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Optimization Of Scanning Electron Microscopy and Serial Block Face – Scanning Electron Microscopy for Investigating Bacteria and Whipworm Egg Interactions

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Soil transmitted helminths (STH) are parasitic worms that infect the intestine of the host and are transmitted from person to person via contact with contaminated soil. These infections are among the most common worldwide, affecting nearly 1.5 billion people [1]. One of the major species of STH, *Trichuris trichiura*, can cause severe symptoms in people with heavy worm burdens including malnutrition, stunted growth, and dysentery [2, 3]. While there is currently a standard therapy available for *T. trichiura* infections, its efficacy is poor with an average cure rate of less than 50% [4]. Therefore, there is a need for the development of better drug treatments for these infections.

The murine pathogen *Trichuris muris* is used to model *T. trichiura* infections in the laboratory. The life cycle of *T. muris* begins with ingestion of embryonated eggs, which then travel through the digestive tract and accumulate in the caecum where the eggs hatch and release L1 larvae [5, 6]. The initiation of this hatching sequence has been shown to occur primarily in the caecum and other groups have demonstrated that this is due to both the passive accumulation of eggs in the caecum as well as the conditions of the caecum/caecal contents being ideal for hatching induction [6]. However, the specific triggers of hatching and how they relate to the physical changes which occur during the hatching process are not fully understood [7].

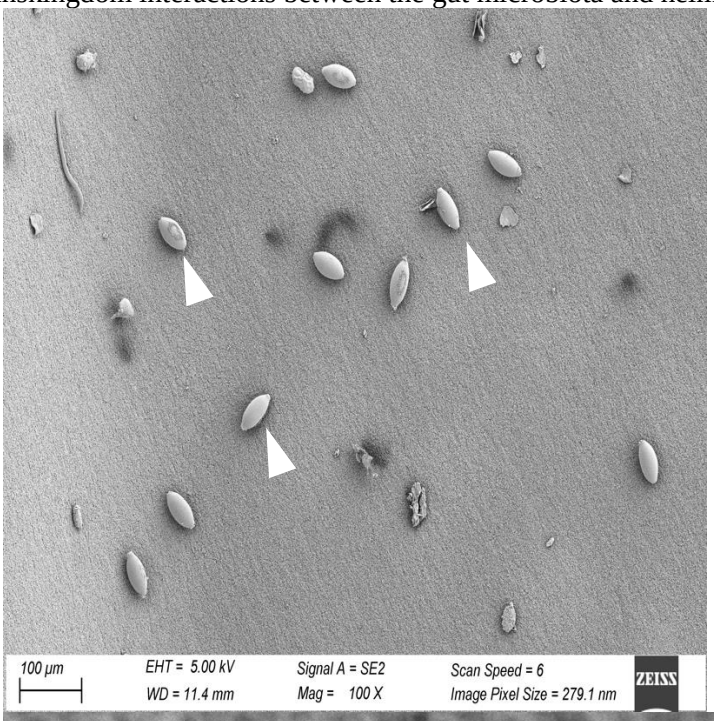
The abundance of bacteria in the caecum led groups to investigate whether bacterial species can serve as triggers of egg hatching. Specifically, it has been shown that *Escherichia coli* cultures can induce egg hatching *in vitro*. Furthermore, they showed that under conditions that trigger egg hatching *in vitro*, *E. coli* preferentially binds to the polar plug region of the egg [8]. The structural changes associated with *E. coli*-mediated egg hatching remain obscure.

Here we report the determination of the appropriate strategy to image eggs and bacteria using scanning electron microscopy (SEM). Given the size (~70 um) and shape of the egg, we determined that the use of transwells was essential for retaining eggs during sample processing, especially at the critical point drying step (Fig. 1A). The SEM images demonstrated that many bacterial cells bind to the polar plug region of the egg (Fig. 1B). We also used serial block face- scanning electron microscopy (SBF-SEM) to reveal the 3-dimensional architecture of the polar plug region and its relative spatial orientation to other organelles inside the egg. The low efficacy of chemical penetration of the eggshell led us to test several different sample preparation strategies. We concluded that microwave assisted sample processing was more effective than the high pressure freeze and freeze substitution methods for preserving the contents of the whipworm egg for ultrastructure analysis. The use of this method allowed us to gain novel insight into the ultrastructural landscape within the egg during the *E. coli* induced hatching process.

In conclusion, SEM and SBF-SEM are important technologies that can be used to gain a deeper understanding of the changes that occur during the hatching process, which may provide some insight into the molecular

mechanisms that take place. Increased knowledge of molecular mechanisms that are important for hatching might also lead to the development of more effective treatments for these infections. As current therapies available only target adult worms, a therapy that instead targets the vulnerable egg hatching stage might be an important new way to tackle this global health burden. Furthermore, these types of studies will allow us to gain more insight into how transkingdom interactions between the gut microbiota and helminths occur.

