FT-IR Microspectroscopy of Cancer Cells and Extracellular Vesicles

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Nevertheless, that cancer cells abnormally secrete large quantities of extracellular vesicles (EV), it is a time-consuming and relatively expensive process to collect samples with high enough concentration to record qualitative FT-IR spectra. Sample preparation for IR-spectroscopy is another principally important step and option. In our previous studies, EV of cancer-derived cells were analyzed using FT-IR spectra recorded using HTS-XT. It was shown that spectrum of HEPES buffer overlaps with that of cells or EV, and thus proteins of cells or EV cannot be identified properly. Therefore, cells or EV were suspended in PBS buffer but 0,9% NaCl also can be used. The aim of this study was to find a method for FT-IR spectroscopy analysis of significantly lower counts of cells or EV. Under study were colorectal cancer cell lines derived from a primary - SW480, and metastatic - SW620, tumour cultured under hypoxic or normoxic conditions and their derived EV. Samples were suspended in PBS buffer, driedon a glass window, to obtain a homogeneous film, and 500 – 1000 micron sample pressed using diamond compression cell. Spectra were recorded using Hyperion 2000 with15x IR objective; the analysed sample area was 100x100 microns. This approach allows to analyse significantly lower amount of sample compared to that of HTS-XT. For example, to gain a good quality spectrum using HTS-XT are required ~200000 cells whereas ~ 2000 cells using a diamond compression cell. FT-IR spectra of cells and EV recorded by HTS-XT or Hyperion correspondingly were qualitatively equal with similar absorbance. This study showed that compression cell is a valuable tool for studies of micro-biosamples by FT-IR microspectroscopy.

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