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FUNGAL GROWTH ON MEDICAL DEVICES: IS *CANDIDA ALBICANS* CAPABLE OF FORMING BIOFILM ON A POLYSTYRENE SURFACE?

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Candida albicans is a fungus that normally inhabits human microbiota without causing symptoms. When the fungus overgrows, the human host develops local yeast infections or other serious diseases like invasive candidiasis. Majority of infections caused by *C. albicans* involve adherent cell communities called biofilms, which attach to medical devices such as catheters, dentures, and prosthetic joints. Biofilms resist anti-fungal compounds and are a gateway to re-occurring infections. The purpose of this study was to investigate if *C. albicans* could develop biofilm on the clinically used plastic, polystyrene. It was hypothesized that *C. albicans* would adhere to and develop quantifiable biofilm on a polystyrene surface.

Using a polystyrene-coated microtiter plate, *C. albicans* was allowed to adhere for 90 minutes by incubation at 37º C and develop over a period of 118 hours. Biofilms were quantified using a Crystal violet assay at 24, 48, 72, and 118 hours. The data supported the hypothesis; *C. albicans* cells adhered and developed biofilm on the plastic surface. Biofilms only developed for 24 hours, but persisted on polystyrene until 118 hours. It may be concluded that in clinical settings, *C. albicans* can produce persistent biofilms on polystyrene-made clinical devices.

Abbreviations: C albicans — Candida albicans

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INTRODUCTION

Fungi are commonly found in the environment, and serve as decomposers of organic matter. They are associated with human diseases such as opportunistic infections in immunocompromised individuals, and various allergic disorders. Fungal infections are a prodigious public health concern, and affect 1 billion of the world's population. 1.5 billion people die annually from complications related to fungal disease (www.microbiologysociety.org, 2016). Increased understanding of fungal infections can enable the development of treatment and prevention strategies for individuals and those who are at high risk. The fungal species *Candida albican*s is the focus of this study. Specifically, this study is investigates if *C. albicans* can adhere and grow as a biofilm on a polystyrene-coated solid surface.

Candida albicans

Candida asymptomatically inhabits human microbiota in the gastrointestinal tract, reproductive tract, oral cavity, and skin of the majority of individuals (Nobile, Johnson, 2015). *Candida* is a type of "yeast", and is a single celled organism. An overgrowth of *Candida* may result in a symptomatic disease. Over twenty species of *Candida* cause infection in humans, the most common species being *C. albicans*. This species causes 90% of reported yeast infections (Centers for Disease Control and Prevention, 2016) (Sahley, 2008) (Bennington-Castro, Sinha, 2014). An overgrowth and entry of *C.*

albicans into the bloodstream results in invasive candidiasis, or candidemia. Approximately 46,000 cases of invasive candidiasis occur yearly, and these are acquired in healthcare settings (Center for Disease Control and Prevention, 2015).

Biofilms

Biofilms can be defined as a community of adherent cells, which stick to a solid surface with the help of extracellular polysaccharides, unlike free-floating cells. They have a unique ability to endure high concentrations of anti-fungal compounds. The reason biofilms can resist being terminated by anti-fungal therapy is because anti-fungal compounds target individual cells and not adherent cell groups. Additionally, the polysaccharide substance produced by cells in a biofilm can block the action of anti-fungal (Nett, 2016). Biofilms grow on artificial surfaces like medical devices including catheters, dentures, and prosthetic joints. They can also grow on biotic or biological surfaces like mucosa. Majority of *Candida*-caused infections involve biofilms on artificial or biological surfaces (Nett, 2016). The National Institutes of Health reported that biofilms are responsible for 80% of all microbial infections in America (Nobile, Johnson, 2015). *C. albicans* is known to produce highly structured biofilms with multiple cell types. They have an intricate design with yeast, pseudohyphal, and hyphal morphologies implanted in an extracellular matrix (Förster, Mogavero, Dräger, Graf, Polke, Jacobson, Hube, 2016).

HYPOTHESIS AND VARIABLES

It is reasonable to hypothesize that *C. albicans* will adhere to and develop as a quantifiable biofilm on a polystyrene surface. The growth environment for *C. albicans*, the chosen surface (polystyrene) for biofilm formation and Crystal violet based assay conditions for biofilm quantification were all maintained as controlled variables in the study. The independent variable was the time after initiating the formation of *C. albicans* biofilm on the polystyrene plate, and the dependent variable was the absorbance at 595 nm, which was indicative of the amount of *C. albicans* biofilm.

