Fast, Walk-away, Automated Processing of Mammalian Tissue for LM and TEM

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Four types of mammalian tissue; kidney, liver, skeletal and cardiac muscle, were processed for transmission electron microscopy (TEM) using the mPrep ASP-1000 automated specimen processor (ASP). For comparison, tissue samples were also prepared using conventional manual reagent exchanges in vials with rotator agitation. Both ASP and manual processing produced well-prepared tissues.

Mammalian tissues were perfusion-fixed on site with buffered 4% paraformaldehyde and 1% glutaraldehyde. Then, ~2 mm tissue samples were excised and oriented in mPrep/s capsules in the electron microscopy lab. Thereafter, each tissue sample remained in its own labeled capsule throughout all subsequent processing. The capsules, in sets of eight, were attached onto the ASP robotic arm (Figure 1). The ASP robotic arm then automatically moved capsule-loaded specimens to each sequential reagent arrayed in standard trough plates (Figure 1). The ASP then aspirated (pipetted) each reagent into the capsules with rapid agitation provided by aspiration and dispense cycles as quick as 2 seconds per reagent exchange. Tissue processing typically required 130 minutes after attaching capsules to the ASP until epoxy infiltration was complete. Processing time was further reduced with some tissues to only 45 minutes. Resin curing was performed by transferring epoxy filled mPrep/s capsules to the curing oven. Sectioning was accomplished by directly mounting the mPrep/s capsules into the microtome chuck, thus preserving orientation and specimen labeling.

Once inserted into mPrep/s capsules and loaded onto the ASP, muscle samples were washed in cacodylate buffer, while liver and kidney samples were washed in phosphate buffer. All samples were then post-fixed in 1% osmium tetroxide in the corresponding buffer. Samples were then washed in distilled water and dehydrated using graded ethanols, transitioned into acetone and embedded into Embed 812. Once infiltrated, the mPrep/s capsules (with tissue specimens) were removed from the ASP and cured overnight at 60°C. One micron sections were obtained for light microscopy and 70 nm sections were obtained for TEM. Sections were negative stained with 8% uranyl acetate in 50% ethanol and Reynolds lead citrate. OsO_4 post-fixation, dehydration, solvent transition, and resin infiltration were confirmed to be complete using light microscopy of toluidine blue stained 1 μm sections (not shown). No artifacts due to incomplete processing were observed. A Philips CM120 Electron Microscope with a BioSprint camera running AMT Capture Engine V700 was then used to image the 70 nm sections.

Summary: Ultrastructural preservation was publication quality with both ASP prepared and manually prepared comparison samples. With the comparison samples prepared in scintillation vials, only one sample could be processed at a time to achieve a reagent exchange rate that could approach the speed of the ASP. All samples were cured and sectioned using the same methods. ASP prepared kidney, liver,

and skeletal muscle illustrate excellent preparation quality (Figures 2-4), as did cardiac muscle (not shown due to space limits.) The ASP sample preparation was much easier than conventional processing because the process was automated and since specimens were never removed from the mPrep/s capsules, thus eliminating messy and error-prone handling. Additionally, reagent processing was achieved in ~2 hours or less, compared to typical day-long conventional reagent exchange protocols.

