Preparation of Renal Tissue

Tips & Tricks for Viewing on the Transmission Electron Microscope Using a Mirror

The Centre for Ultrastructural Imaging (CUI) at King’s College London has been preparing renal tissue for viewing by transmission electron microscopy (TEM) as part of the renal pathology service for over ten years. TEM remains an important diagnostic tool in renal pathology, indeed, guidance issued by the UK’s Royal College of Pathologists cites and supports the Association of Clinical Pathologists Best Practice No. 160, which states that ‘Many respondents [to a review of laboratory practice in renal pathology] expressed the opinion that to carry out evaluation of renal biopsy specimens without at least having the availability of electron microscopy is negligent’ [1].

There are different methods used for processing material to resin, Diagnostic Electron Microscopy edited by John W. Stirling, Alan Curry and Bryan Eden has a good explanation of the various methods [2]. The CUI uses phosphate buffer as cacodylate contains arsenic and a standardized protocol for the preparation of resin infiltrated tissue for TEM (tab. 1). Steps and processing times have been optimized to support a diagnostic EM workflow, where a biopsy is taken in a clinical setting the material divided for pathology services and for diagnostic TEM transferred to the CUI for subsequent processing. A biopsy undergoes primary fixation and transfer into the wash/holding solution within the Histopathology unit. Fixed samples are then mailed to CUI in wash solution. CUI undertakes processing from the secondary fixative stage. This necessitates that the protocol be robust and allow for periods, where material will be held prior to progressing to the next step in the processing workflow (tab. 1).

Over those years we have tried various techniques to simplify and “speed-up” the locating of glomeruli within renal tissue, the structure within which many of the critical diagnostic features are found. Using a Messacut2 Structure Viewer mirror (Leica Microsystems) when trimming resin blocks of renal biopsy speeds up locating of the area of interest. Trimming, rough cutting and messaring of the resin block on the same machine with glass knives also reduces preparation time (fig.
1). This has lead to the development of an optimal sectioning protocol.

Ideally the processed blocks are 1 mm in diameter (some variation may exist due to the use of different needles for taking the biopsy). Individual tissue biopsy of between 2 mm – 3 mm in length and 1 mm diameter are embedded longitudinally in a coffin mould so that any glomeruli seen are on the side of the block and thus not cut first. During processing the block is osmium fixed giving it contrast when cutting the solid polymerized block.

Once the tissue block is cured, excess resin is trimmed from the polymerized block with a single edged razor blade so the it sits flat in the ultra microtome chuck; the angle of the block in the chuck is adjusted so the tissue within is as flat as possible and perpendicular to the knife. In the trimming block the excess resin is trimmed off with a single edged razor blade; some resin is left surrounding the tissue so if the glomerulus is at the edge it will be supported on the grid when collected.
A glass knife is prepared and set up in the ultra microtome and manually advanced until cutting the block. The Messacut Structure Viewer (mirror) aligned so the real image and the mirror image are visible the eyepiece of the ultra microtome (fig. 2). Focusing on the mirror image the block is cut until the appearance of the structures of the tissue is visible in the image. Rough sections of 2 µm – 3 µm are cut moving to a new bit of knife when necessary checking the mirror image in the eyepiece following every 20 – 30 sections cut. An air-duster can be used to clear sections from view. Rough sections of the block are collected at regular intervals to confirm the interpretation of what is seen in the mirror and as proof that no glomeruli was found as sometimes happens. Rough sections are collected in a water drop on a glass slide, dried on 60°C hotplate until no water remains. The dried sections on the hot plate are covered with a few drops of Toluidine Blue Stain (1 % w/v, 5 secs) then rinsed with distilled water in a sink. The slide is then dried again before being mounted with a coverslip and styrolite. The stained slide can be viewed on a compound light microscope using a X40 lens.

Pathology textbooks describe the glomerulus as being between 50 – 150 µm in size though it is said to have a mean diameter of approximately 200 µm in an adult. The glomerulus has a circular to ellipsoid shape with “dots and lines inside” when viewed in the mirror on an ultra microtome or on a glass slide by light microscope. The outside of the glomerulus is often darker than the outside of the tubules surrounding it. Identifying glomeruli is critical to the pathological determination of underlining renal disease. Biopsy is a medically invasive procedure and as such the available tissue is limited and highly valuable. It is imperative for the patient that the smallest necessary biopsy is taken and that the best use of the material be made. Using the mirror during messaring allows the spotting of glomeruli in the block face as trimming progresses (fig. 2). The microtomist can then expand the block face to include additional glomeruli or to cut the more viable glomerulus therefore reducing the amount of ultrathin cutting required. Ideally a messared block face should be trapezoid to rectangular with the widest part at the bottom and longer than it is wide over coming compression when being cut on a diamond knife and maximizing the amount of tissue to be viewed in the TEM.

Sections are collected on 150 mesh copper TEM grids dipped in a weak pioloform/chloroform solution (0.12 g in 10 ml). The addition of the pioloform film makes the grids sticky and further supports the sections during post sectioning staining. The contrasting agents used by the CUI are Uranyl Acetate (0.25 g in 15 ml 50% Ethanol) and Lead Citrate (0.02 g in 0.1M NaOH carbonate free). The 150 mesh size grid gives a good compromise between the area of the tissue to view and the stability/support of the section under the electron microscope beam. Sometimes
the glomerulus is larger than the square size, which can be a visual prompt for the Pathologist when considering a diagnosis. The CUI has used the messa mirror with Reichert Ultracut E, Leica microsystems UC6 and UC7 microtomes and RMC Powertome Ultra microtomes and it is of use in preparing other types of tissue for TEM viewing.

References


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