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A NOVEL HYDROGEL-BASED BIOSAMPLING APPROACH

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14. ABSTRACT (200 WORDS OR LESS)
Available surface sampling approaches (e.g., swabs, biological sampling kits, and wipes), especially from porous surfaces, are generally ineffective. Hydrogel is a water-based gel, which is applied as a thick viscous material on contaminated surfaces and allowed to dry into a thin film within a few hours, depending on ambient conditions. The dried film is then peeled off the surface. During the drying process, the gel encapsulates the bioagent and other contaminants. In this study, biohydrogel was used to examine the sampling efficiency and preservation of materials taken from contaminated surfaces. The biohydrogel proved to be a superior sampling tool; recovery efficiency from 3D objects (e.g., screws) and pinewood coupons was 80% greater than that of existing sampling devices for the recovery of *Bacillus anthracis* (ΔSterne) spores. Biohydrogel proved to be as good a sampling tool for painted steel, polycarbonate, pinewood, and screws as available sampling techniques. Because the biohydrogel recovered <1% of *Staphylococcus aureus* cells from painted steel, polycarbonate, pinewood, and screws, some components of the biohydrogel were concluded to be bactericidal. Spores of *B. anthracis* ΔSterne strain were well preserved at 4 °C for over 5 weeks. Over 5 logs of such spores lost their viability within 2 weeks at 25 and 37 °C, indicating non-optimal preservation at such temperatures.

15. SUBJECT TERMS
Sampling *Bacillus anthracis* (ΔSterne) Biohydrogel *Staphylococcus aureus* Preservation

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PREFACE

The work described in this report was authorized under the U.S. Army Edgewood Chemical Biological Center Seedling Program. The work was started in June 2015 and completed in August 2015.

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CONTENTS

FIGURES

A NOVEL HYDROGEL-BASED BIOSAMPLING APPROACH

1. INTRODUCTION

Biological sampling from environmental surfaces is a complex area of intensive, ongoing research. After a biorelease event, sampling is at the core of all pre- and post-decontamination analyses. Easily available surface sampling approaches (e.g., swabs, biological sampling kits [BISKits], wipes, and vacuum socks), especially those used on porous surfaces such as concrete, are generally ineffective. Brown et al. (2007a) examined the recovery efficiency of sterile polyester–rayon blend gauze wipes used on contaminated stainless steel and painted wallboard surfaces. Their findings showed <35% recovery efficiencies. In a different study, Buttner et al. (2004) investigated the use of BISKits, which yielded a percent recovery of 18.4%. Many studies have tested the use of swabs as biological samplers, and their results have yielded percent recoveries of <55% (Hodges et al., 2006; Hodges et al., 2010; Probst et al., 2010 and Rose et al., 2004). Vacuum socks were used on large surface areas, but the recovery efficiencies by vacuum sampling of spores were <36% (Brown et al., 2007b). Furthermore, such devices are only useful for sampling from smooth two dimensional (2D) surfaces and not from complex 3D structures. Sample collection protocols are often unsuited to long-term preservation, forensics, and diagnostic analyses.

This study investigates the use of hydrogel as (1) a biological sampler and (2) a method for spore preservation. Hydrogel is a water-based gel that is applied as a thick viscous material to a contaminated surface and allowed to dry into a thin film within a few hours. The dried film is then peeled off the surface and analyzed for encapsulated spores in the hydrogel. It was determined that encapsulated spores were present in the hydrogel; thereby, proving that it is possible to use hydrogel as a biological sampler (biohydrogel). To determine that biohydrogel can be used effectively as a sampler for all surface types (i.e., even on 3D surfaces), it was tested on the following four surfaces:

- pinewood,
- polycarbonate,
- painted steel, and
- screws.

