerous cells [1,2] and among different cell lines [3,4]. In this study, we present a comparative analysis of the FT-IR spectra of three distinct cell lines: SH-SY5Y (a human neuroblastoma cell line), MCF-10A (a non-tumorigenic human mammary epithelial cell line), and HepG2 (a human liver carcinoma cell line).

Material & Methods

FT-IR measuring: For IR absorption spectra of the cell samples were obtained using a Spectrum One FTIR spectrometer equipped with a Perkin Elmer Multiscope system infrared microscope and an MCT (mercury–cadmium–telluride) FPA (focal plane-array) detector. The measurements were performed at room temperature on cells grown on MirrIR (25 x 25 mm²) slides in transfection mode. For each experimental condition, three slides were prepared. Spectra were acquired within an aperture of 100 x 100 μm². Different regions were investigated on every slide, and three spectra were acquired for each position. All spectra were collected using 64 scans in the range from 4000 to 600 cm⁻¹ with a 4 cm⁻¹ spectral resolution

Cell line growth conditions: The cell lines of SH-SY5Y and HepG2 were cultured in DMEM supplemented with 10% heat-inactivated FBS, 100U/mL penicillin, 100 μg/mL streptomycin, and 1% L-glutamine. Cell line of MCF-10A was cultured in DMEM/F12 (Gibco 3133-028) supplemented with 5% horse serum (Gibco 26050-070), 20 ng/ml epidermal growth factor (EGF, Sigma SRP3027), 10 mg/ml insulin (Sigma I6634), 0.5 mg/ml hydrocortisone (Sigma H-0888), 100 ng/ml cholera toxin (Sigma C-8052), and antibiotics (Pen/Strep, Gibco 15070-063). The cells were grown at 37 °C in a humidified atmosphere of 95% air and 5% CO₂.

Data analysis: The spectra were preliminarily processed by subtracting the background signal that was acquired from a free-cell zone of the slide, and a baseline were performed on the whole data and operating a piecewise baseline correction. The spectra were then normalized using a vector normalization procedure in order to have comparable intensities adopting a Standard Normal Variate (SNV) method [5].

Results

The absorption bands observed in the SH-SY5Y spectra include amide I (1651 cm⁻¹) that dominant peak arising from C=O stretching vibrations, primarily associated with proteins. The amide II (1546 cm⁻¹), arising from N-H bending and C-N stretching vibrations. Lipid bands (2852-2922 cm⁻¹) that attributed asymmetric and symmetric stretching vibrations of CH₂ groups. Phosphate bands (1237 cm⁻¹ and 1087 cm⁻¹), indicative of nucleic acids and phospholipids. In the HepG2 spectrum, notable absorption bands include amide I and amide II bands that are similar to those seen in SH-SY5Y, but with variations in position and intensity of their peaks, suggesting differences in protein secondary structure or concentration. Prominent peaks in the 2851-2922 cm⁻¹ region, indicate high lipid content, typical of liver cells.

In addition, peaks at 1233 and 1084 cm⁻¹ indicate variations in nucleic acid and phospholipid content. Amide I and Amide II bands of MCF-A10 are consistent with those of SH-SY5Y and HepG2 but with different relative intensities. Peaks in the 2850-2922 cm⁻¹ region, though less pronounced than in HepG2, reflect lower lipid content typical of non-cancerous cells. These findings provide valuable insights into the biochemical composition and functional differences among these cell lines, highlighting the potential of FT-IR spectroscopy in studying cellular mechanisms and disease processes in various biomedical applications. The differences mentioned above reflect each cell type's unique biochemical environment and metabolic states. This distinction can help identify different cell lines.

