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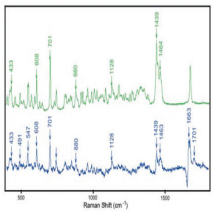
Raman Spectroscopy

Investigating the Atherosclerosis Process by Monitoring Lipid Deposits Including Cholesterol and Free Fatty Acids



Application Note

Biology RA60

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Introduction

Atherosclerosis is the disease caused by the hardening of blood vessel walls involving the deposition of lipid, cholesterol, and calcium on the inner lining of vessels. Plaques contain lipid cores, fibrous caps and calcification, causing vessel lumen narrowing that reduces blood flow, and vessel rupture leading to thrombosis, which results in heart attack or stroke.

Apolipoprotein E (ApoE, the proteinaceous component of high density lipoproteins) enables lipids like cholesterol and triglycerides to move within the water based solution of the blood stream. In healthy individuals, about thirty percent of blood cholesterol is carried by high density lipoproteins (HDL). HDL is involved in transport of cholesterol and triglycerides. A mouse model for human atherosclerosis has been developed in which the animals lack ApoE; these animals are called ApoE knock-out mice.

Results

The aorta from an ApoE knock-out mouse (lumen side) was examined with a Raman microscope (Figure 1). The presence of globular deposits of the order of 3-8 µm was observed. Spectra were recorded from the globule (green), fat (red) and proteinaceous material (blue). The band at 1735 cm^{-1} (marked with a red arrow) is a carbonyl stretching band, and absent in the globule spectrum. This is attributed to the presence of free fatty acid, instead of triglycerides (esters derived from glycerol and fatty acids), in globules. In fact, fatty acids exist predominantly as dimers, in which vibrational motions of two carbonyl groups are coupled. Only the symmetric stretching (two carbonyl groups vibrating in phase) is Raman active and its frequency tends to appear below 1700 cm^{-1}.

The additional sharp bands, especially the one at ca. 700 cm^{-1} (marked with a green arrow) is recognized as representative of cholesterol dissolved in free fatty acid. A model measurement was made in which cholesterol was dissolved in oleic acid, and its Raman spectrum was recorded. Figure 2 shows the spectra of pure oleic acid (red), cholesterol crystals (green) and cholesterol dissolved in oleic acid (blue).

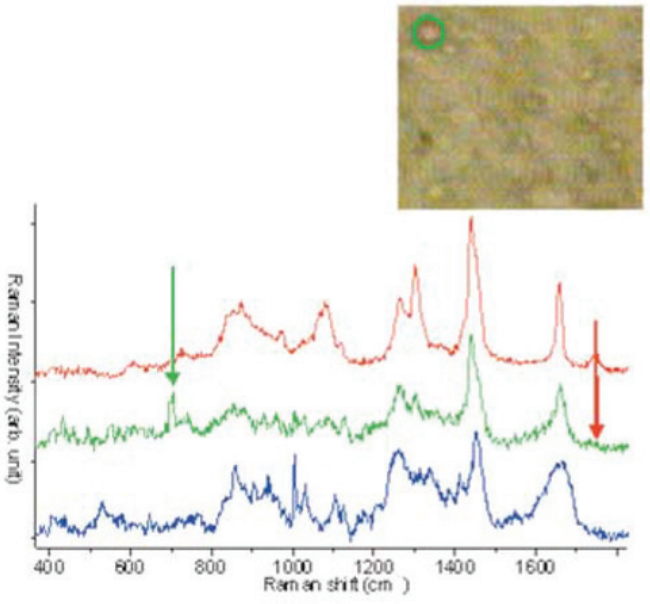

Figure 1. Optical micrograph of the lumen side of an aorta from an ApoE knock-out mouse. A globule is marked with a green circle. Raman spectra of a globule (green), a fatty region (red), and a proteinaceous-rich region (blue) are shown below.