Preservation of biological samples is a major concern after a biological incident. The Centers for Disease Control and Prevention (CDC) standard methods for shipping biological samples dictate that samples must be stored between 2 and 8 °C and must be used within 48 h of sampling. Thorne et al. (1994) determined that if samples are not refrigerated right after they are taken, they lose viability. Krumnow et al. (2009) preserved *B. subtilis* spores in acacia gum, which is a natural polymer, for 64 days at 5, 15, 25, and 40 °C. They showed that *B. anthracis* Sterne and methicillin-resistant *Staphylococcus aureus* spores had a higher level of protection when acacia gum was added to the sampling material (i.e., cotton and wool). Hydrogel was used to preserve biological samples after they were encapsulated until they could be processed. Sorokulova et al. (2012) tested hydrogel on two different surfaces (painted steel and

polycarbonate). They stored the samples at 5, 25, and 37 °C and conducted the sampling weekly for up to five weeks to determine if hydrogel could be used to preserve biological material.

2. MATERIALS AND METHODS

2.1 Test Organisms and Reagents

The *B. anthracis* (ΔSterne) spores used in this study were cultured by Michael Kim in the BioTechnology Branch (U.S. Army Edgewood Chemical Biological Center). The *S. aureus* vegetative cells used in this study were procured from American Type Culture Collection (number 6538).

The biohydrogel, a biodegradable polymer, contains a chelating agent (>35% water) and organic solvents. It was procured from Metis Scientific (Richardson, TX).

The pinewood, polycarbonate, and painted steel sample coupons (3×1 in.^{2}) and screws were procured from Home Depot (Bel Air, MD). The coupons were autoclaved in sterile petri plates before use.

Tryptic soy agar (TSA) plates were used for culturing the samples and 0.01% Tween 80 was used as the extraction media.

2.2 Methods

2.2.1 Sampling Experiments

Samples from each surface type were processed separately. Three coupons from each surface type and three screws were set aside to be used as control samples. Biohydrogel was not applied to these samples.

The remaining coupons were divided equally into two batches. One batch was inoculated with 50 µL of *B. anthracis* ([ΔSterne] 1×10^7), and the other was inoculated with 50 µL of *S. aureus* (1×10^7). Similarly, the remaining screws were divided into two batches, and each batch was treated with one of the inoculums. After the samples dried, the biohydrogel was overlaid onto the coupons and placed in a biosafety hood to dry. Once the biohydrogel dried into a thin film, it was peeled off each coupon and put into 20 mL of 0.01% Tween 80. The gel from each coupon was treated as a separate sample. The actual surface coupon was discarded and the only thing from each sample that was analyzed was the biohydrogel. The samples were incubated at 37 °C for 2 h until the biohydrogel was rehydrated in the 0.01% Tween 80. After rehydration, serial dilutions were performed for each sample, and then the dilutions were plated on TSA plates and incubated at 37 °C overnight.

The control coupons and screws were placed in 20 mL of 0.01% Tween 80, serially diluted, and plated.

2.2.2 Preservation Experiments

Only the polycarbonate and painted steel coupons were used for the preservation experiments. These coupons were inoculated with 50 µL of *B. anthracis* (ΔSterne) and dried overnight in a biosafety hood. When they were dry, the coupons were overlaid with biohydrogel and replaced in the biosafety hood to dry overnight. Once the gel dried, the coupons were exposed to the following temperatures:

- 18 polycarbonate and 18 painted steel coupons exposed to 4 °C;
- 18 polycarbonate and 18 painted steel coupons exposed to 25 °C; and
- 18 polycarbonate and 18 painted steel coupons exposed to 37 °C.

The coupons were sampled at six different times: at 24 h and at 1, 2, 3, 4, and 5 weeks. Three coupons of each surface type were taken out at the appropriate time point, and the gel was peeled off the coupons and put into 20 mL of 0.01% Tween 80. These samples were incubated at 37 °C for 2 h until the gel was rehydrated. After the rehydration process, serial dilutions were conducted for each sample. The dilutions were plated on TSA plates and incubated at 37 °C overnight.

