

Application Note

Leica EM TP – Embedding of murine fibroblasts grown on Aclar plastics for TEM

Courtesy of: Siegfried Reipert, Department of Molecular Cell Biology
Mac F. Perutz Laboratories; University of Vienna
A-1030 Vienna, Dr. Bohr-Gasse 9

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Leica EM AMW Application Note

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Tissue culture

Epithelial cells (PtK2) or fibroblasts (3T3) were grown in DMEM at 37°C and 8% CO₂. They were plated either on glass coverslips, 12 mm in diameter, or on Aclar plastics (EMS, Science Services, Munich, Germany) of the same size. The substrata were cleaned by HCl treatment for 2 h and subsequent washing in distilled water and 70% ethanol. Before use, they were incubated in culture medium at 37°C over night.

Prefixation

Prefixation was performed outside of the TP, either for 30 min with 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3 at room temperature, or by microwave fixation in 0.5% glutaraldehyde, as described in detail elsewhere (Reipert et al., 2008).

Use of the TP for Postfixation, Dehydration and Embedding in Epoxy Resin

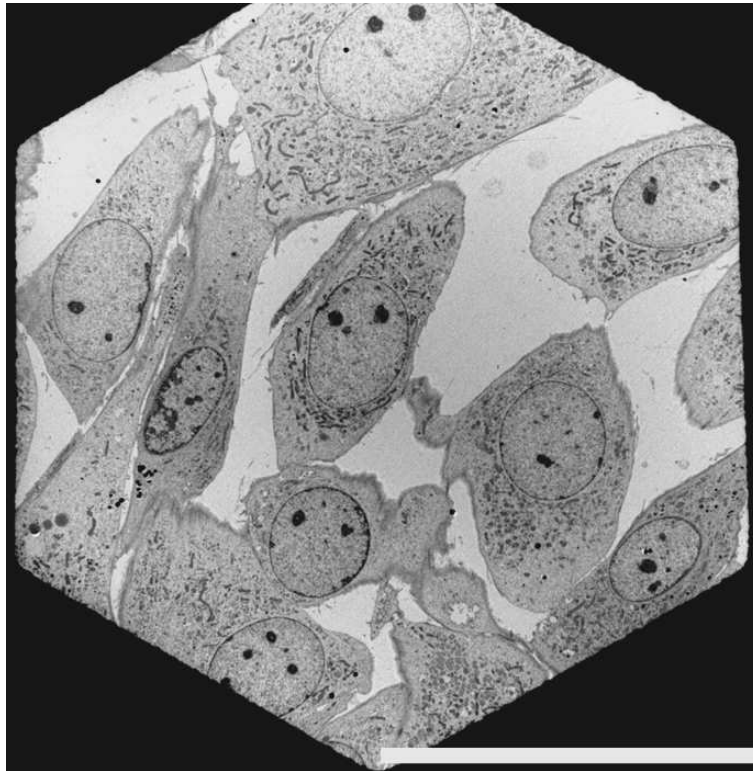
Subsequent processing steps for epoxy resin embedding were performed or in an automatic tissue processor (LEICA Microsystems, Vienna, Austria). Four baskets (large size, 3 divisions) of the tissue processor were arranged in pairs of two at the stem assembly to give space for the uptake of 6 coverslips in vertical position with the cells facing towards the stem. If necessary, further 6 coverslips might be added by placing them with their back sides opposite to the coverslips already loaded.

