Recently, we described optimized process of gradual hemolysis for isolation of erythrocyte ghosts from slaughterhouse blood [1], which can be used for their drug loading as well. Future studies regarding the encapsulation of drugs in the ghosts will require powerful microscopy technique, such as atomic force microscopy (AFM), to provide information of relevance to cellular biophysical chemistry research. For this reason, two drying methods were examined to optimize the preparation of bovine and porcine erythrocyte and empty ghosts for AFM observation.

In order to preserve lipid and protein composition of erythrocytes and isolated ghosts, the samples were prepared for AFM by introducing next protocol: 500 µL of erythrocytes and ghosts were allowed to settle onto poly-L-lysine-coated cover glasses at 4°C overnight. Samples were fixed in 2% glutaraldehyde in PBS and 2% osmium tetroxide for 2h each, washed and dehydrated through a graded series of ethanol solutions (10, 30, 50, 70, 95 and 100%) for ten minutes each. The second procedure differed in adding dehydration step consisting of three times washing in acetone. AFM measurements were performed in semi-contact mode at room temperature and under ambient conditions, using the NTEGRA Prima system from NT-MDT (NT-MDT Co. Moscow, Russia). NSG01 probes from NT-MDT with a typical tip curvature radius of about 6 nm and a typical force constant of 5 N/m were used.

For both samples of erythrocytes and ghosts, the preparation procedure without final dehydration in acetone, could not allow AFM observation. Possibly, remained water induced erythrocyte membrane skeleton thermal fluctuation and/or it was deformed with the force applied by the AFM probe [2]. AFM topography images of dried bovine samples (Fig. 1 a, b) prepared with second procedure confirmed the results on the morphology and size of ghosts obtained by FE-SEM [1]. Fig. 1 (c, d) showed the altered surface texture with invaginations in porcine ghosts as well, probably formed due to isolation process. Besides, AFM of porcine ghosts revealed the fraction of lipoprotein deposits having diameter of ~200 nm (Fig. 1 (e, f)). These fractions were not observed in the sample of bovine ghosts. Successfully demonstrated preparation procedure for ghosts AFM observation will further provide more significant qualitative and quantitative AFM measurements of single ghost mechanics, especially drug loaded ghosts, by acquisition of force-deformation profiles and extraction of Young’s moduli.


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Fig. 1: Three-dimensional AFM images of erythrocytes and resulting ghosts attached to glass surfaces: (a) erythrocytes from bovine and (c) porcine slaughterhouse blood (b) resulting bovine and (d) porcine erythrocyte ghosts (e) fragments of lipoprotein nature derived from porcine erythrocyte ghosts (f) enlarged fragments given in (e)