High resolution atomic force microscopy as a tool for topographical mapping of surface budding

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Our paper is focused on building up an Atomic Force Microscopy (AFM) based methodology to characterize extracellular vesicles (EVs) morphology and distribution over the cell surface in different physiological conditions, while validating the efficacy of the AFM technique against conventional Electron Microscopy (EM) methods. To the best of our knowledge, the use of AFM-derived morphometric parameters to describe EVs trafficking and its variations under certain physiological conditions, has not yet been investigated. We take advantage of the capability of the AFM to provide, with a single exposure, high-resolution imaging, coupled with important information about biophysical and biomechanical properties of the specimens, such as the surface roughness. We demonstrate that both microscopy techniques were capable to obtain compatible average values of vesicles size as well as their distribution, resulting increased after H2O2 treatment. However, AFM analysis allowed to obtain a more accurate topography and distribution of plasma membrane budding throughout the cell surface and to appreciate details not accessible from the EM, principally due to the AFM easier specimen's preparations requirements. Taken together, our results may provide interesting perspectives to implement a morphometric approach for studying and understanding the pathophysiological state of the cell related to EVs trafficking.