3. RESULTS

3.1 Efficacy of Biohydrogel

To determine if biohydrogel was an effective sampler, the percent recovery of the *B. anthracis* (ΔSterne) spores was determined by dividing the average colony-forming units (cfus) of the test biohydrogel by the average cfus of the positive controls of the screws and each coupon type and then multiplying by 100 (Figure 1).

$$\text{Percent Recovery} = \frac{\text{Average cfus of test biohydrogel} \times 100}{\text{Average cfus of positive controls}}$$

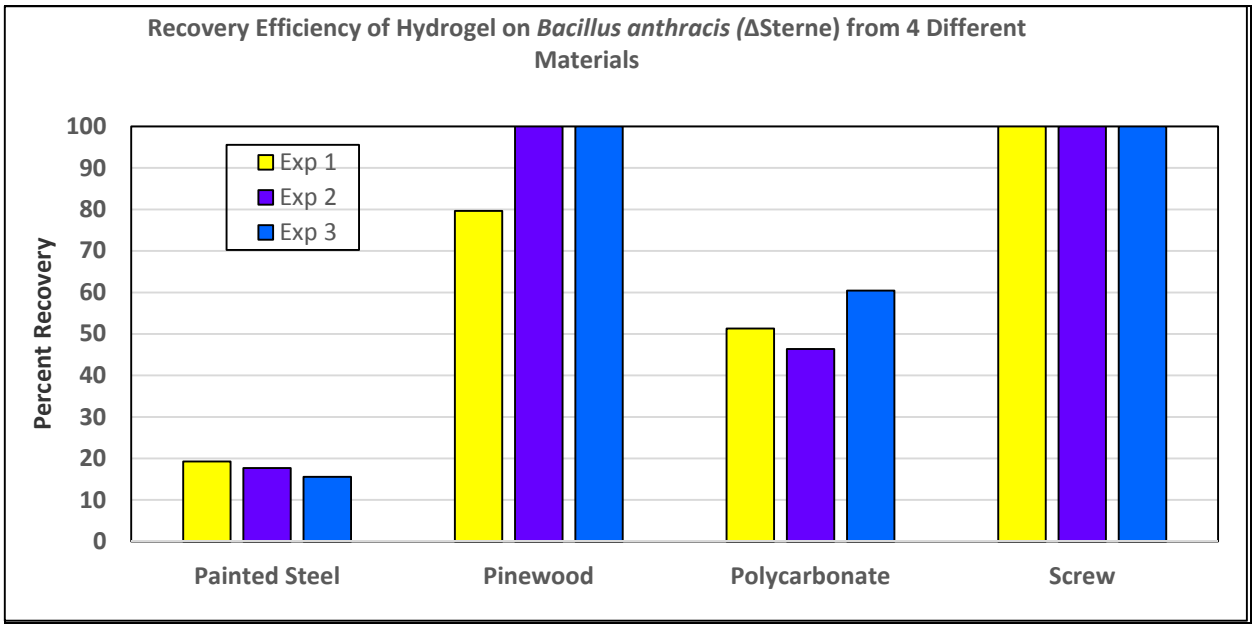

Figure 1. Recovery efficiency of biohydrogel on *B. anthracis* (ΔSterne) from screws and painted steel, pinewood, and polycarbonate coupons.

The biohydrogel proved to be a better sampling tool than other existing devices for pinewood and 3D objects (i.e., screws) when used for the recovery of *B. anthracis* ([ΔSterne] >80% recovery). For painted steel and polycarbonate, the biohydrogel seemed to be as effective as other available samplers, such as wipes, swabs, and BISKits (15–60% recovery), for the recovery of *B. anthracis* (ΔSterne) spores. Biohydrogel can be sprayed with paint so that it is easy and efficient use in a large area.

Biohydrogel was used to recover from 0.35 to 0% of *S. aureus* spores from painted steel, polycarbonate, pinewood, and screws (data not shown). This biohydrogel seemed to be bactericidal for vegetative cells; therefore, we will be focusing on developing a "designer" gel, without bactericidal solvents, as an effective sampler for vegetative bacterial cells.

