



5-2009

The Influence of Biofilm on the Antifungal Activity of Amine Oxide

Jonathan Michael Hart
University of Tennessee Health Science Center

Follow this and additional works at: <https://dc.uthsc.edu/dissertations>



Part of the [Prosthodontics and Prosthodontology Commons](#)

Recommended Citation

Hart, Jonathan Michael , "The Influence of Biofilm on the Antifungal Activity of Amine Oxide" (2009).
Theses and Dissertations (ETD). Paper 108. <http://dx.doi.org/10.21007/etd.cghs.2009.0126>.

This Thesis is brought to you for free and open access by the College of Graduate Health Sciences at UTHSC Digital Commons. It has been accepted for inclusion in Theses and Dissertations (ETD) by an authorized administrator of UTHSC Digital Commons. For more information, please contact jwelch30@uthsc.edu.

The Influence of Biofilm on the Antifungal Activity of Amine Oxide

Abstract

Denture-related stomatitis (DRS) is the most common oral disease affecting denture wearers today, and is characterized by inflammation of the denture bearing mucosa. In a search through historical dental literature for the most often suspected culprit of DRS, one will repeatedly find first listed the fungus *Candida albicans*. While *C. albicans* can be isolated from the fitting surfaces of dentures, and the supporting mucosa of patients suffering from DRS, it is also found in the oral cavity of 65% of the human population—most of which is free from oral disease. A truth puzzling to investigators, the discovery of a self-produced, protective matrix present around colonies of communal bacteria and fungi (or biofilm) began to shed light on that troubling finding.

Various investigators have shown that fungal cells in a biofilm community tend to be more resistant to anti-fungal agents than freshly grown cells. Further, it has been shown that organisms thrive in a biofilm environment, and can behave differently than when they are out of the community. It is not clearly understood, however, why these changes occur. Regardless, *C. albicans* and its cell components can elicit stimulation of inflammatory cytokines by human monocytes, *in vitro*, and it is believed that this interaction with monocytes likely leads to the gingival inflammation evident in DRS.

Post-infection therapies for DRS are known to the profession. Prescription antifungal creams alone or in combination with tissue conditioning are tried-and-true methods of treating DRS, however, effective *preventive* interventions are not readily available. If found, preventive therapy initiated before the onset of DRS could prevent the most common disease affecting denture wearers today.

The current study focused on the influence of the surfactant amine oxide (AO) on fresh *C. albicans* and *C. albicans* cultured from biofilms, and the subsequent impact of that interaction on human monocyte production of IL-1 β and TNF- α . This study also addressed the impact that AO has on biofilm formation by examining the viability of cells cultured from that AO-modified matrix. If found effective, AO may be valuable in preventing or reducing the incidence of DRS mediated by the fungal pathogen *C. albicans*. Elucidating the differences between the interaction of fresh and biofilm grown *C. albicans* with human monocytes will help clarify the specific host response to this fungal pathogen and shed light on its effect on the chemical mediators of DRS.

Currently, chemical biocides are used to control microbial growth and plaque accumulation. Those agents include sodium hypochlorite and various surfactants. As previously mentioned, this study assessed the antifungal activity of the surfactant AO. The hypotheses of this study are: (1) AO is fungistatic and fungicidal against *C. albicans*; (2) AO will prevent or reduce fungal biofilm formation on denture acrylic surfaces; and (3) biofilm grown *C. albicans* will stimulate human monocytes to secrete a greater amount of inflammatory cytokines than cells derived from fresh culture.

Results of this study indicate a significant difference in both viability and monocyte stimulatory ability following exposure to AO between both fresh and biofilm-cultured cells. The degree of AO activity was found to be time and concentration dependent as higher concentrations of AO and higher exposure times to AO were found more effective at killing fungal cells. Further, AO was found effective in altering some protective characteristic of biofilms. The presence of the surfactant during matrix formation rendered inhabitant cells more subject to AO's antifungal activity than cells cultured from biofilms allowed to develop outside the presence of AO.

