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Chemical characterization of cell-death-dependent calcification in aortic valve stenosis by Raman mapping on sections and matching against histochemical counterparts

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Raman micro-spectroscopy is a qualitative and quantitative non-destructive analytical technique in which a monochromatic radiation emitted from a laser source is focused on a sample through microscope optics, and the light inelastically scattered by the sample is thus collected and analyzed by means of an optical spectrograph. The bands observed in Raman spectra correspond to molecular vibrations of the biochemical species constituting the sample, with each species contributing with a specific molecular "spectral fingerprint". Exploiting this analytical capability, "chemical images" showing the distribution of different biochemical species are produced by collecting spectra from each point of the sample: an approach called "Raman mapping". With diagnostic purposes, Raman mapping is being increasingly exploited to characterize pathological tissues. 30-mμ-thick cryosections of samples from aortic valve leaflets surgically excised from patients affected by calcific aortic valve stenosis (CAVS) were mounted on calcium-fluoride-slides for Raman-analysis. Nearly adjacent 8-mμ-thick cryosections were mounted on histological slides for histochemical analysis, including von Kossa silver staining. The localization of prominent hydroxyapatite quantities closely correlated with von Kossa staining at level of calcific nodules and single neighbouring dead cells and cell debris. In addition, Raman imaging revealed great amounts of phspholipids and cholesterol around the calcific nodules, where a distinct cell degeneneration occurs, as previuosly ultrastructurally described (Ortolani et al., Histol. Histopathol. 22, 261-272, 2007), which includes increasing in cell membranous component, its subsequent dissolution, and release/clustering of lipid-containing material acting as major hydroxyapatite nucleator. Such a lipid distribution in Raman-maps was superimposable to specific reactivity for von Kossa and Cuprolinic blue staining on histological sections. Interestingly, Raman-maps also showed further co-localization of carotenoids with lipid moiety and in particular cholesterol. In conclusion, more detailed chemical characterization was provided of the calcific process underlying CAVS by implementing histochemical and ultrastructural procedures with Raman micro-spectroscopy. In addition, considering that Raman spectra could be obtained in-vivo upon shining the laser light on the valvular tissues via an optical fiber, the intense and characteristic signal of carotenoids might be used as an optical marker for precocious diagnosis of valve calcification.

Keywords: Raman-imaging, aortic valve stenosis, soft tissue calcification