Reliable protocol for sample preparation to observe nanomaterial adherence to the surface of biological cells by using scanning electron microscopy.

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Background. When assessing the interaction between nanomaterials and cells, an important step is to image the effects induced by the material to the cell membrane. In order to evaluate the results of the interaction process, scanning electron microscopy (SEM) is typically used. Commonly, an important step during the preparation of biological cell samples for SEM is represented by the critical point drying, which involves the replacement of the alcohol used for dehydration with an inert gas. This conventional drying method is not only hard to accomplish, but it can lead to sample destruction if specific parameters are not met. On the other hand, when assessing nanomaterials adhered to the cell membrane, the integrity of the cell is not necessarily important, so a little cell deflation doesn't affect the intended purpose of the evaluation. Here, we describe a simple and more cost efficient method to prepare biological samples for SEM imaging that preserves cell integrity and can be used to describe nanomaterials interaction with cell surface.
Methods. Cells were grown on sterilized silica chips, after which the evaluated nanomaterial was added to the cell culture media at least 24h for incubation. Afterwards, the samples were washed to eliminate non-adhered nanomaterials, fixed with glutaraldehyde and osmium tetroxide, and dehydrated with increasing concentrations of alcohol. The silica chips were then air dried in the biological safety hood and in vacuum, followed by a sputter coat film of 5 nm of gold. The samples were imaged with a scanning electron microscope.
Results. We were able to obtain well preserved biological cell samples, both with and without nanomaterials adhered to the cell membrane surface. Nanomaterials such as magnetic nanoparticles and magnetic nanowires were easily traceable on cell surface. Furthermore, the nanomaterials were clearly observed in the images obtained, while the cell surface was not affected by the drying process applied. Although the samples obtained using our method were characterized by a slight deflation of the cells, the morphology of the cells is well preserved and the method is suitable for the evaluation of the interaction between nanomaterials and cell surfaces.
Conclusions: We have described a novel cost efficient and easy to perform method for processing biological samples for SEM imaging that preserves cell morphology and can be used for analyzing nanoparticle and nanowires interaction with cell surface.
Keywords. SEM imaging, nanoparticles, nanowires, cell samples