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Natiera Magnuson

University of Nevada, Las Vegas

Manomita Patra

University of Nevada, Las Vegas

Maria Elena Reynaga

University of Nevada, Las Vegas

Ernesto Abel-Santos

University of Nevada, Las Vegas

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The Release of Calcium in Bacillus anthracis Pathogenicity

Natiera Magnuson, Manomita Patra, Maria Elena Reynaga, and Ernesto Abel-Santos

Department of Chemistry, University of Nevada Las Vegas



Abstract *Bacillus anthracis* spores form in response to starvation and can withstand extremes of heat, radiation, and chemical toxins, making *B. anthracis* spores ideal vehicles for infections.¹ The resistance and dormancy of bacterial spores are dependent on a largely dehydrated core.² The spore core is not only devoid of water, but contains between 0.8 to 1M calcium complexed with 2,6-pyridinedicarboxylic acid (dipicolinic acid, DPA). The DPA-calcium complex (CaDPA) helps protect DNA, RNA, and the metabolic enzymes needed for the establishment of a vegetative cell cytoplasm.³ An anthrax infection starts with the germination of *B. anthracis* spores in a macrophage.⁴ The germinated spore can then produce toxins that eventually kill the macrophage.⁵ During *B. anthracis* spore germination, large concentrations of calcium ions (Ca²⁺) are released.³ Calcium ions act as a second messenger in macrophages, and it is possible that the release of these ions interfere with the macrophages ability to detect newly germinated *B. anthracis* spores.⁶ In this project, we will investigate the role of calcium release on infected macrophage viability.

Introduction

Bacillus anthracis is a gram-positive, spore-forming, rod-shaped bacterium.⁷ *B. anthracis* spores are resilient, surviving extremes of temperature, low-nutrient environments, and harsh chemical treatment.¹ The DNA in the spore core is protected by the calcium dipicolinic acid complex (CaDPA).³ When spores are inhaled, ingested, or come into contact with a skin lesion on a host the spores may reactivate and multiply rapidly. The vegetative form of *B. anthracis* releases the lethal anthrax toxin.⁵ Macrophages are white blood cells within tissues that phagocytize cellular debris.⁸ When a macrophage phagocytizes a *B. anthracis* vegetative bacterium, the bacterium is killed. However, when a macrophage phagocytizes a *B. anthracis* spore, the spore is not killed. Instead, the spore is able to germinate into a vegetative bacterium. Toxin production is not detected until three hours after the onset of germination.⁹ During these three hours, the newly germinated *B. anthracis* cell is vulnerable to its external environment, and yet it is not destroyed. Calcium ions act as secondary messengers in macrophages. A secondary messenger is a molecule that relays signals from receptors on the cell surface to target molecules inside the cell, in the cytoplasm, or nucleus.⁶ When a *B. anthracis* spore germinates, it releases calcium ions.¹ It is possible that the sudden influx of calcium ions inside the macrophage, once the spore starts to germinate, can confuse the cell signaling system. This confusion may allow the newly germinated bacterium to go undetected; it then can release the anthrax toxin to kill the macrophage. The purpose of this study is to determine the effect of calcium ion release on the macrophage.

