**SEM Visualization of the Lyme Producing Bacterium, *Borrelia burgdorferi***

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**Spring 2009 Scanning Electron Microscopy Course Project**

**Summary**

Electron microscopy is a powerful tool which has led to numerous breakthroughs in biological research. While it has contributed to many areas of research the area of interest to my project was visualization of *Borrelia burgdorferi*, the causative organism of Lyme disease, using Scanning Electron Microscopy (SEM). A significant human pathogen close range visual analysis of the organism can provide important clues on the roles of certain genes in motility and pathogenicity. Understanding the role of these genes in turn can lead to potential improvements in detection and treatment of patients suffering from Lyme disease. However, the preparation and visualization of microorganisms pushes the abilities of Transmission Electron Microscopy and Scanning Electron Microscopy (SEM) to their limits. For this reason well designed protocols have developed over time for studying such small fragile specimens.

**INTRODUCTION**

*B. burgdorferi* is an unusual bacterium responsible for causing Lyme disease. Unlike most bacteria which generally tend to fall on a continuum between coccii (spherically shaped) and bacillus (not shaped) *B. burgdorferi* is a spirochete, a bacterium with an unusual wave or spiral body plan. Other medically relevant spirochetes include *Treponema pallidum*, which causes syphilis, and *Treponema denticola*, which is a cause of periodontal (gum) disease. This unusual body plan is due to a unique quality of these organisms' flagella.

**METHODS**

Preparation: Poly-Llysine-coated Thermaco coverslips were covered in a few drops (approximately 50-150x) of BSK containing late log phase *B. burgdorferi* for one hour. Following a gentle wash with Sorensen’s phosphate buffer (0.15M, pH 7.3) specimens were fixed with 2.5% glutaraldehyde (in same buffer) for one hour. Following three washes with buffer, specimens received a secondary fixation with 1% osmium tetroxide in buffer for one hour, and washed three times in buffer. Specimens were then dried with increasing concentrations of ethanol up to 100% concentration and treated three times with hexamethyldisilazane. Each drying step lasted ten minutes. Remaining solution was pipetted off and the coverslip was allowed to dry at room temperature in a fume hood inside a Petri dish (to protect from dust). Great care was taken at all steps due to the delicacy of the specimen. Specimens were sputter coated with gold/palladium prior to examination. Spirochetes were examined at 30KV spot size 4 under high vacuum conditions. They were imaged at a relatively high magnification. Three typically associate with one another forming collections or clumps, this is a particularly large example.

**RESULTS AND DISCUSSION**

SEM was successful however a significant number of specimens were lost or distorted (loss of spiral structure) by the preparation procedures. Greater care and caution reduced this loss dramatically, nonetheless some distorted specimens remained. Figures 7 distorted, Figure 4 normal cell. Future preparations can include Environmental SEM (ESEM) in which cells may or may not be preserved and remain undamaged. This potentially involves the least amount of specimen preparation and may result in minimal artifacts. This class project involved SEM which is quite useful for examining whole spirochetes, visualization of flagella for future studies might best include TEM-negative staining as well as ESEM.

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