The electron microscopy and atomic-force microscopy study of the structure of cells VERO, RK-13 and human fibroblasts infected by the viruses of Togaviridae family.

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The Atomic Force Microscope (AFM) is the instrument that allows obtaining the images with very large dynamic range, much higher than in optic or electron microscopes. This instrument is used now for investigation of the biological microobjects like DNA, proteins viruses etc. At the same time we consider as very perspective approach to use the AFM in combination with the electron microscopy (EM) for cytological studies.

The aim of our work is to examine the structure of the cells VERO, RK-13 and human fibroblasts infected by the viruses of Togaviridae family (VEE, rubella virus and else) under the different conditions of fixation of the samples for AFM and with there observation in different media (water or air); more over we tried to apply both EM and AFM techniques for examination of ultra fin sections of cellular samples

Materials and Methods: The cell cultures VERO, RK-13 and fibroblast culture and the same cultures infected by the viruses were fixed in different times using 0,2 or 0,02% of glutar aldehid in water. After the fixation the samples were stored in Tris buffer or were passed through the series of alcohol solutions of 50, 70, 96 and 100% and than were stored at 4%. We used the standard test glaces with cells cultures; the observations were done using Solver-Bio equipment (NT – MDT). The samples were unstained. The ultra fin sections were prepared in Arladit medium according to standard method. For the scanning of samples we used contact mode and taping mode of the microscope that involves the superposition of modulation over the SPM scanning system.

The results of the AFM examination revealed that the cells surface is almost identical on fixed and non-fixed samples, except for 0,2% glutar aldehid fixation that produces more granulated surface. The control samples differ from the infected ones by more round cells shape and by increase of granulation of the surface. We were unable to detect in any case the budging of the viruses in spite of the fact that this process was observed on some cells by EM. The AFM analysis of the ultra fin sections allows distinguishing the main cellular organelles like nucleus or cytoplasm but we were not able to identify the zones of virus reproduction. We are working now on adjustment of the AFM technique to obtain higher resolution of the cellular samples.