

Cryo lift-out technique to study host-pathogen interaction on cell monolayer

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Background:

Cryo lift-out technique is an advanced sample preparation technique for cryo-TEM microscopy. Here, a bulk from the sample surface is lifted out in a vertical orientation using a cryo-needle and thinned using FIB milling, allowing the study of tissue or thicker samples in TEM. In addition to that, the recent development of serial lift-out technique would allow us to understand the information along the volume of the sample. However, in cell biology research, traditional cryo-FIB that yields horizontal lamella is used, which poses a bottleneck to study the vertical structures in the cells (missing wedge issue). Lift-out is not possible on cell monolayers because of the smaller thickness of the samples. Therefore, we developed a sample preparation technique using hydrogels to grow cell monolayers, followed by high-pressure freezing (HPF) and then lift-out to study the vertical (apico-basal oriented) structures in cells.

Methods:

Optimized hydrogel preparation is vital for performing lift-out on cell monolayer. Here, we used acrylamide gels that have better water content which suits cryo-microscopy. HUVEC cells are used as a model because of our interest in studying a novel (unpublished) actin cytoskeleton structure that is formed from the apical side of the cell to the basal (through the cell volume), due to an extracellular bacterial infection. The hydrogel-Cell-Bacteria sample is frozen using HPF technique, and the lift-out and FIB thinning were performed on Aquilos-2 FIB SEM. Tomograms were acquired using Glacius or Titan TEM.

Results:

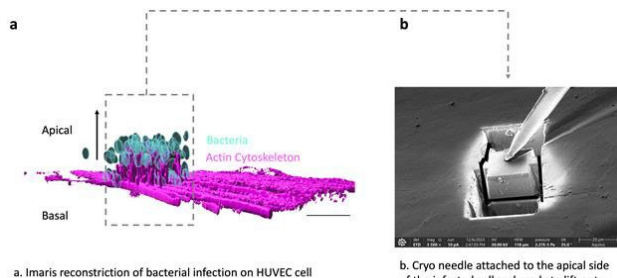
Hydrogel preparation, including parameters of polymer concentration, height, and width of the gels, is optimized. The lift-out, which is a completely manual process, has been semi-automated with the help of the IFLM module to perform correlation microscopy using the Maps software. At present, we are replacing the half-moon grids with a rectangular track to perform serial lift-

out with the cell samples. The ongoing efforts are to obtain the region of interest with the help of Cryo-CLEM and IFLM techniques to build tomograms.

Conclusion:

Cryo-EM's contribution to cell biology research provides a huge potential to understand the proteins' structures, arrangements, and their microenvironment in their native state. On the contrary, apico-basal polarity and corresponding structures are common in cell biology. For instance, structures such as cell-cell junctions, intestine villus, pedestals, podosomes have specific functions and unique orientation. To study these structures at their native orientation at TEM is physically not possible, due to its alignment along the beam path and missing wedge issue. Therefore, we have developed a sample preparation technique that would allow the researchers to use cell monolayers to perform lift-out techniques to study the vertically aligned structure in a horizontal lamella.

Graphic:



Keywords:

Cell monolayer, Hydrogel, HPF, Lift-out

Reference:

Reference:

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