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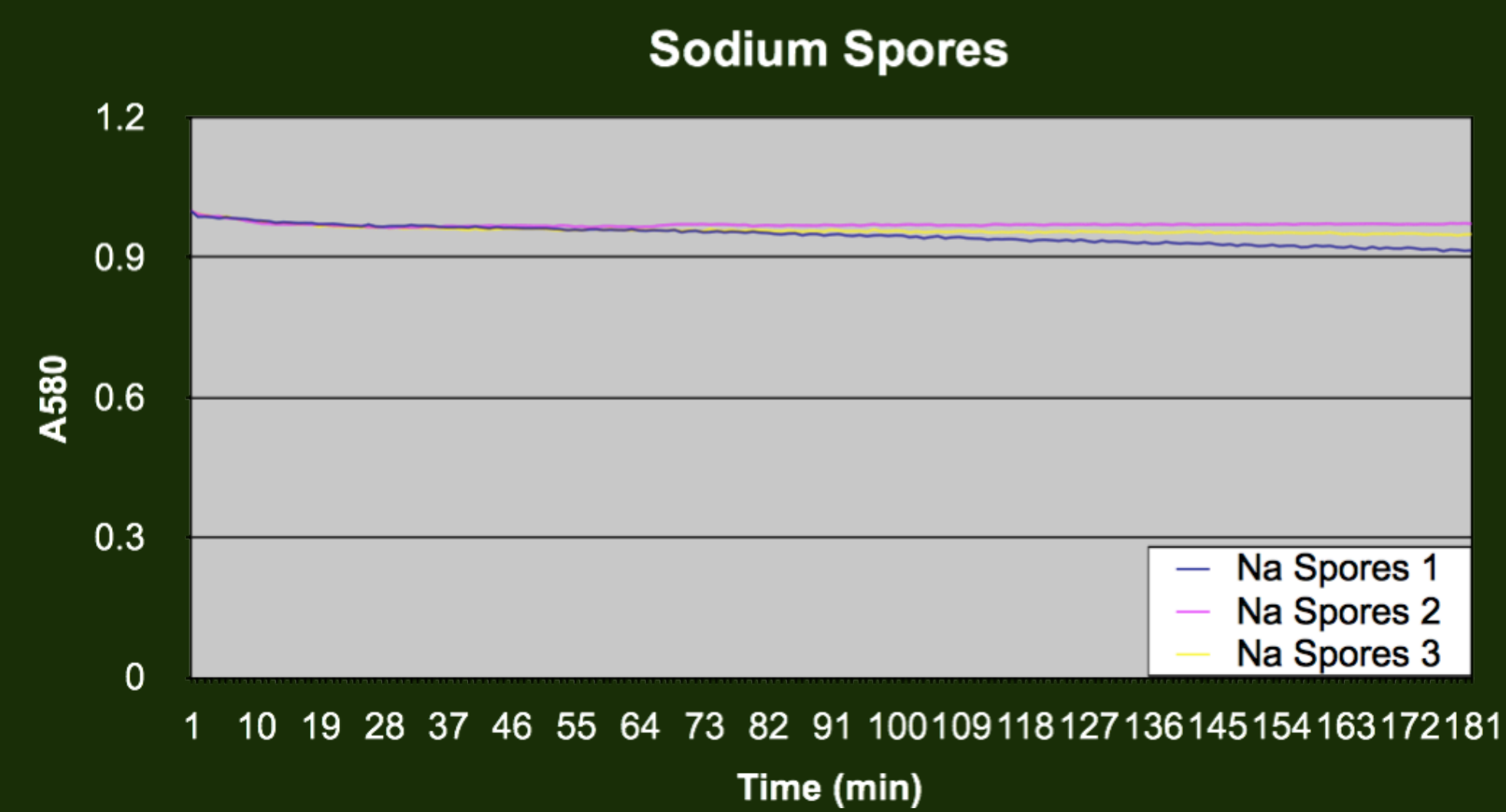