3.2 Sample Log Reduction

The log reduction for each sample was calculated to determine if spores were preserved over time at each of the three different temperatures. The log reduction was calculated by taking the average log of the positive controls (no gel) and the average log of the biohydrogel coupon type at a specific time and temperature. Spores of *B. anthracis* (ΔSterne) were preserved in the biohydrogel at 4 °C for more than 5 weeks on painted steel and polycarbonate coupons. This was longer than the usual time samples can be stored before they have to be processed. *B. anthracis* (ΔSterne) did not survive as well in biohydrogel at 25 and 37 °C (Figures 2–5). We believe that the spores germinated at 25 and 37 °C, which caused the cells to die in the dried biohydrogel. After a new designer hydrogel without bactericidal agents is developed, preservation of samples at 25 and 37 °C could be further investigated. If spore viability remains stable in the biohydrogel at these two temperatures for more than 5 weeks, sample refrigeration, which is not always possible, would not be necessary before processing.

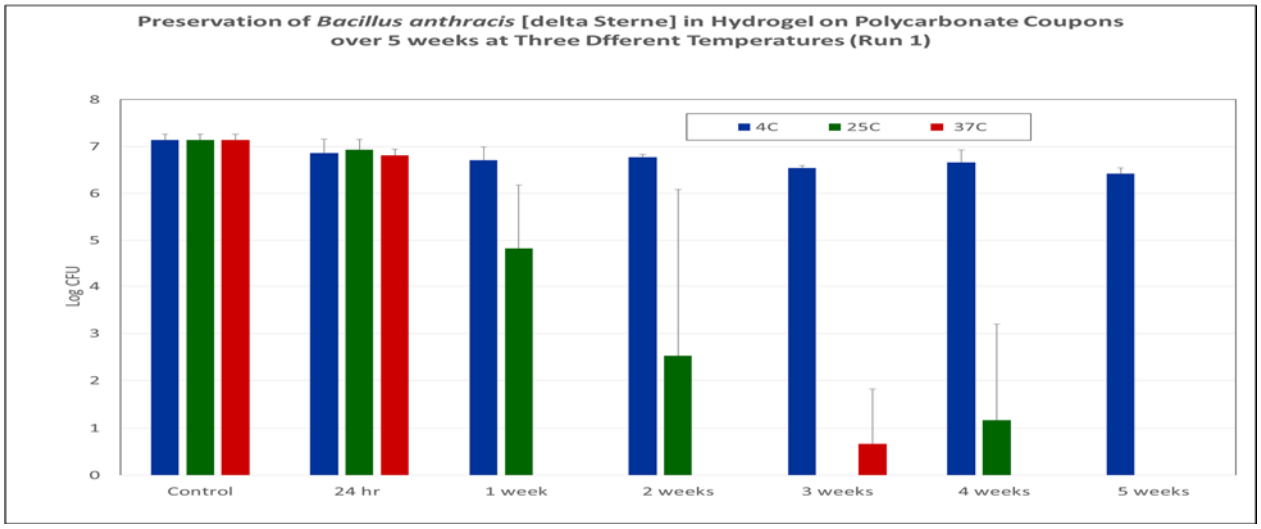

Figure 2. Preservation of *B. anthracis* (ΔSterne) in biohydrogel on polycarbonate coupons over 5 weeks at three different temperatures, Run 1.

