Abstract

The development of new technologies and machinery has made advances in many industries. These advances are becoming commercially available to the standard electron microscopy facility. We set out to determine if one of these aids, the Poly III, available from Electron Microscopy Sciences, was an efficient replacement of hand processing.

Materials and Methods

Mouse kidney and muscle tissues were removed in cubes of approximately 1.0 mm³ and fixed for 24 hours with 2.0% paraformaldehyde, 2.5% glutaraldehyde (Electron Microscopy Sciences, Hatfield, PA) in 0.1 M Sodium Cadyculate buffer (pH = 7.4). After rinsing in 0.1 M Sodium Cadyculate buffer, the tissues were postfixed in 1% OsO₄ (Steven’s Metallurgical, New York, NY) in 0.1 M Sodium Cadyculate buffer followed by washing in distilled water and en bloc staining in 2% uranyl acetate (SPI, West Chester, PA). Dehydration was carried out using a 30–100% graded ethanol series, followed by two changes in propylene oxide.

The tissues were separated into those for hand processing and those for the Poly III. Hand processed tissues were placed into a 1:1 resin:PO mixture, followed by 3:1, then 3 100% resin changes. Poly III processed tissues were placed into individual BEEM capsules in 1:4 resin:PO mixture, and put into the machine using Program #3, which is designed for use with Propylene Oxide as the intermediate solvent. All samples were embedded in LX112 resin (Ladd Research Industries, Burlingotn, VT) and polymerized in a 60° oven.

Ultrathin sections (80 nm) were obtained with a diamond knife (Diatome, Hatfield, PA) on a Leica UC7 (Leica Microsystems, Wetzlar, Germany), stained with uranyl acetate-lead citrate, and observed with JEOL 1200EX and JEOL 1400Plus transmission electron microscopes (JEOL USA, Peabody, MA) at an accelerating voltage of 80 kv.

Results

Samples were compared via ease of sectioning and morphological appearance in the electron microscope [1]. All blocks sectioned without wrinkles and easily formed ribbons in the boat. Kidney morphology was evaluated using the following items: red blood cell smoothness, podocyte morphology, slit membrane visibility, ribosome visibility, and mitochondrial cristae membrane definition. Muscle morphology was evaluated using the following: t-tubule system visibility, mitochondrial cristae membrane definition, muscle fibre visibility and Z-banding.

As shown in Figures 1 & 2, the kidneys are well preserved and infiltrated, the podocytes are well defined with an easily discernible plasma membrane and the slit membrane is visible in both hand and machine embedded samples. Figures 1 & 2 show the similarities between the hand and Poly III processed muscle tissues. The muscle tissue shows well preserved structure in the mitochondria and the t-tubule system is clearly visible. There are no visible morphological differences between hand and machine processed kidney and muscle tissues.

An additional advantage of the Poly III unit is a significant reduction in reagent volume per sample. Table 1 shows that the volume of resin needed per hand processing sample, including filling the BEEM capsule prior to polymerization, increases rapidly as the number of samples increases. The Poly III system removes the intermediate solvent from the sample without the need to make multiple resin changes. The Poly III only requires 500ul of solution per BEEM capsule. The resins used in electron microscopy are toxic and may cause skin irritation, so reducing exposure to the researcher may be better for the health of the technician [2].

<table>
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<th>Hand Processing</th>
<th>Poly III Processing</th>
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| Infiltration volume + BEEM capsule volume = total volume
| 5 samples 115 ml + 75 ml = 190 ml
| 10 Samples 135 ml + 75 ml = 210 ml
| 20 Samples 270 ml + 150 ml = 420 ml |
| Infiltration volume + BEEM capsule volume = total volume
| 7.5 ml + 75 ml = 82.5 ml
| 15.5 ml + 150 ml = 165.5 ml
| 10.5 ml + 150 ml = 160.5 ml

In conclusion, the Poly III is a suitable replacement for hand processing of murine kidney and muscle tissue, saving reagents and time for the electron microscopy facility. The tissues chosen for this experiment were two readily recognizable samples that are routinely processed by hand and evaluated in many EM facilities. The machine processed samples were adequately infiltrated with the standard PO program in the Poly III. This machine does allow for time, temperature, and vacuum levels to be adjusted by the end user, allowing a variety of customized programs that can be added to the standard programs supplied with the unit. This variability allows the end user to optimize the program for the tissue of interest.

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References


Conclusions