MATERIALS AND METHODS

Candida albicans growth

Candida albicans (American Type Culture Collection (ATCC #18804), Manassas, USA) was purchased as lyophilized powder and re-suspended in 5 ml of sterile water. 100 µl of re-suspended *C. albicans* was aliquoted into 1.5 ml microcentrifuge tubes. Stock yeast cultures were grown on yeast-peptone-dextrose (YPD) agar and incubated at 37º C. Before beginning the biofilm assay, liquid cultures of *C. albicans* were initiated by inoculating 30 ml of YPD media with a loopful of *C. albicans*. The cultures were grown on a shaker at room temperature for ~38 hours to allow yeast cells to reach logarithmic growth phase. For biofilm formation, cells were counted using a hemocytometer, and a suspension with 10^7cells/ml was prepared.

Biofilm Adhesion Assay

For the adhesion assay, 100µl of cell suspension was added to 60 wells of a 96 well polystyrene-coated microtiter plate (Fisher Scientific Inc., Asheville, NC). After 90-minute incubation at 37°C, the free-floating cells contained in the media were removed by discarding the media. The cells were washed with 200 µl of sterile Phosphate Buffered Saline (PBS; Gibco Laboratories, Gaithersburg, MD). 100 µl of 1% w/v Crystal violet was added to each well and incubated at 37°C for 20 minutes. The plate was then washed twice with 200 µl of sterile PBS. 200 µl of 95% ethanol was added to each well as a solvent for crystal violet that remained attached to the yeast cells. 100 µl of 95% ethanol was then transferred to a new microtiter plate reader for the absorbance to be measured at 595 nm using a Synergy HT plate reader (BioTek Instruments Inc., Winooski, Vermont, USA). 100 µl of 95% ethanol was maintained as a blank during absorbance measurements.

Biofilm Development Assay

For the development assay of the *C. albicans* biofilm, the cells were allowed to adhere for 90 minutes by incubation at 37°C, as previously described. After 90 minutes, the free-floating cells were removed by discarding the media, and fresh YPD media was added to each well. The biofilm was allowed to develop for up to 118 hours by incubation at 37°C, and the media was replaced daily. The amount of biofilm was quantified using a Crystal violet assay (as previously described) at 24, 48, 72, 118 hours. The readings were averaged and compared, and the p value < 0.05 for all designated times.

RESULTS

C. albicans cells adhered as biofilm to the surface of the microtiter plate after 90 minutes. Quantifiable biofilms continued to adhere and develop between 90 minutes and 24-hour time periods. Subsequently, the biofilm growth did not progress as shown in both **Figures 1 and 2**. The experiment and data collection lasted 118 hours.

DISCUSSION

The purpose of this study was to investigate if *C. albicans* could adhere and grow on a polystyrene surface. It was hypothesized that *C. albicans* would adhere and continue to grow as a biofilm on the previously noted surface. The data collected from this study supported the hypothesis, as proved in **Figures 1-2**. Development of *C. albicans* biofilm ceased progression after 24 hours. Biofilm may proliferate longer than 24 hours, but not on a polystyrene surface. The data concluded is important for a continuation of this investigation on biofilm development in relation to clinical surfaces. This study is phase one in an experiment involved with the investigation of biofilm and medical devices. The clinical relevance of fungal biofilms is substantial as a growing number of people are developing diseases that are resisting anti-fungal medication. Studies conducted on these cell communities are essential in improving treatment for diagnosed people and for disease prevention.

