Abstract

The aim of this work was to analyze the physical properties of lipid vesicles at the nanoscale. To achieve this, a methodology was developed to study extracellular vesicles using atomic force microscopy (AFM) and to compare these results with data obtained by nanoparticle tracking analysis (NTA). This type of research may be useful for the application of extracellular vesicles in diagnostic procedures and for their use as drug carriers.

Data and Methodology. Extracellular vesicles from PC3 prostate cancer cells cultured at the Department of Biomedical Physics, University of Warsaw, were studied, as well as reconstituted, lyophilized extracellular vesicles from the same cell line. In addition, results were compared with those for artificially created nanovesicles—liposomes. Experiments were conducted using AFM and NTA, and the experimental results were compared with computational modeling. Two computational models were used: the first, developed by our team was used to describe the shape and stiffness of a vesicle attached to a mica surface and was based on Canham–Helfrich theory. The second described the settling of vesicles on a substrate, based on the theory of Brownian motion in a liquid droplet.

Results. A procedure for imaging extracellular vesicles by AFM in liquid was developed, including an effective method for obtaining a contaminant-free mica substrate with a positive surface charge. The vesicle-substrate contact angle was also studied and it was observed to decrease with increasing vesicle diameter. A new method for studying lipid membranes was proposed, based on the geometric parameters of nanovesicles and informed by computational modeling. An increase in the average diameter of extracellular vesicles was observed over time after the sample was deposited on the substrate. A comparison of vesicle sizes obtained by different methods showed that the hydrodynamic diameters (from NTA) were larger than the geometric diameters (from AFM).

The principal achievements and innovative results obtained during my doctoral work included: establishing a methodology for imaging extracellular vesicles with AFM, observing and modeling the increase in geometric diameters over time after sample deposition on the substrate, proposing an AFM–NTA scaling factor as a characteristic of a given vesicle type, observing and modeling the relationship between the increasing contact angle and the related height / radius of curvature (H/R_c) ratio for smaller

vesicles, and proposing a rapid procedure for estimating the bending modulus based on vesicle geometry and a limited number of force spectroscopy measurements. It is hoped that this work will contribute to enriching the methodology for studying nanovesicles using AFM and NTA and to a better understanding of the properties of extracellular vesicles.

Keywords: extracellular vesicles, liposomes, atomic force microscopy, AFM, nanoparticle tracking analysis, NTA, bending modulus