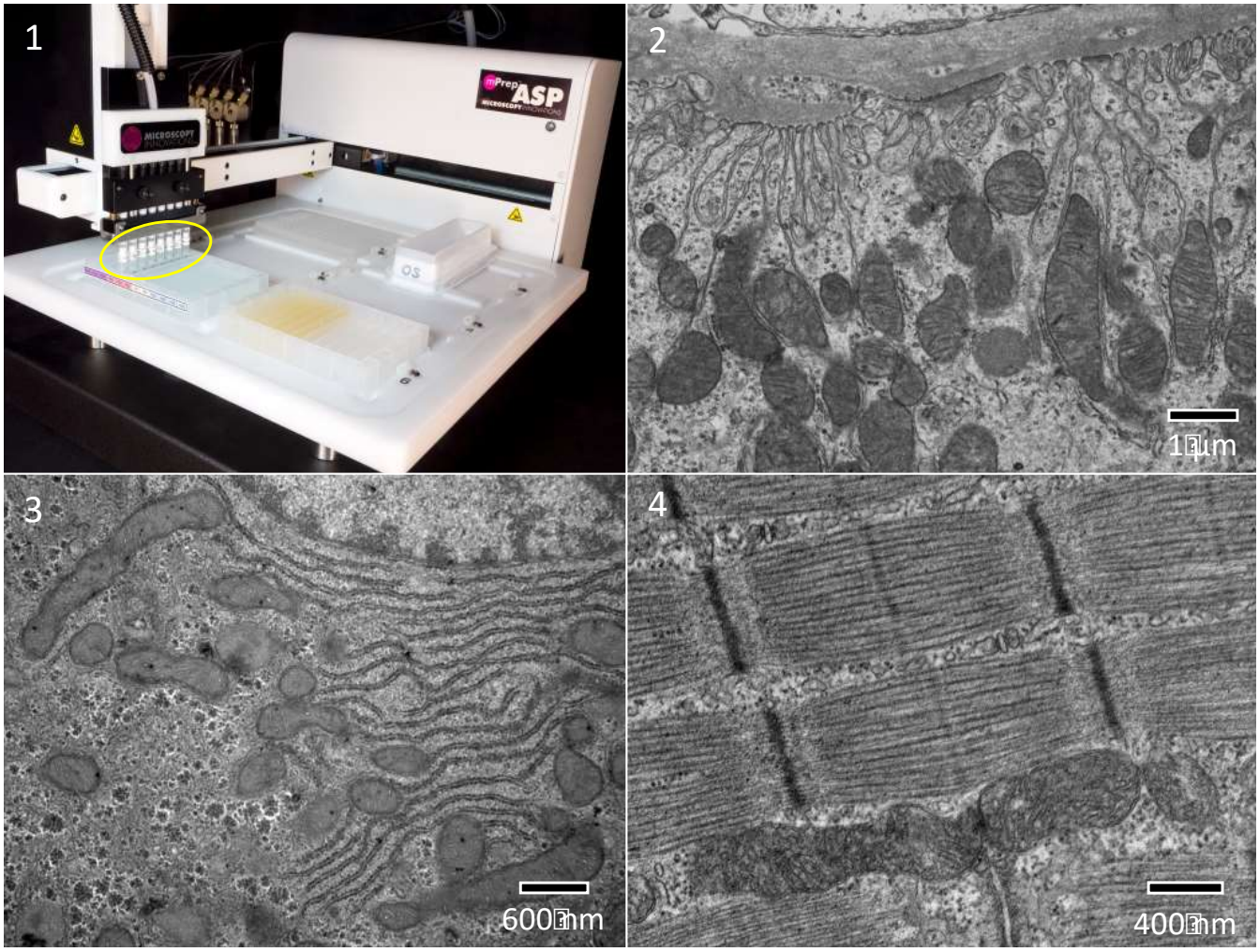

Figure 1: ASP-1000 automated processor with tissue specimens in mPrep capsules on robotic arm (circled). Reagents are drawn into capsules from 12-row trough plates and reservoirs on the platform.

Figure 2: Proximal convoluted tubule cell in rat kidney along the cells basolateral side shows invaginating membrane folds with many long mitochondria. Note fine mitochondrial detail including outer mitochondrial membranes and cristae.

Figure 3: Rat liver hepatocyte in the sinusoid shows well preserved layers of rough endoplasmic reticulum, glycogen alpha particles (electron dense rosette-like clusters) mitochondria and a portion of the cell's nucleus. Note well-preserved nuclear envelope, outer mitochondrial membrane and cristae.

Figure 4: Rat gastrocnemius (skeletal) muscle shows a myofibril with sarcomeres along with subsarcolemmal mitochondria and sarcoplasmic reticulum. Clearly seen are the sarcomere Z-lines, M-lines and mitochondrial cristae.