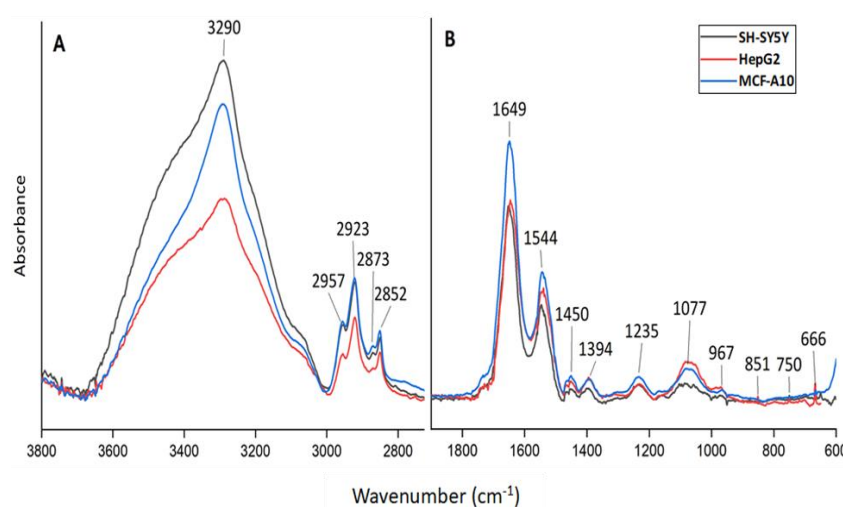
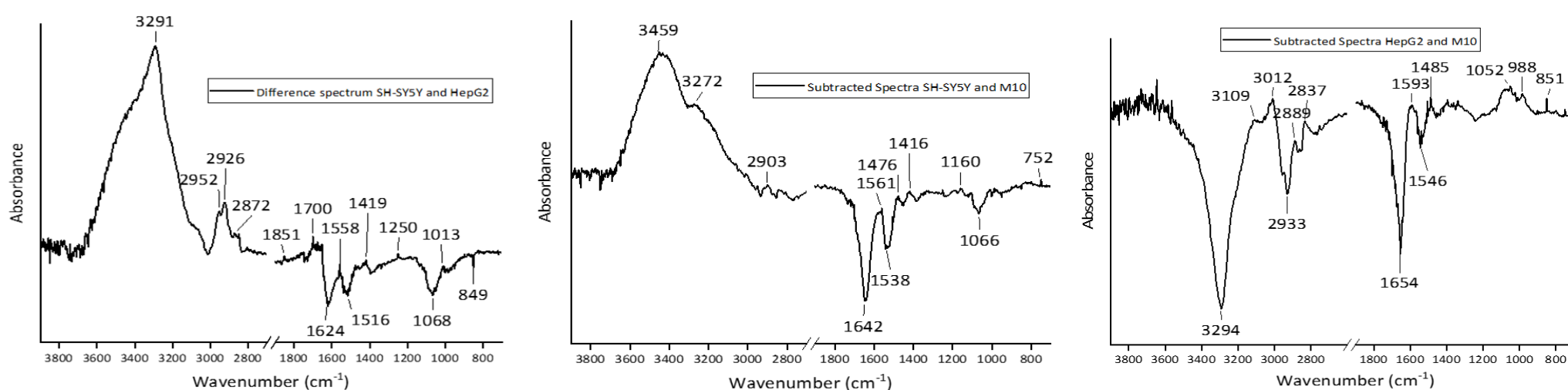


Figure 1. Average FT-IR spectrum obtained in transfection mode from SH-SY5Y, MCF-A10 and HepG2 cells.

Figure 2. Difference spectra from SH-SY5Y, MCF-A10 and HepG2 cells.



Conclusions

These findings provide valuable insights into the biochemical composition and functional differences among these cell lines, highlighting the potential of FT-IR spectroscopy in studying cellular mechanisms and disease processes in various biomedical applications. The differences mentioned above reflect each cell type's unique biochemical environment and metabolic states. This distinction can help identify different cell lines. Understanding these spectral differences can provide insights into the molecular basis of cellular functions and aid in the development of cell-specific therapeutic strategies.

References

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