Serial Block Face SEM Visualization of Tuberculosis Infected Macrophages

Jonas A. Actor[1,2], Shen-An Hwang[3], William Monroe[4], Dustin Morado[3], Angel Paredes[4], Jun Liu[1,3], Jeffrey K. Actor[1]

[1]UT-Medical School Summer Research Program in Biomedical Sciences, [2]University of Chicago, [3]Department of Pathology, University of Texas-Houston Medical School, [4]U.S. Food and Drug Administration*, Jefferson, AR

UTHealth | Medical School
The University of Texas Health Science Center at Houston

ABSTRACT

Tuberculosis is the most common infectious disease worldwide; its etiological agent *Mycobacterium tuberculosis* (Mtb) is present in more than a third of the global population. While much is known about the overall immune response, it is still unknown as to why granulomatous Mtb will cause necrotic tissue damage, develop into latency, or will heal to form a Ghon complex. Current efforts focus on whether these outcomes are determined by the initial innate immune response elicited during Mtb infection. We aim to examine the trafficking within an infected macrophage host during the initial stages of infection by three-dimensional (3D) modeling of the Mtb bacteria within macrophage host cells during the innate immune response, using the groundbreaking serial block-face scanning electron microscopy (SEM). Such an approach has previously been impractical because of the low noise-to-contrast ratios in other microscopy techniques at this scale. J774A.1 macrophages were exposed to Mtb or non-virulent BCG and then prepared with an osmium tetroxide stain. A macrophage monolayer was then embedded in Epoxy resin (LX112). Scanning electron microscopy using Gatan 3View SEM took ~600 cross sections of 50nm thickness at unprecedented resolution. Together with advantaged image processing and segmentation, we revealed novel cellular models of macrophages infected with mycobacteria. This also provided structural evidence of an increase in vesicle formation in Mtb infected macrophages compared to the avirulent BCG organisms. These results highlight the usefulness of 3D models generated by this novel application of SEM. Future research will expand this model to quantify the number and the volumes of vesicles that appear post-infection as a measure of the cellular immune response induced by Mtb.

INTRODUCTION

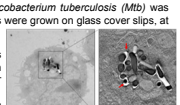
- *Mycobacterium tuberculosis (Mtb)* is the etiological agent of tuberculosis (TB) infection [Hunter, 2009]. We hypothesized that there are unique qualitative differences in intracellular trafficking due to levels of mycobacterial glycolipid found on the surface of virulent *Mtb* that differ in content from those associated with the *M. bovis* vaccine strain [, and that the intracellular trafficking ultimately affects disease pathogenesis [Indrigo, ; Yamagami, 2001; Hunter, 2014].

METHODS

Infection of mouse J774A.1 cells with *Mycobacterium tuberculosis (Mtb)* was completed as described [Indrigo, 2003]. Cells were grown on glass cover slips, at 37°C, 5% CO_2.

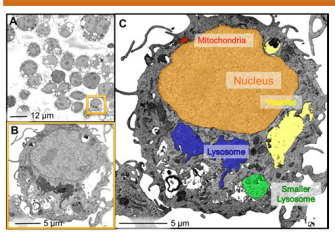
Processing of infected cells was accomplished after 4 hrs, using formalin fixation. Cells were transferred to matrix for embedding.

Serial Block Face SEM. Infected and control cells were processed for serial block face sectioning using the procedure developed by the NCMIR, University of California San Diego [Deerink]. Serial images were recorded using the Gatan 3View2 SBF apparatus mounted on a Zeiss Merlin FEG SEM operating at 1.4 kV and 3000x magnification. Segmentation of the structures was accomplished using the Amira software package. (FEI Company).

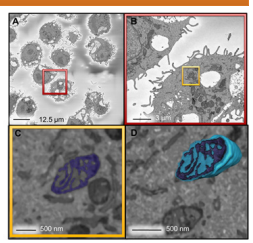


Conventional CryoEm of Mouse J774A.1 monocytes with MTB. Cells prepared at 4 hrs post infection. Right = high power resolution of inset. Note the presence of trehalose 6,6'-dimycolate glycolipid surrounding organisms (arrows). CryoEM courtesy of Jun Liu, Ph.D.

