“On-Profile” Cryo-Sectioning and Immunolabeling

Tips & Tricks for Preparation of Monolayers Grown on Support Membranes

Many methods have been described for the preparation of cultured cells for immuno-gold labeling and further investigation by Transmission Electron Microscopy (TEM). However, it is widely accepted that the antigenicity of most molecules is often compromised by the chemicals used during the processing of such samples for TEM. Furthermore, embedding material in resins can limit access to antigen binding sites by antibodies.

By using Tokuyasu’s technique [1,2] extensive chemical treatments can be avoided and antigenicity not compromised, additionally, the approach does not involve resin infiltration proffering greater access to antigen sites by labeling antibodies. In brief; Tokuyasu technique requires the sample to be lightly fixed, cryo-protected, frozen, cut at low temperatures (-80°C to -110°C) and thawed before subsequent immunolabeled with antibodies tagged with colloidal gold.

However, when the sample is a monolayer of cells grown on a membrane support and it is essential that the orientation of the cells be maintained a new series of obstacles must be overcome. Maintaining the three-dimensional organization of a monolayer is key for many experimental systems (i.e. studies on transport across membranes or on cell polarity, etc.), thus, the orientation in which the sample is sectioned is critical. In this report we describe a simple method to prepare such samples when the orientation of the samples is paramount to the topic under investigation.

Sample Preparation

A monolayer grown on a support membrane is fixed with 4% paraformaldehyde, 0.1% glutaraldehyde in 0.1M phosphate buffer pH 7.2, thoroughly rinsed with phosphate buffer and cut out of the plastic well frame. The free membrane is now ready for sandwich-embedding (fig. 1A). First, prepare a glass slide by covering it with a piece of thin flexible Aclar film (Agar Scientific, UK). Then aliquot a generous amount of 12-20% gelatin in PBS (melted at 37°C) on top of the Aclar. Working quickly to avoid setting of the gelatin, transfer the entire membrane onto the gelatin and sink it gently until it is suspended within the gelatin pool.
In order to remove the excess of wash buffer before sinking the membrane inside the gelatin it is recommended to touch the edge of the membrane onto filter paper. Use a second piece of Aclar film to cover the gelatin and finally use another glass slide to sandwich it all (fig. 1B) Press gently so the whole assembly sets flat and allow setting at 4°C for at least 1 hour. After solidification, remove the top and bottom glass slides and carefully peel off the two sheets of Aclar. Using a very sharp scalpel or a razor blade cut small blocks of gelatin including small portions of the monolayer membrane (1-2 mm cube) (fig. 1C) and cryo-protect by immersion in a 2.3 M sucrose solution. Incubate in sucrose for at least 24 h at 4°C. After cryo-protection, the gelatin blocks containing the membranes are mounted on aluminum cryo-pins in such way that the profile of the membrane is perpendicular to the sectioning plane. Mounted samples can now be cryo-fixed by plunging into liquid nitrogen and stored in liquid nitrogen until ready for cryo-sectioning. This method has been optimized to generate samples that have to be cut “on-profile”. Given the undulated nature of the support membrane, “on-face” sectioning of the monolayer will be very difficult since only very small areas of the membrane will be perfectly flat when mounted on the cryo-pins (fig. 2).

References

Authors & Affiliation
Gema Vizcay-Barrena and Roland Fleck

1 Centre for Ultrastructural Imaging, Kings College London, London, UK

Contact
Gema Vizcay-Barrena
Centre for Ultrastructural Imaging