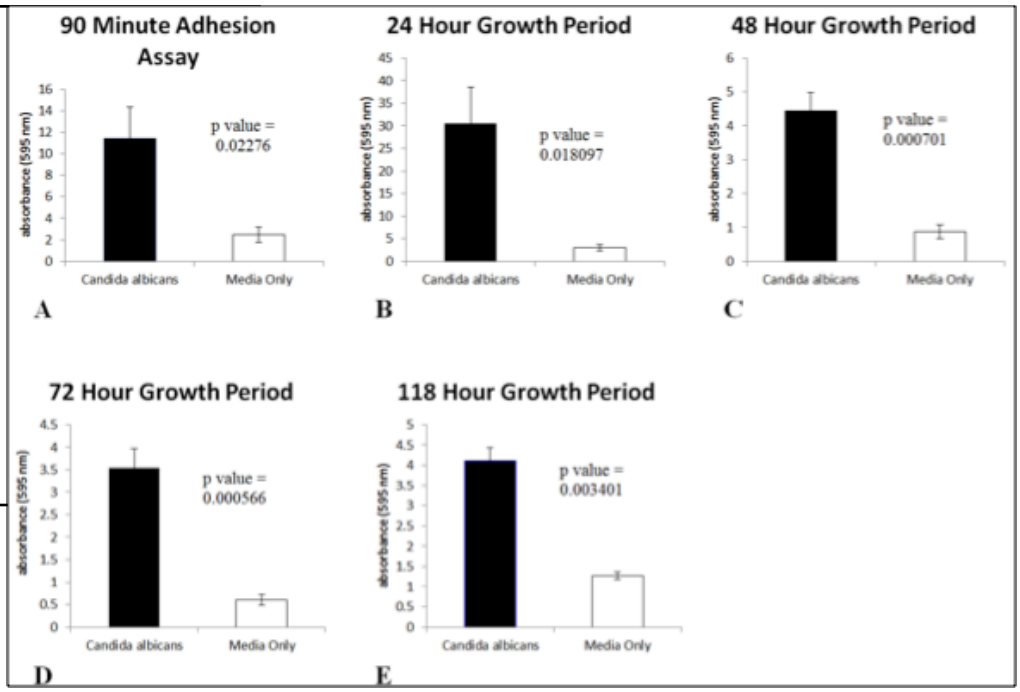
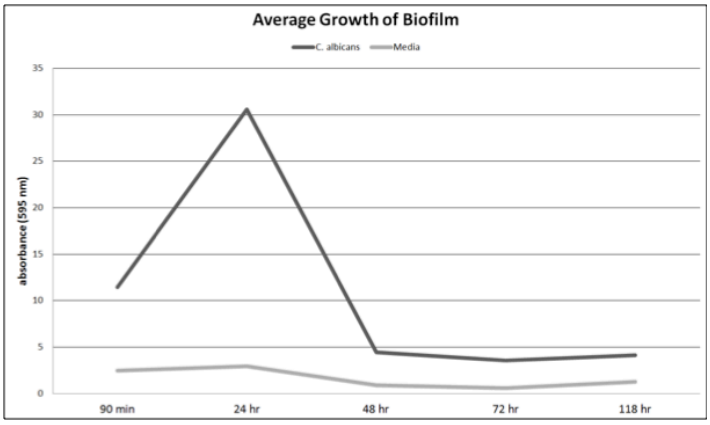


Figure 1: The absorbance was quantified at time periods labeled (A-E) of *C. albicans* cells and compared to regular media cells. Chart A shows significant adhesion to a polystyrene surface. Charts B-E show minimal development after a 24-hour period.

Figure 2: *C. albicans* cells adhered to a polystyrene surface after a 90-minute adhesion assay. Peak growth is between the 90 minutes and 24-hour time. The biofilm did not develop after 24 hours, but still inhabited on the microtiter plate.



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MATERIALS INDEX

1. Growth Medium:
- a. Yeast-Peptone-Dextrose Agar and Broth: Ingredients **Broth consists of same ingredients except agar*
  - i. Bacto-Agar-20g
  - ii. Bacto-peptone-20g
  - iii. Bacto-yeast extract-10g
  - iv. Distilled water-1L
  - v. Glucose-20g
- b. Sabouraud-Dextrose Agar: Ingredients
  - i. Agar concentrated power-65g
  - ii. Distilled water-1L
- c. Materials
  - i. aluminum foil
  - ii. balance
  - iii. beaker
  - iv. 1L bottle- autoclavable
  - v. flask
  - vi. petri dishes
  - vii. steam autoclave
  - viii. spatulas
  - ix. stir bar
  - x. weigh boats

2. Hemocytometer Counts
- a. Automatic pipet- specifically p 200
- b. Distilled water **interchangeably used with non-inoculated media*
- c. Hemocytometer
- d. Inoculated medium (*C. albicans*)
- e. Manual tally counter
- f. Microscope- 40x

3. Spectrophotometer Tests
- a. Absorbance spectrophotometer
- b. Automatic pipet
- c. Chemical wipes
- d. Computer program to read data ** this study used Logger Pro ver. 3.11*
- e. Cuvettes
- f. YPD broth

4. Biofilm
- a. Initiating *C. albicans* culture
  - i. Hemocytometer
  - ii. Lab shaker
  - iii. YPD media in petri dish
  - iv. YPD broth in 50ml centrifuge tube
- b. Adhesion Assay/Development Assay
  - i. Automatic pipet- specifically p 200
  - ii. Cell culture
  - iii. Crystal violet solution- 1% w/v
  - iv. Ethanol- 95% v/v
  - v. Phosphate Buffered Saline (PBS) - sterile
  - vi. Non-sterile microtiter plate- 96 well reader
  - vii. Polystyrene microtiter plate - 96 well
  - viii. Synergy HT plate reader

5. Biosafety cabinet, cold room, incubator