VISUALIZATION OF MACROPHAGE INTRACELLULAR STRUCTURES USING SERIAL BLOCK SEM

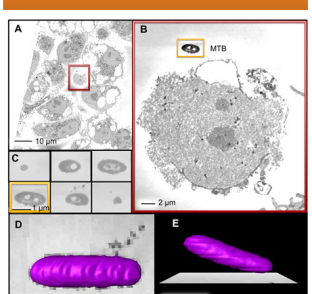


Detailed visualization of J774A.1 Macrophage using Serial Block Face SEM Methodology. A. Complete 8000x8000 image of MTB-infected mouse macrophages after 24 hours; boxed cell expanded to B. Using segmentation, we can highlight individual internal cellular structures, including individual organelles, as seen in C.



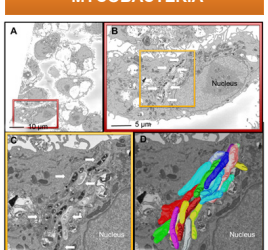
Technique Potential: Visualization of Known Cellular Structure. Full 8000x8000 cross section of infected macrophages after 4 hours; highlighted area in B details a subsection of a macrophage, where interior organelles are clearly visible. C demonstrates how much detail is preserved by using this SEM technique; segmentation of the inner membrane shown. D renders the mitochondria as a 3D surface, preserving the inner structure. The slide background is a cross section about ~300nm below the mitochondria.

RECONSTRCTUCTION OF EXTRACELLULAR MYCOBACTERIUM



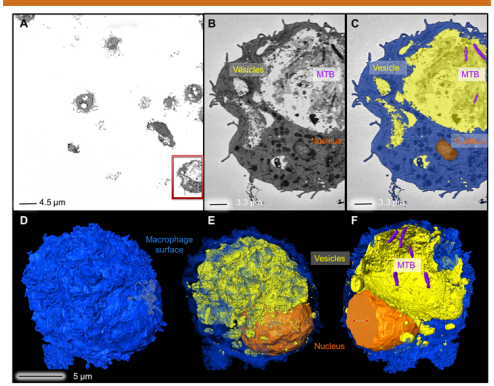
Reconstruction of Captured Extracellular Mycobacterium. A. SEM micrograph of MTB infected macrophages after 4 hours. Cellular (ghost) remnant and extracellular MTB focused upon in B, with enhanced contrast. 6 of the 28 cross sections of the bacteria are shown in C, with sections about 250 nm apart. View is rotated in D to see the bacillus along its largest axis, with its surface rendering in E. Scale bar in A is 10 µm, B 2 µm, C-E 1µm.

INTRACELLULAR CORDED MYCOBACTERIA



Identification of Corded Mycobacteria within Infected Macrophage. A. SEM micrograph of MTB infected macrophages; boxed macrophage expanded to B, where several mycobacteria are seen (arrows). Highlighted area in B is focused upon in C and D to detail organisms, rendered in different color in D, using segmentation and surface rendering from Amira 5.6.0.

VESICLE LOCALIZATION OF INFECTIOUS MYCOBACTERIA IN MACROPHAGES POST CHALLENGE



Full Rendering of Infected J774A.1 Macrophage with Mycobacteria Localized to Intracellular Vesicle. A. Full 8000x8000 SEM cross section of infected macrophages at 24 hours post-infection. Bottom-right macrophage is enlarged in B (contrast edited). Segmentation is shown in C, highlighting the vesicles (yellow), nucleus (orange), and three MTB (purple). Surface rendering is presented in D, viewing axis along x=z. Surface is made transparent for E, allowing a view displaying vesicles and the nucleus. Cell is rotated 180° and cut away in F, revealing MTB within the main vesicle, as seen in the cross-sections in B-C. Diameter of macrophage in D-F is approximately 16 µm. White bar on the nucleus surface in F is 3 µm in the XY plane.

SUMMARY/CONCLUSION

- **A method was successfully established to visualize *Mycobacterium tuberculosis* infected macrophages using serial block face SEM techniques. This allows unparalleled visualization of organisms within cellular compartments, and a unique way to identify and track infection within a single cell.**

- **Organisms were successfully identified in intracellular compartments of infected cells. This establishes a unique tool to understand the trafficking events initially post infectious challenge.**

- **This technique of three-dimensional (3D) modeling of the mycobacteria within macrophage host cells will assist in understanding the early responses elicited during the innate immune response.**

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