LR White sections as a support films for transmission electron microscopy; further applications

Jan Hobot and Chris von Ruhland

Electron Microscopy Unit, Central Biotechnology Services, School of Medicine, Cardiff University, Cardiff, U.K.

Email, hobot@cf.ac.uk
Keywords: support film, LR White, transmission electron microscopy

Support films for transmission electron microscopy have been prepared from a number of materials including formvar, celloidin, carbon and silicon nitride. In the case of films prepared from organic polymers, carbon coating is essential. We have recently demonstrated the utility of LR White acrylic resin sections as slot grid support films for the uninterrupted examination of thin sections of biological material by transmission electron microscopy. In particular, we emphasized the robustness of the film, even without carbon coating, compared to commercially available, carbon-coated alternatives [1]. The resin support film not only allowed ultrastructure to be viewed clearly, but demonstrated its immense stability when epoxy resin sections were immunolabelled with colloidal gold following prolonged treatment with sodium periodate. In these experiments, the grids were immersed for long periods in various reagents. No adverse effects were observed on both acrylic or epoxy sections, and very low background counts recorded [1].

Here we describe further applications of this simple technology for negative staining and for analytical electron microscopy.

Grids with a resin support film were prepared as follows. Hard grade LR White acrylic resin was heat polymerized at 60°C for 24 hours. The block was trimmed to a trapezium, measuring approximately 1.5 x 2.5 mm, and 60nm thick sections were cut with either glass or diamond knives and collected onto 150 mesh gold or nickel grids.

For negative staining exponentially growing cells of the bacterium *Pseudomonas aeruginosa* were used. Grids were incubated on drops of the following solutions: bacterial culture, 1% glutaraldehyde (in nutrient broth), nutrient broth (x3), distilled water (x6), 4% uranyl acetate, air dried.

For analytical electron microscopy, exponential cultures of *P. aeruginosa* were prepared, the controls left untreated, and silver nitrate added to others. Embedding was in LR White, and sections were picked up on the support grids described above. X-ray microanalysis was performed on both. To further investigate the utility of LR White support films, gadolinium-containing iron oxide nanoparticles were suspended by ultrasonication in distilled water and air-dried onto the LR White support film.

Negative staining of bacteria, and other specimens, has been done traditionally on grids having either a formvar/celloidin film coated with carbon or carbon alone. Excellent results have been obtained, including bacterial flagella [2]. Using the resin support grids, this excellence is maintained (Fig. 1). Additionally, in comparison to traditional coated grids, the resin support grids remain very stable and do not tend to break during the extensive procedures of negative staining, where the grids are passed from solution droplet to solution droplet.

A problem associated with X-ray microanalysis is the stability of the specimen and that of the embedding resin when subjected to various spot sizes, sometimes at quite high magnifications. The resin support grids are ideal, for they provide both added stability and a low background signal, such that spectra from untreated and treated cells of *P. aeruginosa* can be analysed, with silver deposits being detected in the treated cells. These results were identical with earlier data obtained from similar cells, but where the sections had been mounted on carbon coated celloidin films [3]. In the case of metal nanoparticles, the support film remained stable and intact throughout the analysis even when X-ray signal acquisition times were prolonged.

Grids with a resin support film are therefore ideal to use for all manner of electron microscopic techniques. They are stable under a variety of conditions within the microscope, they are hardy to various chemical reagents, withstand constant handling, and are easy to prepare. One or two blocks

of LR White resin will provide a practically endless supply of material for films, and, importantly, where budgets are tight, are very much cheaper than their commercial counterparts!

References

[1] von Ruhland, C. J. and Hobot, J. A. Journal of Microscopy **240** (2010), p130-134
[2] Hobot, J. A. in “Molecular Medical Microbiology”, ed. M Sussman, (Academic Press, London), p7-32.
[3] Hobot, J. A. et al Journal of Electron Microscopy **57** (2008) p67-75.

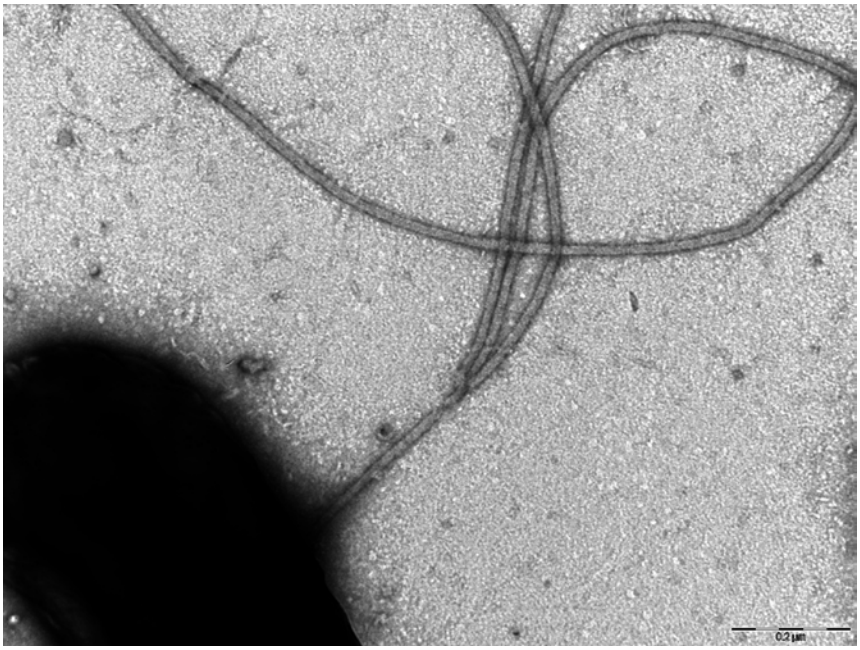

Figure 1. *Negative staining of P. aeruginosa showing bacterial flagella. The technique was performed using grids with a resin support film. Scale bar is 0.2 microns.*