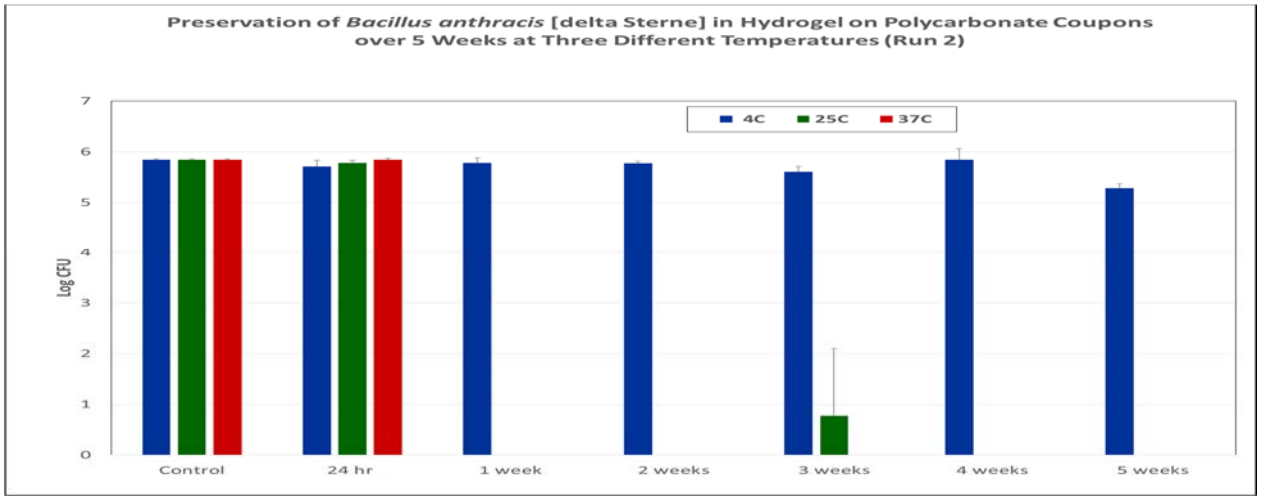

Figure 3. Preservation of *B. anthracis* (ΔSterne) in biohydrogel on polycarbonate coupons over 5 weeks at three different temperatures, Run 2.

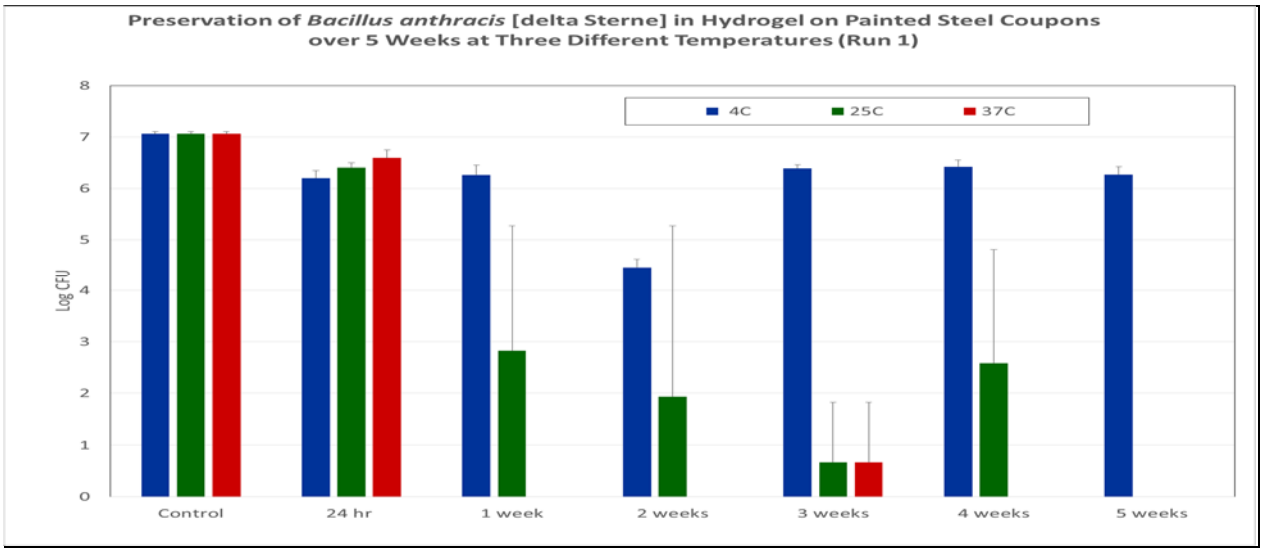

Figure 4. Preservation of *B. Anthracis* (ΔSterne) in biohydrogel on painted steel coupons over 5 weeks at three different temperatures, Run 1.

