Improved method for sequential study of an isolated glomerulus by scanning electron, transmission electron, and light microscopy

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The use of isolated glomeruli was first described by Arakawa in 1971,¹ and since then these have been employed by several workers²⁻⁹ for studying human glomerular diseases in the scanning electron microscope. As Ng *et al*⁹ pointed out, however, the use of scanning electron microscopy alone is of little diagnostic value for such study. This is because the surface changes of various disease states are not well understood and documented. So far, the information provided by scanning electron microscopy is complementary to transmission electron and light microscopy. In order to establish firm scanning electron microscopy diagnostic criteria for glomerular lesions, it would be most helpful if direct correlation of scanning electron microscopy observations with the well documented transmission electron and light microscopy studies on the same sample was possible for every biopsy specimen.

We have recently reported a method for the correlative study of an isolated glomerulus by scanning electron, transmission electron, and light microscopy.¹⁰ It is particularly suitable for percutaneous needle biopsy specimens, which are usually too small to provide adequate material for the various studies. An improved method is described below, in which a resin support is used instead of silver tape for holding the critical point dried glomerulus for scanning microscopy. It requires less time and offers the advantage of easy manipulation in the subsequent processing of the specimen for transmission electron and light microscopy.

Material and methods

PREPARATION OF RESIN SUPPORT

The resin support was prepared in advance by adding a thin layer of a 3:1 mixture of epoxy resin

and propylene oxide into a disposable aluminium dish or the cap of a plastic bottle. After overnight polymerisation of the resin in an oven at 70°C, the hardened resin slice was removed from its mould and small squares of convenient size (about 6 × 6 mm) were cut out. Each resin square was glued on to an aluminium stud and a small amount of colloidal carbon was applied to connect the resin and the stud for electrical conduction.

PREPARATION OF GLOMERULI FOR SCANNING ELECTRON, TRANSMISSION ELECTRON AND LIGHT MICROSCOPY

Kidney tissue from a normal Sprague-Dawley rat was employed. Fixation, isolation, and processing of glomeruli for scanning electron microscopy were carried out as described previously.¹⁰ In brief, several glomeruli were isolated from glutaraldehyde fixed kidney tissue using fine needles under a dissecting microscope. After osmication and buffer wash, they were pipetted on to a piece of Chinese lantern paper, which was then folded into a small package and processed for critical point drying. Each dried glomerulus was mounted on a resin support using a small amount of quick drying glue. The specimen was coated with gold-palladium and viewed in a Cambridge Stereoscan 150 scanning electron microscope at 20 kV.

Each resin support with the attached glomerulus was then removed carefully from the stud. The glomerulus was processed *in situ* on the resin for transmission electron and light microscopy according to the following schedule:

- 1 Absolute ethanol, 5 min.
- 2 Propylene oxide, 1 min.
- 3 Poly/Bed 812 resin-propylene oxide 1:1 mixture, 15 min.
- 4 Poly/Bed 812 resin-propylene oxide 3:1 mixture, 30 min.
- 5 Poly/Bed 812 resin, two changes in 30 min.

A 12 well porcelain plate was used for the above process. After adequate infiltration each resin support with a glomerulus on top was embedded in a thin layer of fresh epoxy resin in an aluminium dish. Alternatively, a gelatin capsule was filled with epoxy resin and inverted over the infiltrated glomerulus. The resin was then polymerised in an oven at 100°C for 1½ h. Ultrathin sections were prepared subsequently, double stained with uranyl acetate and lead citrate, and examined in a Philips 300 transmission electron microscope at 60 kV. Semithin sections were cut immediately after ultrathin sectioning. They were stained with toluidine blue or other special stains for light microscopy.

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Fig. 1

Fig. 2

Fig. 3

Fig. 1 Surface topography of a glutaraldehyde fixed isolated glomerulus shown by scanning electron microscopy.

