CONVENTIONAL CHEMICAL FIXATION OF MAMMALIAN CELLS FOR IMMUNOFLUORESCENCE LABELLING LEADS TO SIGNIFICANT ULTRASTRUCTURAL CHANGES

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It is known that chemical fixation with formaldehyde and glutaraldehyde at room temperature leads to changes in the cellular ultrastructure. Various constituents of the cell are not optimally preserved. These well-known preparation artifacts are still mostly neglected even after the introduction of super-resolution light microscopy techniques. We believe that the sample preparation must be reviewed and addressed not only for conventional light microscopy, but especially for super-resolution microscopy. Morphological changes of the ultrastructure of the sample becomes a relevant issue for super resolution imaging. To develop an alternative fixation technique that meets all the requirements for maintaining the ultrastructure in a near native state while still allowing immunofluorescence labelling, we investigated the ultrastructure of cells after standard chemical fixation for light microscopy using transmission electron microscopy. We cultured adherent cell cultures on sapphire disks and fixed them by 4% formaldehyde and 0.05 – 0.1% glutaraldehyde, the commonly used protocols for immunofluorescence labelling. To assess the ultrastructure by transmission electron microscopy without subsequent morphological changes of the cells, we immobilized these chemically fixed samples by vitrification using high-pressure freezing followed by freeze-substitution and Epoxy resin embedding. This procedure is considered least susceptible to artifacts for analysis of the ultrastructure in transmission electron microscopy at room temperature and allows assessing artifacts of the chemical fixation protocol [1]. We could show that fixation using formaldehyde leads to severe nucleo- and cytoplasmic extraction and disruption of various organelles. In particular, mitochondria showed a considerable morphological deviation from cryo-prepared samples after formaldehyde fixation, i.e. selective swelling, extraction of cristae and local ruptures of its membrane. Our findings indicate that even smallest amounts of glutaraldehyde in addition to 4% formaldehyde exert a significant favourable effect on the morphological maintenance of mitochondria, other organelles, and components of the cytoskeleton. Since aldehydes generally bind to terminal amino groups, they do not react with undenatured nucleic acid, carbohydrates, or lipids. Thus, in conventionally fixed samples, proteins are fixed while leaving a large part of other cellular constituents unfixed [2]. These conventional fixations do not meet the ultrastructural preservation at a degree in the low nanometer range, that is accessible using super-resolution microscopy techniques. At present, there is no satisfactory protocol which solves the fixation issue for super-resolution light microscopy. For this reason, we are investigating potential solutions such as substituting primary fixation with high-pressure freezing and freeze-substitution, followed by rehydration to enable immunofluorescent labelling, while enhancing the preservation of the ultrastructure.

[1] J. Dubochet, "The physics of rapid cooling and its implications for cryoimmobilization of cells," *Methods Cell Biol.*, vol. 79, pp. 7–21, 2007.

[2] R. A. Fleck and B. M. Humbel, *Biological Field Emission Scanning Electron Microscopy*. Wiley & Sons, 2019.