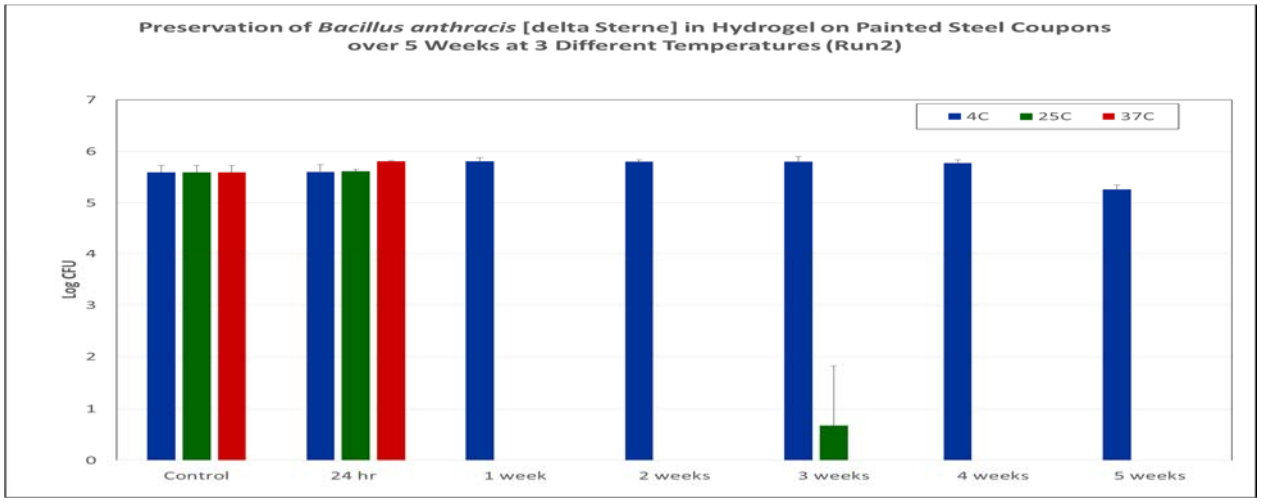

Figure 5. Preservation of *B. anthracis* (ΔSterne) in biohydrogel on painted steel coupons over 5 weeks at three different temperatures, Run 2.

4. CONCLUSIONS

Biohydrogel has proven to be an effective tool for sampling bacterial spores. It is as good, or even better, than currently available samplers and can be used in large-scale biological incidents. The current form of biohydrogel cannot be used to sample *S. aureus* spores because it has proven to be bactericidal; therefore, a designer gel with no or a significantly reduced amount of organic solvents is required. The experiments with *S. aureus* (and other vegetative cells) will be repeated in another study.

Biohydrogel was successfully demonstrated to be an effective sampler on pinewood coupons and screws when used for the recovery of *B. anthracis* (ΔSterne) spores. We will be performing more experiments using biohydrogel sampling on other 3D objects.

The current form of biohydrogel can be used for preserving spores up to 5 weeks, if samples are stored at 4 °C. Although CDC protocol dictates that samples should be used within 48 h of sampling and stored between 2 and 8 °C, samples can be stored longer with no spore loss if biohydrogel is used as a sampling tool. In this study, spores exposed to temperatures of 25 or 37 °C for more than 5 weeks did not survive. At these temperatures, the spores may have germinated in the biohydrogel, in which case, the vegetative cells would have died because biohydrogel has been shown to be bactericidal. Further preservation tests will be performed with a new designer gel (i.e., hydrogel with no bactericidal agent) to determine if spores can survive at 25 or 37 °C for more than 5 weeks. The designer gel will be used to determine the preservation of samples containing vegetative cells.

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