Imaging of lipids in atherosclerotic lesions by FT-IR spectroscopy and hierarchical cluster analysis

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Three groups of lipids (free fatty acids, triglycerides and cholesteryl esters) and cholesterol were measured by means of ATR FT-IR spectroscopy. Each group was composed of palmitic, oleic and linoleic acid derivatives, compounds found to be involved in development of atherosclerosis. Spectral analysis of these compounds allowed clear discrimination between free fatty acids and the other groups based on carbonyl band position (the 1699-1710 cm^{-1} range). Next, we applied IR imaging of lipids followed by Hierarchical Cluster Analysis (HCA) on the tissue of C57Bl/6J ApoE/LDLR $^{-/-}$ female atherosclerotic mice and a C57Bl/6J control mice (fed with normal chow diet). The measurements were completed with an FT-IR spectrometer equipped with a 128x128 FPA detector.

Unsupervised hierarchical analysis of the normal and diseased sections of the aorta lumen provided clear spectral features that discriminate development of the atherosclerotic plaque, including appearing the C=O stretching band, increasing intensity of the phosphate bands, and changing $\nu(CH_2/CH_3)$/Amide A, Amide I/Amide II ratio. Moreover, a class of atherosclerotic plaque, divided into 2 sub-classes, was found in the diseased tissue. One of them shows higher content of proteins and cholesterol, while the second class is rich in cholesteryl esters. This part of our work was performed on cross-sections of the aorta tissue mounted on Ag/SnO_2 coated infrared reflective slides (Kevley Technologies). In turn, HCA of tissues deposited on the glass slides, hence limited to the 2200-3800 cm^{-1} spectral range, was performed and compared with a standard Oil Red O histological staining. Our experiments revealed a very good correlation between IR imaging and histological procedure showing that IR imaging in the high-wavenumber region coupled with HCA is sensitive enough to probe development of atherosclerotic lesions in blood vessels.

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