Figure 3: Absorbance analysis of the remineralized sodium spores

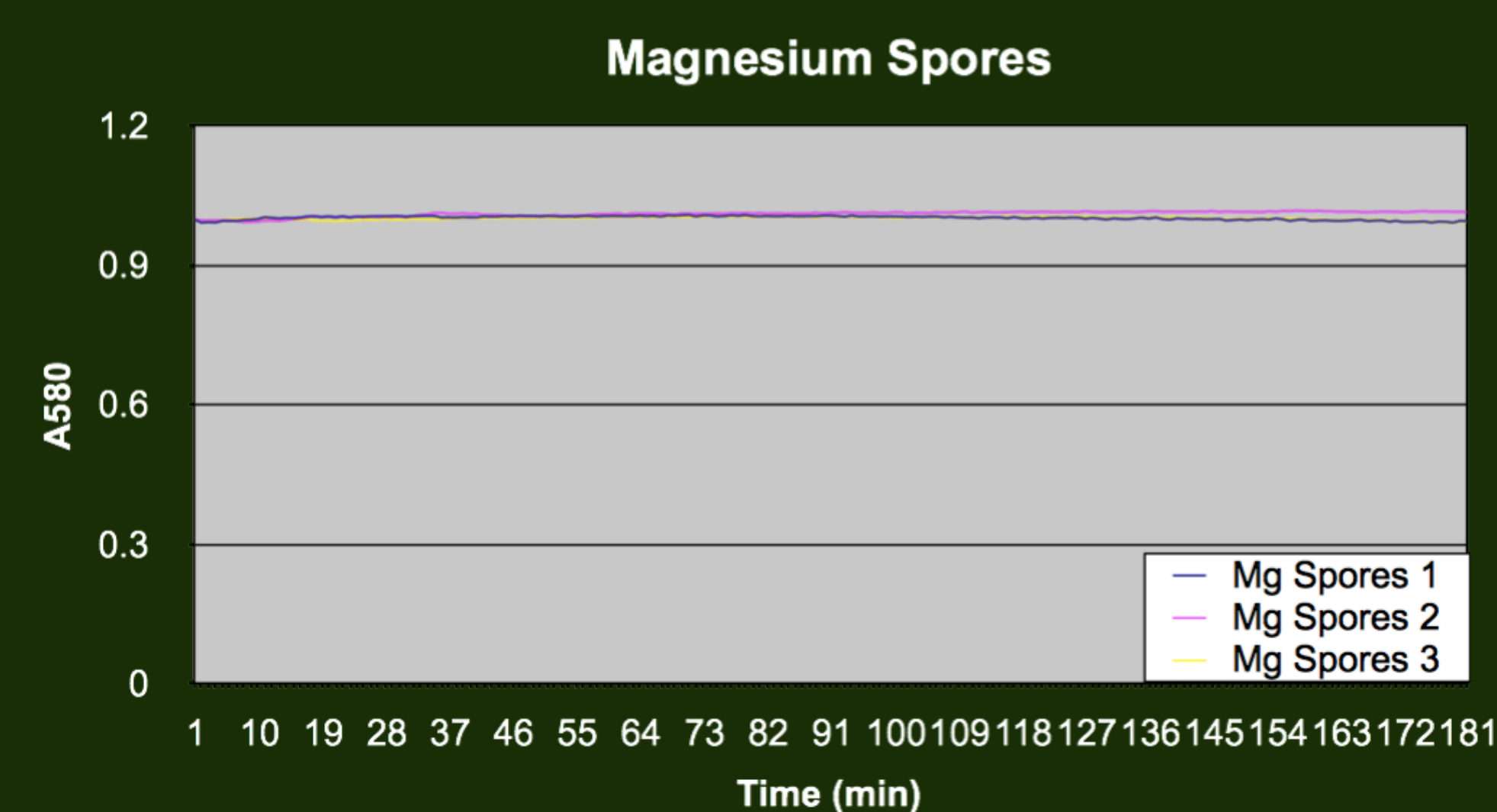


Figure 4: Absorbance analysis of the remineralized magnesium spores

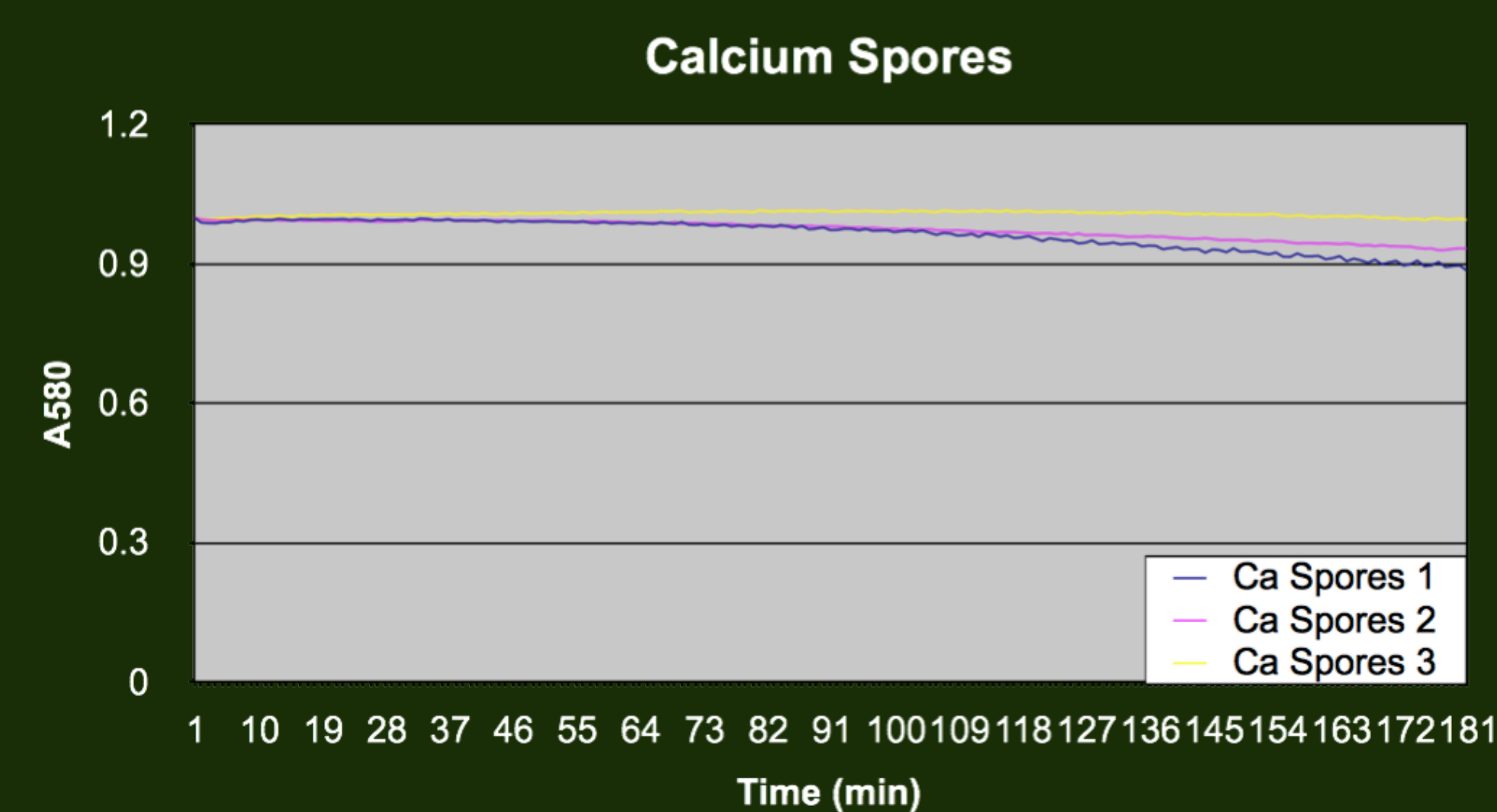


Figure 5: Absorbance analysis of the remineralized calcium spores



Figure 1: A living macrophage (opaque) and a dead macrophage (blue)

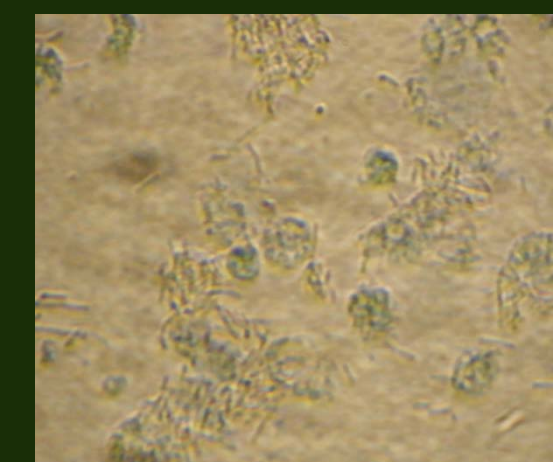


Figure 2: Germinated spores with killed macrophages

Hypothesis

The release of calcium ions during *Bacillus anthracis* spore germination impacts anthrax-mediated macrophage death.

Methods

- **Spore Preparation**
 - The remineralized spores were prepared using a modified method outlined in Igura et al
- **Cell Culture**
 - Murine macrophages were cultured in a HyClone HBS medium that included fetal bovine serum
- **Infection**
 - Remineralized spores were added to the macrophages and incubated
 - Then the macrophages were washed several times and the medium, now including an antibiotic, was re-added
- **Microscopy**
 - Macrophages were stained with 0.04% Trypan Blue
 - Macrophages were visualized with light microscopy
- **Absorbance**
 - Germination was measured as a decrease in optical density (A₅₈₀) using the Tecan M200 and the coordinating iControl computer program

Results

The remineralized spores took over 18 hours to germinate and kill the macrophage. This may be due to the lack of *B. anthracis* germinants in the medium. Of the three remineralized spores analyzed here, the remineralized calcium spores were most efficient at germination and macrophage killing. Through this, it can be inferred that calcium is important to spore germination and possibly to the eventual lysis of the macrophage.

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