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# Use of Quantomix™ Chambers for Backscattered Electron Imaging of Immunoperoxidase-Labeled Intracellular Antigens in Hydrated Cells

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Electron imaging of immune-labeled intracellular antigens is an integral tool for understanding molecular cell biology and pathogenesis. Recently Quantomix™ Ltd. began producing vacuum containment chambers and related products enabling SEM of volatile materials using backscattered electron imaging. These chambers incorporate an array of membrane-covered viewing windows that allow transmission of primary incident and elastically-scattered electrons, and x-ray photons [1].

To determine whether the chambers might be useful for studying intracellular host-parasite interactions we compared immune-histochemically stained preparations of HeLa cells, infected with *Chlamydia trachomatis* by TEM, and by SEM in the Quantomix™ chambers. For TEM, samples were prepared as previously described [2], using immunoperoxidase labeling followed by traditional fixation, embedment, and sectioning. For SEM we followed the TEM procedures with the following modifications. Chamber membranes were pre-coated with collagen to promote cell adhesion. When reaction product was evident in infected cells by light microscopy, labeling was halted by rinsing in buffer and post-fixing with Karnovsky's fixative. Samples in the chambers were then washed twice in 0.1 M phosphate buffer, pH 7.2, treated with 0.1% OsO4 in phosphate for 5 min, washed three times 5 min with water, stained with 0.5% uranyl acetate in water, washed three more times, filled with Quantomix™ imaging buffer, and examined in an Hitachi S4500 FESEM using a Robinson backscattered electron detector. Care was taken to avoid contacting and disrupting the chamber viewing-window membranes.

Labeled inclusions and chlamydial reticulate bodies were observed within infected cells by both methods (Fig. 1). By TEM, dense reaction product was evident on chlamydiae probed with specific primary antibodies (Fig 1A), but not on samples labeled only with the secondary conjugate (Fig. 1B). Similarly by SEM, relatively intense backscattered electron signal was apparent on chlamydiae (Fig 1C, D). No immune labeling was evident on the secondary antibody control samples (Fig. 1E) or on uninfected samples (not shown). No chamber membranes burst during examination of these samples. We measured point-to-point resolution of approximately 50 nm between intracellular structures within the chambers. It is likely that working distance, gain settings, and aberrations caused by electron passage through the viewing membrane and aqueous sols contributed to resolution loss. Although, immuno-SEM using Quantomix™ chambers provides a rapid alternative to TEM embedment and sectioning, the resolution of intracellular structures was limited in these experiments. With careful handling, the chambers may prove effective for containing infectious agents such as prion protein preparations that resist inactivation by aldehyde fixatives.

References

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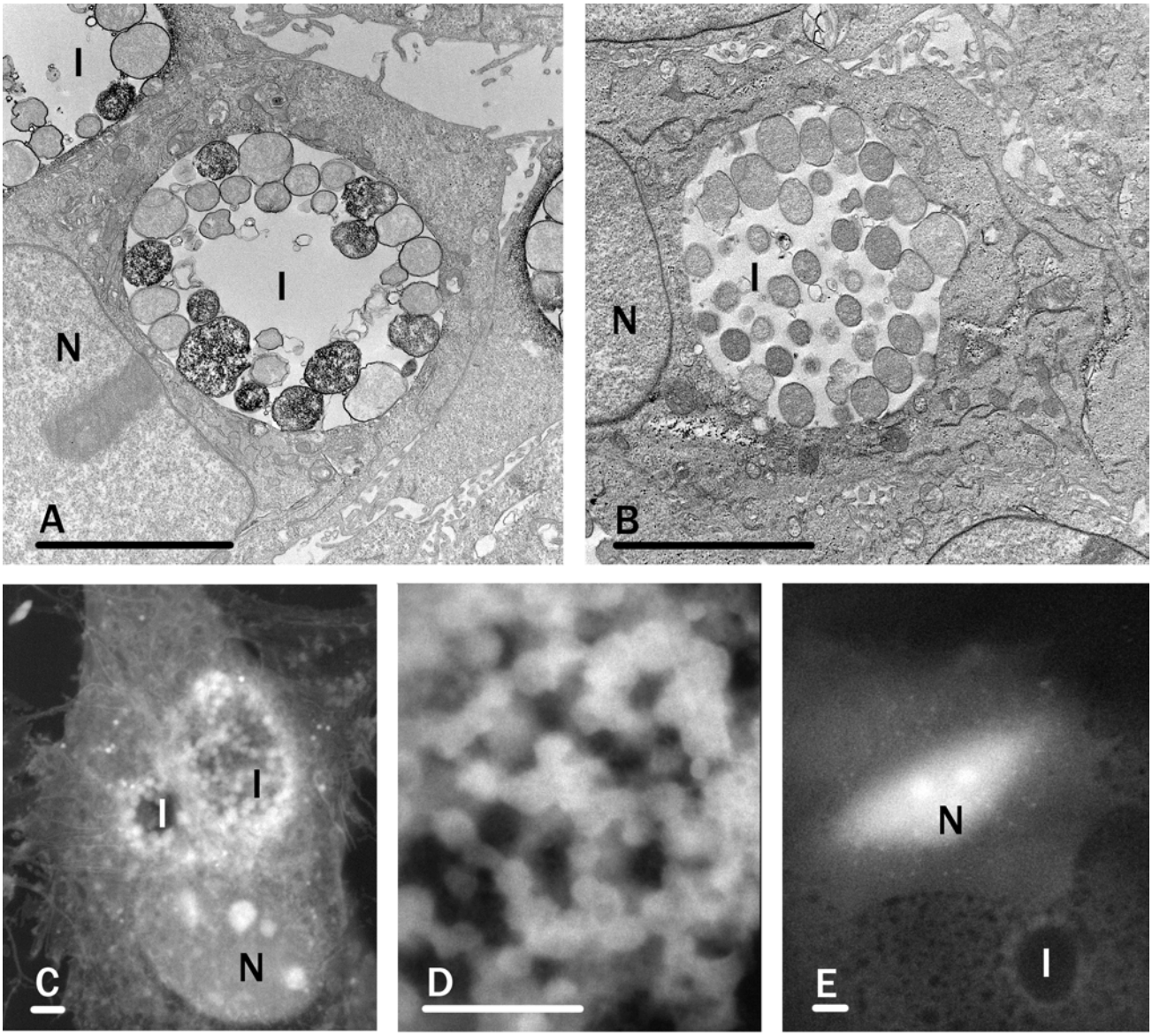


Fig. 1. Comparison between immune histochemical TEM in thin sections, and SEM in Quantomix™ chambers. A. Thin section of Chlamydia-infected HeLa cells, probed with primary anti-chlamydial antibodies and horseradish peroxidase-labeled secondary antibodies. Dense DAB reaction product is evident on intracellular bacteria. B. Thin section of an equivalent preparation probed with the secondary conjugate alone. C. and D. Immuo-SEM of *Chlamydia*-infected HeLa cells and imaged using backscattered electron detection in Quantomix™ chambers. Relatively intense backscattered signal was associated with bacteria within intracellular chlamydial inclusions. E. Immune SEM preparation probed with the secondary conjugate alone. Abbreviations: N, nucleus; I, chlamydial inclusion. Bars, 5 μm.

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