A Study on the Ability of an Organo-Selenium, Attached to a Cotton Dressing, to Inhibit *Candida albicans* Biofilm

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ABSTRACT

Statement of Purpose: *Candida* species are fungal pathogens known for their ability to cause superficial and systemic infections in the human host. These pathogens are able to persist inside the host due to the development of pathogenicity and multidrug resistance traits, often leading to the failure of therapeutic strategies. One specific feature of *Candida* species pathogenicity is their ability to form biofilms, which protects them from external factors such as host immune system defenses and antifungal drugs. The following research was carried out to determine the ability of organo-selenium compounds attached to a cotton dressing, to block the attachment and potential biofilm formation of *Candida albicans* onto the dressing.

Methods: Cotton wound dressings were prepared by covalently attaching organo-selenium (OS) to the dressing. It was shown that this OS treated dressing can catalyze the formation of superoxide formation (**Figure 1**) by the utilization of a luminometer..

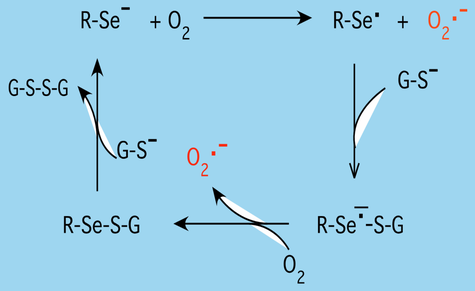

Figure 1. Equation showing the catalytic mechanism of superoxide production by selenium [1]

Superoxide formation in the dressing, is what is required to block the biofilm formation of the microorganisms. These dressings were then treated with a multi-antifungal drug resistant *Candida albicans* in vitro. After 48-hours of growth, the amounts of bacteria growing in the cotton wound dressings were determined by a colony forming unit (CFU) assay. and imaged by scanning electron microscopy. To test for stability, the dressings were soaked for over 2 years in phosphate buffered saline (PBS) and then tested by the CFU assay.

Results: Utilizing colony forming unit (CFU) assays, over 8 logs of inhibition (100%) were found for the *Candida albicans* strain 3147 on the OS materials containing 1% and 8.0% concentrations of selenium compound. The dressing containing 0.5% selenium showed over 4 logs of inhibition when compared with the control (untreated) dressing. These results are shown in **Figure 3**. Scanning electron microscopy (**Figure 4**) was also carried out on the dressing samples to confirm the results obtained with the CFU assays. Stability study (**Figure 5**) of dressing with selenium after soaking for 2 years in PBS.

Conclusion:

1.Cotton textile materials can be incorporated with selenium monomers.

2. Textiles attached with 1.0% and 0.8% Selenium inhibits over 8 logs of *C. albicans* biofilm formation.

3. Attaching Organo-Selenium to textiles is stable for over 2 years soaking in PBS and still exhibits inhibition of biofilm formation.

INTRODUCTION

Organo-selenium is unlike other biocidal agents, such as silver ions, in that selenium can be covalently attached to various materials with no loss of catalytic activity which is the ability to catalyze the formation of superoxide radicals ($O2^{\bullet -}$). These superoxide radicals play an important role in the killing of microorganisms [2]. Selenium can continuously produce superoxide radicals by giving an electron to oxygen and taking one from sulfur compounds that are present in body fluids. *Candida albicans* is a normal member of the host microbiota, colonizing the oral cavity, skin, gastrointestinal tract, and female genitourinary tract [3]. In healthy individuals, *C. albicans* growth is generally innocuous, however, overgrowth due to changes in the host microbiota, local environment and the host immune response, can cause superficial dermal and mucosal infections, such as diaper rash, vaginal yeast infections, and thrush [4]. In immunocompromised individuals, *C. albicans* can act as a life-threatening pathogen, invading the bloodstream and many of the major organs [5]. These infections are very difficult to treat due to the characteristics of these species: resistance to antifungal drugs, expression of virulence factors, and

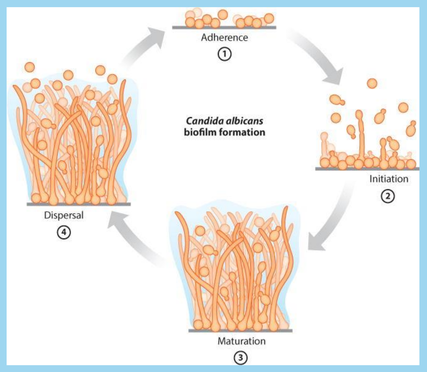

Figure 2. Figure 1 Stages of *Candida albicans* biofilm formation. ① Adherence of yeast-form cells to a surface. ② Initiation of cell proliferation, forming a basal layer of anchoring cells. ③ Maturation, including growth of hyphae concomitant with the production of extracellular matrix material. ④Dispersal of yeast-form cells from the biofilm to seed new sites [7].