Postfixation with 0.5% OsO₄, washing in cacodylate buffer, dehydration in a series of ethanol, mediation of the resin infiltration by propylene oxide, as well as sample infiltration in mixtures of propylene oxide and Agar 100 (Agar Scientific, Cambridge, UK) were performed according to the following protocol:

Programme step/ vial	Time	Reagent	Agitation	Temperature
1	5 min	0.1 M cacodylate buffer, pH 7.3	3	20 °C
2	5 min	0.1 M cacodylate buffer, pH 7.3	3	20 °C
3	5 min	0.1 M cacodylate buffer, pH 7.3	3	20 °C
4	45 min	0.5% OSO ₄ in 0.1 M cacodylate buffer, pH 7.3	1	20 °C
5	10 min	0.1 M cacodylate buffer, pH 7.3	3	20 °C
6	10 min	0.1 M cacodylate buffer, pH 7.3	3	20 °C
7	10 min	0.1 M cacodylate buffer, pH 7.3	3	20 °C
8	10 min	30% ethanol	3	20 °C
9	10 min	50 % ethanol	3	20 °C
10	10 min	70 % ethanol	3	20 °C
11	10 min	95 % ethanol	3	20 °C
12	10 min	100% ethanol	3	20 °C
13	10 min	100% ethanol	3	20 °C
14	10 min	propylene oxide	3	20 °C
15	10 min	propylene oxide	3	20 °C
16	15 min	Agar 100 : propylene oxide 1:3	3	20 °C
17	15 min	Agar 100 : propylene oxide 1:2	3	20 °C
18	45 min	Agar 100 : propylene oxide 2:3	1	20 °C

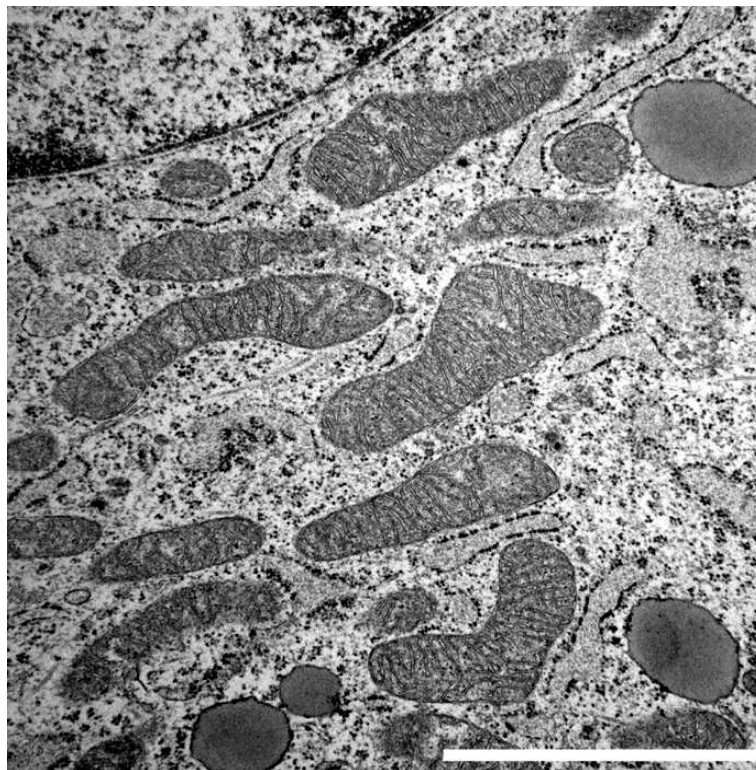
Subsequently, the coverslips were taken out of the baskets for flat embedding in pure resin Agar 100 (Agar Scientific, Cambridge, UK). Cells were infiltrated in a drop of resin for 2 h or over night. After infiltration, an Eppendorf tube with the bottom and lid cut off was placed above the sample. After an initial heat polymerization at 60°C for 3 h the Eppendorf tube was filled up with resin and polymerization was continued over night.

Reipert S, Kotisch H, Wysoudil B, Wiche G. (2008) Rapid Microwave Fixation of Cell Monolayers Preserves Microtubule-associated Cell Structures. J Histochem Cytochem. Apr 14. [Epub ahead of print]



Bar, 50µm

Fig. 1: MW-fixed and TP-embedded 3T3 fibroblasts (overview)



Bar, 2 µm

Fig. 2: MW-fixed and TP-embedded 3T3 fibroblasts (detail)