A)



B)

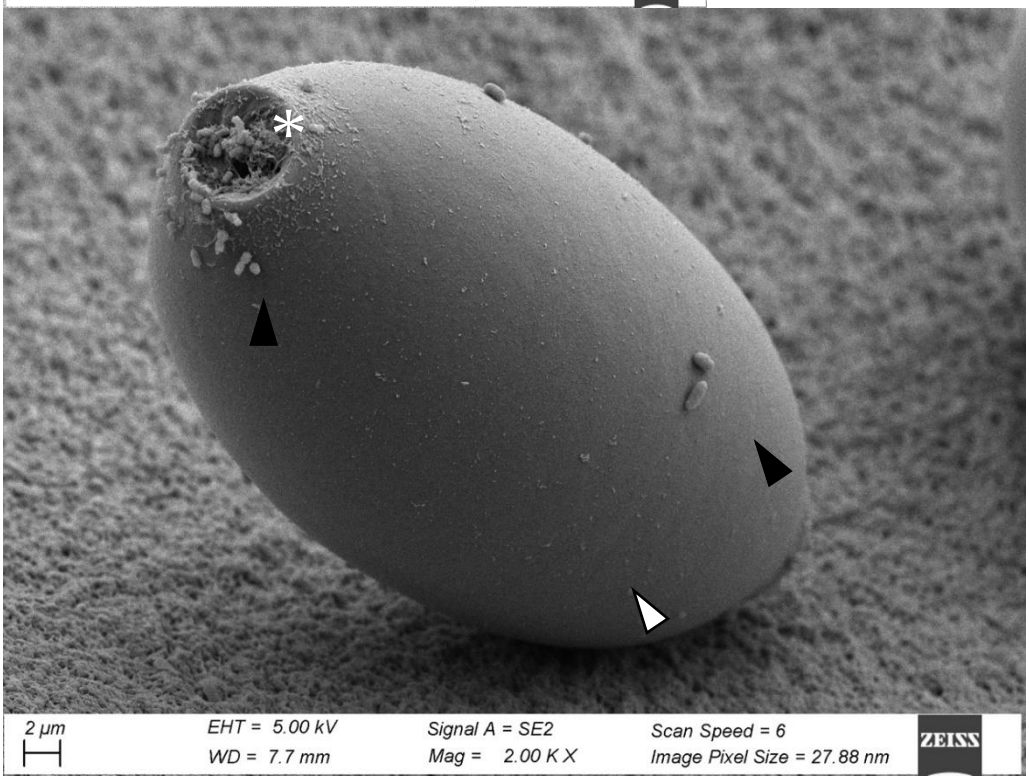


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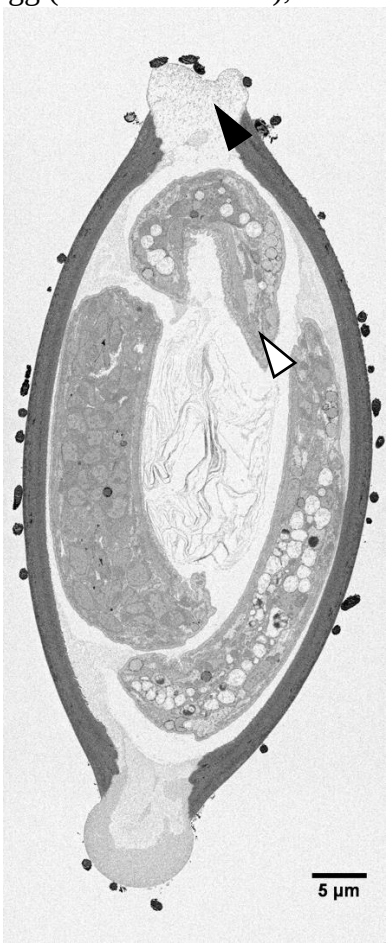
Figure 1. SEM of *T. muris* eggs and *E. coli* after a 1.5 hr incubation. (A) Low magnification SEM image showing multiple *T. muris* eggs (arrowheads). (B) High magnification SEM image of a single *T. muris* egg (white arrowhead), associated bacteria (black arrowheads) and polar plug (white asterisk).



A) B)

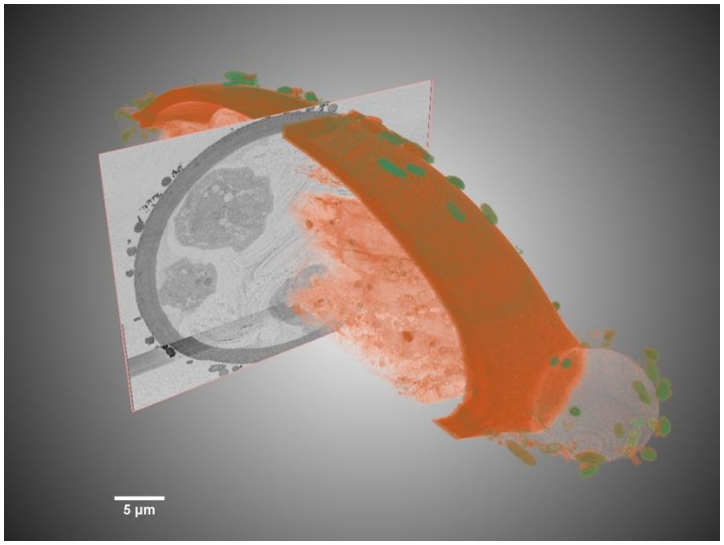


Figure 2. SBF-SEM of *T. muris* eggs and *E. coli* after a 1.5 hr incubation. (A) A single slice SBF-SEM image showing well-preserved interior of the egg (white arrowhead) and bacteria in close association with the polar plug region of the egg (black arrowhead). (B) 3D rendering of the stack of SBF-SEM

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images showing the egg (orange) in close association with bacteria (green) at the polar plug region (white arrowhead).

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