Unveiling Extracellular Vescicles diverse morphology with Cryo-Electron Microscopy

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Extracellular Vescicles (EV) are a heterogeneous population of phospholipid bilayer-enclosed vescicles recognized to be fundamental mediators of cellular cross-talk once they are secreted from a variety of cells through activation, stress and apoptosis pathways. EV have been mainly characterized by flow-cytometry, whereas morphological information were firstly obtained by their atomic force microscopy studies. Conventional transmission electron microscopy has been used to study morphology of EV isolated from plasma, urine and synovial fluids, the main limit being the standard TEM sample preparation through dehydration, chemical fixation and staining of the biological specimens, that hampers the visualization of their native state. On the other hand, cryo-electron microscopy (cryo-EM) does not require staining or chemical fixation, the sample being directly vitrified on a EM grid. Nowadays vitrification is a cryo-fixation method widely exploited in the sample preparation of biological specimens, that preserve their morphology and allows their near-atomic resolution imaging. This is achieved by flash freezing the sample, preserved in physiological buffer conditions and placed onto a cryo-EM grid, in liquid ethane, thus avoiding ice crystal formation since water is transformed in a glass-like state. Hence, sample morphology is well retained, allowing direct visualization of near-native state extracellular vescicles at the highest possible resolution, both in their empty or cargo-loaded states.