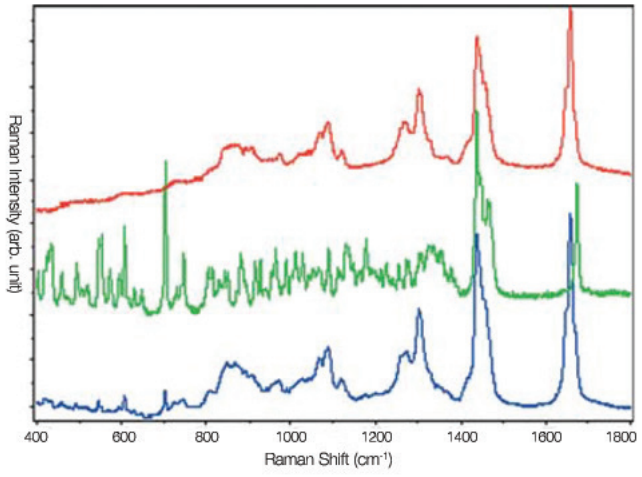

Figure 2. Raman spectra of pure oleic acid (red), cholesterol crystals (green) and cholesterol dissolved in oleic acid (blue).

The oleic acid (solvent) spectrum is subtracted from the solution (cholesterol dissolved in oleic acid) spectrum and compared to cholesterol crystals spectrum (Figure 3). This was to verify that spectral features of cholesterol are maintained in the solution spectrum, even though solute and solution spectra may appear quite different at first glance. All of the cholesterol bands are recognizable in the difference spectrum, even the carbon-carbon double at ~ 1680 cm^{-1} that was hidden under that of the oleic acid.

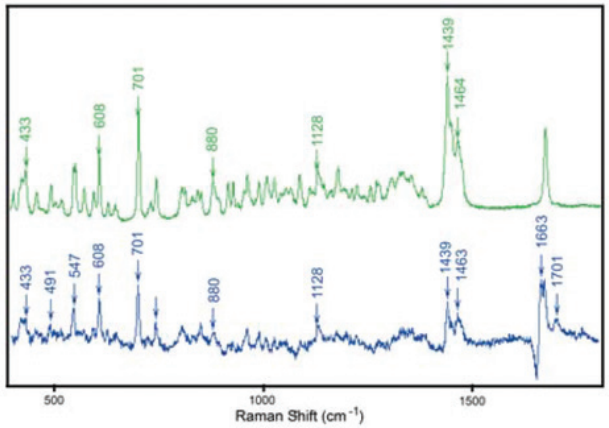

Figure 3. Spectra of cholesterol crystals (green) and the difference spectrum of solution (cholesterol in oleic acid) and solvent (oleic acid) spectra

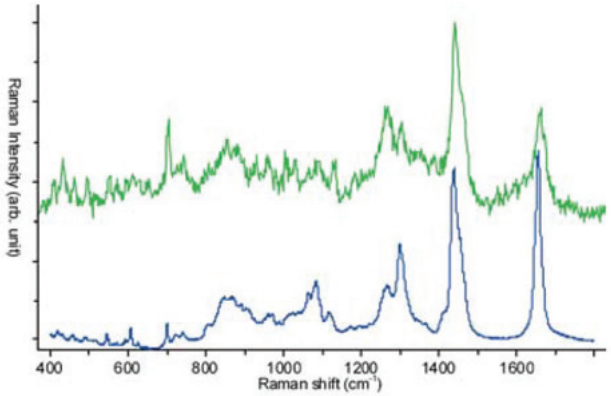

Figure 4. Raman spectra of the globule on the luminal side of the ApoE knock-out mouse (green) and cholesterol dissolved in oleic acid (blue)

In figure 4, the spectrum of the globule of the aorta sample obtained from an ApoE knock-out mouse (from figure 1) is compared to the cholesterol in the oleic acid solution spectrum (from figure 2). The two spectra show a good agreement indicating that the globules are composed of free fatty acids with dissolved cholesterol.

Conclusion

Comparing these two measurements -the results of the aorta of an ApoE knock-out mouse and the results of the chemical study - indicates that the globule deposits contain cholesterol dissolved in fatty acid, and thus can be used as an early indicator of the atherosclerosis process in animal models during drug development.

Acknowledgement

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References

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[2]YA Fadeev and AA Kolesnikova, Determination of quasi-elastic coefficients of intermolecuar interaction in associates of fatty acids and their perfluorine derivatives, J. of Appl. Spect., 62(6) 1032-1034 (1995)

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