

One Simple and Reproducible Sample Prep Protocol Used to Compare the Surface Topography (SEM) of the Mouse and Newt RPE and the Bruch's Membrane

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Retinal pigmented epithelium (RPE) supports the neural retina (NR), and responds to NR injury. When NR is damaged, the adult newt can regenerate an entire functional NR by reprogramming the RPE. In contrast, mammalian RPE (mouse) cells often reprogram and undergo an epithelial-mesenchymal transition resulting in scar formation [1, 2]. Since both the mouse and the newt can reprogram their RPE cells in the face of retina damage, we compared the similarities and differences of the RPE between the regenerative newt model and non-regenerative mouse model. However, current SEM protocols were designed for the whole eyecup, which easily undergoes morphological and structural deformation (fig.1A,B) during critical point drying (CPD) [3]. Here, we combined light microscopy (LM) cryosectioning and modified SEM techniques to generate one simple and reproducible protocol, which we use to compare the surface topography of the mouse and the newt RPE and underlying Bruch's membrane.

Whole eyes with the optic nerve were detached from the ocular muscles of adult newts and mice. The anterior eye with the iris and lens were separated from the posterior eyecup based on published protocols in mice [4]. The posterior eyecups were fixed in 2.5% glutaraldehyde and 2% formaldehyde in 0.05M cacodylate buffer for 4 hours at 4°C. After extended washes, the samples went through a gradual sucrose gradient from 7.5% to 80% at 12hrs each in 4°C before being cryo-embedded in Optimal Cutting Temperature (OCT) medium on dry ice. 25µm cryostat sample sections were collected on gelatin-coated 12mm cover glass. Sections were post-fixed in the above fixative for an additional 12hrs at 4°C. After extended washes with the same buffer, samples were fixed in 1% OsO₄ for 1 hour on ice. After ddH₂O washes, the samples were *en bloc* stained with 1% uranyl acetate overnight at room temperature (RT). After washes, the samples were dehydrated through a gradient of ethanol (EtOH) from 50%-70% on ice, and from 80% to 100% at RT for 60min each. The samples were further dehydrated in 100% EtOH overnight twice. They were then CPD with 1hr CO₂/EtOH exchange time. The samples were viewed under the Zeiss Supra 35 VP FEG-SEM after coated with 20nm of gold.

This protocol resulted in minimal morphological and structural deformation (figs.1A & B vs C & D) during processing and sectioning which permitted reliable measurements of retinal thickness in both newt and mouse eyes. The data of the cryo-sectioned adult C57BL/6 mouse and *Notophthalmus viridescens* newt eyecups showed that the thickness of the mouse NR is 228µm, which is significantly thinner than the newt at 500µm (fig.1C&D). Additionally, under higher mag (35,000x), three intact layers: (1) RPE) (2) BrM) and (3) choroid were identified in each model. Moreover, the samples revealed elongated and obliterated melanosome-like structures preserved as solid bodies. The thickness of the BrM in the two models was between 3.0-4.0µm (fig.2), which is consistent with recent findings [5-7]. Further interspecies comparisons of the ultrastructure of the RPE and the BrM using the cryosectioned samples with the modified TEM preparation is ongoing.

References:

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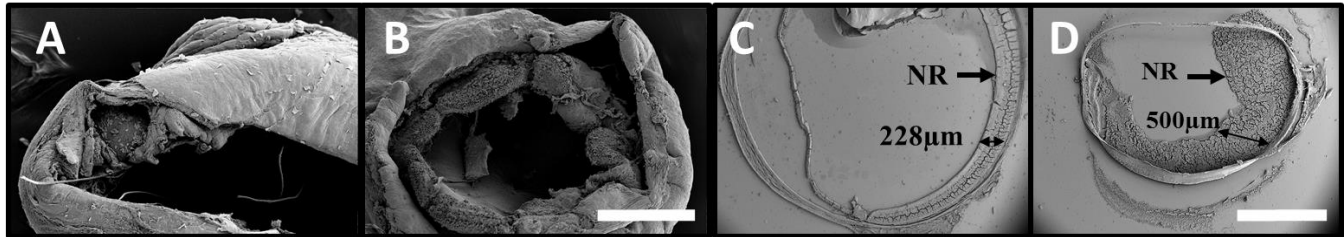


Figure 1. Typical SEM results of mouse (A) and newt (B) whole posterior eyecups. (C-D) are cryo-sectioned adult C57BL/6 mouse and *Notophthalmus viridescens* Newt eyecups. Note that the Neural Retina layer (NR – black arrows) of the mouse eyecup is 228µm, which is significantly smaller than the newt eyecup at 500µm. Imaged at 8.0kv with 26mm working distance for (A-B), and 5.0kv, and 8.0mm working distance (C-D). (Bars = 500µm).

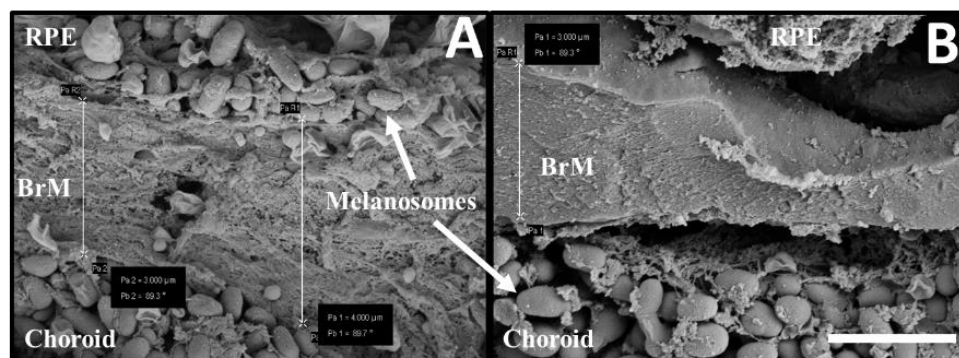


Figure 2. SEM of three intact layers: (1) Retina Pigmented Epithelium (RPE), (2) Bruch's membrane (BrM), and (3) Choroid of the B6/C57 mouse (A) and eastern newt (B). Under high magnification (35,000x), the samples show details of the RPE and the choroid, with the elongate and oblate melanosome-like structures (white arrows) preserved as solid bodies. The thickness of the BrM in two models is between 3.0-4.0µm. Imaged at 5.0kv with 9.5mm working distance (Bar = 2.5µm).