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Raman Spectral Fingerprints of Healthy and Cancerous Human Colorectal Tissues

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Abstract : Colorectal cancer is the third most common cancer diagnosed in Europe, according to the latest incidence data provided by the World Health Organization (WHO), and early diagnosis has proved to be the key in reducing cancer-related mortality. In cases where surgical interventions are required for cancer treatment, the accurate discrimination between healthy and cancerous tissues is critical for the postoperative care of the patient. The current study focuses on the ex vivo handling of surgically excised colorectal specimens and the acquisition of their spectral fingerprints using Raman spectroscopy. Acquired data were analyzed in an effort to discriminate, in microscopic scale, between healthy and malignant margins. Raman spectroscopy is a spectroscopic technique with high detection sensitivity and spatial resolution of few micrometers. The spectral fingerprint which is produced during laser-tissue interaction is unique and characterizes the biostructure and its inflammatory or cancer state. Numerous published studies have demonstrated the potential of the technique as a tool for the discrimination between healthy and malignant tissues/cells either ex vivo or in vivo. However, the handling of the excised human specimens and the Raman measurement conditions remain challenging, unavoidably affecting measurement reliability and repeatability, as well as the technique's overall accuracy and sensitivity. Therefore, tissue handling has to be optimized and standardized to ensure preservation of cell integrity and hydration level. Various strategies have been implemented in the past, including the use of balanced salt solutions, small humidifiers or pump-reservoir-pipette systems. In the current study, human colorectal specimens of 10X5 mm were collected from 5 patients up to now who underwent open surgery for colorectal cancer. A novel, non-toxic zinc-based fixative (Z7) was used for tissue preservation. Z7 demonstrates excellent protein preservation and protection against tissue autolysis. Micro-Raman spectra were recorded with a Renishaw Invia spectrometer from successive random 2 micrometers spots upon excitation at 785 nm to decrease fluorescent background and secure avoidance of tissue photodegradation. A temperature-controlled approach was adopted to stabilize the tissue at 2 °C, thus minimizing dehydration effects and consequent focus drift during measurement. A broad spectral range, 500-3200 cm-1,was covered with five consecutive full scans that lasted for 20 minutes in total. The average spectra were used for least square fitting analysis of the Raman modes.Subtle Raman differences were observed between normal and cancerous colorectal tissues mainly in the intensities of the 1556 cm-1 and 1628 cm-1 Raman modes which correspond to v(C=C) vibrations in porphyrins, as well as in the range of 2800-3000 cm-1 due to CH2 stretching of lipids and CH3 stretching of proteins. Raman spectra evaluation was supported by histological findings from twin specimens. This study demonstrates that Raman spectroscopy may constitute a promising tool for real-time verification of clear margins in colorectal cancer open surgery.

Keywords : colorectal cancer, Raman spectroscopy, malignant margins, spectral fingerprints
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