Fig. 2 Transmission electron micrograph of the glomerulus shown in Fig. 1 showing good preservation of glomerular features. Original magnification $\times 1140$.

Fig. 3 Semithin section stained with 1% toluidine blue to show the light microscopic appearance of the same glomerulus. Original magnification $\times 970$.

Results and discussion

The preparation of the resin support is simple. A 3:1 mixture of epoxy resin and propylene oxide was used instead of pure epoxy resin to facilitate the spreading of the otherwise viscous resin in the aluminium dish. With the evaporation of the volatile propylene oxide in the oven, a thin uniform layer of resin was formed after its polymerisation. Mounting the critical point dried glomerulus on the resin support was not difficult, but it should be done with care. It is important to use only a small amount of quick drying glue to fix the glomerulus, as excess glue tends to cover the glomerular surface. Excess glue will ruin the preparations by obscuring the surface ultrastructure and impede infiltration of the glomerulus by epoxy resin in subsequent processing.

Figs. 1–3 show a single glomerulus viewed

sequentially by scanning electron, transmission electron, and light microscopy. The glomerular surface was intact and devoid of extraneous deposits (Fig. 1) and the fine surface structures were maintained. Transmission electron microscopy of the same glomerulus (Fig. 2) showed that the glomerular features were well preserved. The toluidine blue stained semithin section (Fig. 3) provided better resolution for studying the general morphology of the glomerulus under a light microscope. Other special stains such as methenamine silver and periodic acid Schiff could also be performed on these semithin sections.

In our previous report,¹⁰ an adhesive silver tape secured on an aluminium stud was used to hold the dried glomerulus for scanning electron microscopy. The scanned glomerulus was then carefully detached from the tape using fine needles under a dissecting

microscope. There was a possibility of damaging the glomerulus in this manoeuvre and of specimen loss during fluid exchange in subsequent processing. With the present method, each glomerulus is processed in situ on its resin support. Provided that it is properly mounted the glomerulus remains attached to the resin in the entire process. Handling the specimen during preparation is thus facilitated. Loss and damage of glomeruli are also minimised. The preparation time for transmission electron and light microscopy is shortened so that the entire process can be completed in 3–4 h. The results are comparable with those produced by the previous method. Correlative microscopy is therefore possible within two to three days.

Thus this method is a useful alternative to the silver tape method in the preparation of isolated glomeruli for correlative scanning and transmission electron microscopy as well as light microscopy. We hope that it will extend the role of scanning electron microscopy in the diagnosis of glomerular diseases.

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Letters to the Editor

Peripheral white cell counts in Australian aborigines

Bain *et al*¹ have commented on the lowered white cell count and absolute neutrophil count in blacks of African and West Indian origin compared with north European whites, Indians, and Orientals. Other groups of blacks of different ethnic origin may not show this difference.

We have recently studied the aboriginal community of Yirrkala (population 1022) in North East Arnhemland in the Northern Territory of Australia. The total white cell counts ($\times 10^9/l$) in apparently well adult men and women (aged 12 or over) are shown in Table 1.

Table 1 Total white cell counts ($\times 10^9/l$) in apparently well adult aborigines

Sex	No	White cell count		
		Range	Mean	SD
M	131	6–14	9	2
F	191	5–19	9	2

The mean percentage differential counts in random groups of men and women were

calculated (Table 2). All these were apparently clinically well, but some showed a usually minor haemoglobin or leucocyte abnormality. Thirty five subjects who were well and had "normal" Caucasian values for haemoglobin and white cell count were also examined.

These figures are significantly different from Caucasian percentages only with regard to eosinophils. In the "normal" group, only nine subjects had absolute eosinophil counts of $<500 \times 10^9/l$, 18 had

Table 2 Mean percentage differential counts in groups of aboriginal men and women

	Sex	No	Neutrophils	Lymphocytes	Monocytes	Eosinophils
Random	M	28	57	28	7	8
Random	F	48	57	27	7	9
"Normal"	M & F	35	54	30	6	10