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Cross-sectioning of adherent cells on thin plastic substrate for serial block-face imaging

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Background

The single-celled enteric parasite Giardia lambia (Figure 1A) uses a ventral disc [1], consisting of a spiral-formed microtubule apparatus, for adhesion to their host duodenal epithelium. For analyzing the phenotype of various mutations of the ventral disc proteins, we started with conventional thin sectioning of the parasites cultivated on thin plastic bottom of plastic culture dishes (Figure 1B). The complexity of the observed phenotypes prompted us to consider volume imaging for a more comprehensive analysis of the ventral disc morphology. Instead of performing an additional cultivation and processing for volume imaging, we tested, if the already available sample blocks, prepared with our standard protocol for thin sectioning (osmium tetroxide, tannic acid, uranyl acetate/UA-Zero, Epon) [2], were suitable for serial block-face (SBF) scanning electron microscopy (SEM) at sufficient resolution. To our surprise, the resulting data sets were of sufficient quality (Fig. 1C) to reconstruct the entire ventral disc microtubule apparatus of the parasite in 3D (Figure 1D), but limited in resolution. SBF SEM usually requires a high-contrast en bloc staining of the sample with various heavy metals and reduction of the charging by improving the electrical conductivity (e.g. conductive filler) or by charge compensation (e.g. gas injection). As a consequence, the time of sample preparation usually is much longer for SBF imaging than for standard thin section imaging. Moreover, the introduction of more heavy metals changes the ultrastructural appearance of the sample. With our study, we wanted to find a generic sample preparation workflow for adherent cells which provides sufficient contrast and image quality in SBF SEM.

Methods

For the experiments we used adherent HeLa cell cultures grown in plastic dishes which were equipped with small silicone inserts on their plastic bottom (ibid µ-dishes, with microinserts). To stepwise improve the image contrast and quality, we introduced various simple modifications to our standard embedding protocol: minimal resin embedding, addition of silver colloids, higher processing temperature during heavy metal treatment, higher concentration of osmium tetroxide, additional heavy metal incubation steps (osmium tetroxide, lead aspartate). Cells were embedded in situ on their substrate in Epon, extracted with a hot scalpel and mounted on an aluminum stub using conductive epoxy glue. After trimming to a size of 1x0.25x0.25

mm, SBF SEM was performed with a Teneo Volumescope at high and low vacuum conditions.

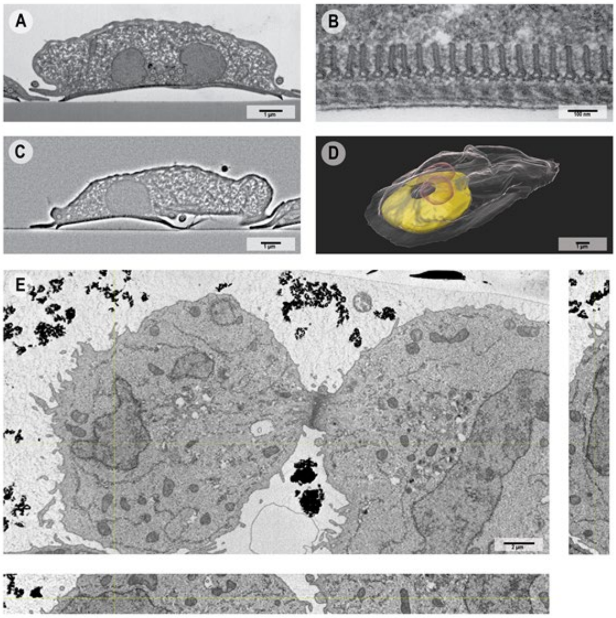
Results

The addition of further incubation steps with heavy metals to our standard sample preparation protocol resulted in improved resolution and image quality in block-face imaging. The contrast of the samples which were prepared with a single osmium tetroxide incubation and an additional en bloc treatment with lead aspartate (Figure 1E) allowed a proper discrimination of the main organelles and cytoplasmic structures already at low magnification and was very similar to the appearance of cells in thin sections taken from samples prepared by our standard protocol after on-section staining. The increased heavy metal load introduced by an additional osmium tetroxide incubation provided slightly higher lateral resolution at the cost of a rather unusual contrast. SBF imaging of adherent cells at low vacuum resulted in more stable image series at higher resolution in all directions than at high vacuum, regardless of the heavy metal load. The addition of silver colloid improved only the imaging at high vacuum with less significance at highest heavy metal load tested.

Conclusion

Our cross-sectioning workflow for SBF SEM of adherent cells on plastic substrate offers the possibility of direct investigation of cell-substrate interactions. The upright mounting of the sample allows us to collect complete volumes of many cells and large total sample volume. Finally, we could find a protocol which provides comparable ultrastructural appearance than in conventional thin section EM and with only limited additional effort compared to our standard embedding protocol.

Graphic:



Keywords:

conductivity, cell culture, sample mounting

Reference:

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