Recently it has been shown that organo-selenium can be attached to different biomaterials and block the biofim formation of bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (8). These ranged from surface coatings on contact lenses, RO membranes as well as cellulose discs. These studies showed that a concentration of organo-selenium in the low micromolar range was sufficient to inhibit bacterial attachment to these materials, and longer periods of exposure to an aqueous environment do not compromise the effectiveness of the coating. In this study, we examined the effectiveness of a selenium attached cotton textile dressings in inhibiting the growth of *Candida albicans*.

MATERIALS & METHODS

Selenium: Our study utilized selenium Se-3321 (OS). This compound was synthesized as a diselenide in order to protect the selenium during polymerization and to ensure that the selenium can be activated later by a thiol addition. This organo-selenium was then mixed with different amounts of the diol that didn't contain selenium and then was attached to the textile material at the concentration of selenium of 0.5, 1, and 8% of selenium.

Biofilm Assay: A 1 cm^2 dressing was placed into each well of a 24-well plate containing YPB media. Both the OS treated cotton textile dressings and the untreated controls were inoculated with 102-103 colony forming units (CFU) of *Candida albicans* strain 3147 (ATCC 10231) and incubated at 37°C for 48 hours. After time had elapsed, the textiles were vortexed in phosphate buffer saline (PBS) to remove the *Candida albicans* biofilms. In order to determine the CFU, we plated 10-fold serial dilutions on YPD agar plates and incubated at 37°C. After 24 hours, the number of recovered *Candida albicans* that are attached to the dressings (CFU, colony forming unit) are determined.

Scanning Electron Microscopy: After 48 h of incubation at 37°C, the samples were freeze-dried overnight .Any attached media was gently wiped and shaken off from the samples. After mounting the samples, a thin layer of Pt was coated onto the samples to improve conductivity. The images were taken by Hitachi S/N-4300 under Environmental mode with 20 kV accelerating voltage and 300 Pa air pressure

RESULTS

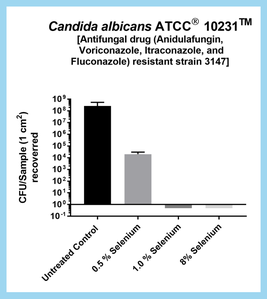

Figure 3.. Cotton dressings coated with organo-selenium, tested for the ability to inhibit biofilm formation by *Candida albicans*. Values represent the means of triplicate experiments ± standard errors.

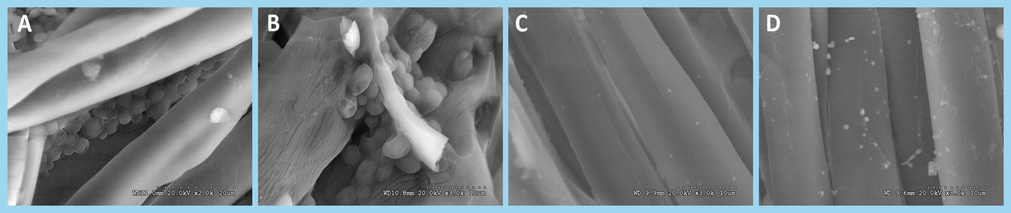

Figure 4. Scanning Electron Microscopy analysis of *Candida albicans* (A to D) biofilm formation on untreated (A) and OS-coated (0.5, 1, and 8% selenium) cotton dressings. Biofilms were allowed to form as described in the Materials and Methods. Three fields of view were examined from randomly chosen areas from the optical surface of each sample at magnification of ×2,000 and ×3,000 for the cotton dressings. Each experiment was conducted in triplicate. Representative fields of view are shown. Bars, 20 or 10 μm.

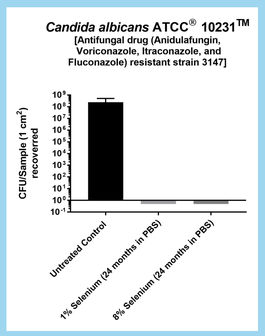

Figure 5. Organo-selenium coating on cotton dressing soaked for 2 years in (PBS). The samples then were dried, sterilized, and tested for the ability to inhibit biofilm formation by *Candida albicans* . Values represent the means of triplicate experiments ± standard errors.

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