Constribution of Transmission Electron Microscopy and Electron Energy-Loss Spectroscopy for characterizing the composition and the initial formation site of incipient Randall's plaque

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We aim at analyzing the composition of Randall's plaques in the early stages of their formation and correlate their nature to their localization in the kidney. The complexity of these biomaterials that combine inorganic and organic phases in a multi-scale structure (ranging from nanometers to macroscopic) requires the use of different characterization techniques: immunohistochemical analysis (cell level), scanning electron microscopy coupled to EDX mapping (microscale), infrared spectroscopy µFTIR (organic compounds), imaging, diffraction and nanoanalysis in (Scanning)TEM (nanoscale and analysis of crystal structure and composition of the nanocrystals).

In this context, we are conducting Electron Energy-Loss Spectroscopy (EELS) experiments coupled to STEM imaging. This technique is of great interest because it allows to perform basic mapping of light elements of biological interest (Ca, Mg, P, N, ...) on a nanometer scale.

A first series of experiments was conducted on ultrathin sections obtained by ultra-microtomy and making use of a nitrogen-cooled sample stage coupled to a highly sensitive camera (also nitrogen cooled for a minimum dark current). A first series of elemental maps coupled to nano-diffraction experiments in conventional TEM allowed to reveal the crystallinity of the nano-calcifications and in some cases to identify the presence of these nano-calcifications within vesicles whose role and nature is to be determined.

Further experiments are ongoing to further characterize these nano-calcifications in order to
- discriminate between the various phases identified by FTIR on these samples: carbo-apatite, whitlockite and their co-existence with amorphous calcium phosphate phases
- establish by EELS the nature of vesicles when they coexist with micro-calcifications.