These results suggest that AO is effective at reducing the number of viable *C. albicans* cells isolated from fresh and biofilm culture, as well as fungal cells located in a biofilm environment. Further, this series of experiments suggest that by reducing the number of viable fungal cells, AO is also capable of indirectly

decreasing the activity of human monocytes to release the inflammatory cytokines IL-1 β and TNF- α . Future investigations should address the intraoral applications of AO, and its effectiveness in a clinical setting.

Document Type

Thesis

Degree Name

Master of Dental Science (MDS)

Program

Prosthodontics

Research Advisor

Jegdish P. Babu, Ph.D.

Keywords

Biofilm, Amine Oxide

Subject Categories

Dentistry | Medicine and Health Sciences | Prosthodontics and Prosthodontology

**THE INFLUENCE OF BIOFILM ON THE ANTIFUNGAL ACTIVITY OF
AMINE OXIDE**

A Thesis
Presented for
The Graduate Studies Council
The University of Tennessee
Health Science Center

In Partial Fulfillment
Of the Requirements for the Degree
Master of Dental Science
From The University of Tennessee

By
Jonathan Michael Hart
May 2009

Copyright © 2009 Jonathan Michael Hart
All rights reserved

ACKNOWLEDGEMENTS

I would like to express my gratitude to everyone who has helped me in my endeavor of pursuing a Master of Dental Science in Prosthodontics. To Dr. Jeg Babu, without his immense knowledge and hard work, this research project would not have been possible. To my committee members, Drs. Dave Cagna, Mark Anderson, David Tipton, Ed Thomas, and Audrey Selecman for their advice and experience. To the American College of Prosthodontists Education Foundation and The University of Tennessee Alumni Foundation for their monetary support of this project. Lastly, I must thank my family for their love and support.

ABSTRACT

Denture-related stomatitis (DRS) is the most common oral disease affecting denture wearers today, and is characterized by inflammation of the denture bearing mucosa. In a search through historical dental literature for the most often suspected culprit of DRS, one will repeatedly find first listed the fungus *Candida albicans*. While *C. albicans* can be isolated from the fitting surfaces of dentures, and the supporting mucosa of patients suffering from DRS, it is also found in the oral cavity of 65% of the human population—most of which is free from oral disease. A truth puzzling to investigators, the discovery of a self-produced, protective matrix present around colonies of communal bacteria and fungi (or biofilm) began to shed light on that troubling finding.

Various investigators have shown that fungal cells in a biofilm community tend to be more resistant to anti-fungal agents than freshly grown cells. Further, it has been shown that organisms thrive in a biofilm environment, and can behave differently than when they are out of the community. It is not clearly understood, however, why these changes occur. Regardless, *C. albicans* and its cell components can elicit stimulation of inflammatory cytokines by human monocytes, *in vitro*, and it is believed that this interaction with monocytes likely leads to the gingival inflammation evident in DRS.

Post-infection therapies for DRS are known to the profession. Prescription antifungal creams alone or in combination with tissue conditioning are tried-and-true methods of treating DRS, however, effective *preventive* interventions are not readily available. If found, preventive therapy initiated before the onset of DRS could prevent the most common disease affecting denture wearers today.

The current study focused on the influence of the surfactant amine oxide (AO) on fresh *C. albicans* and *C. albicans* cultured from biofilms, and the subsequent impact of that interaction on human monocyte production of IL-1 β and TNF- α . This study also addressed the impact that AO has on biofilm formation by examining the viability of cells cultured from that AO-modified matrix. If found effective, AO may be valuable in preventing or reducing the incidence of DRS mediated by the fungal pathogen *C. albicans*. Elucidating the differences between the interaction of fresh and biofilm grown *C. albicans* with human monocytes will help clarify the specific host response to this fungal pathogen and shed light on its effect on the chemical mediators of DRS.

Currently, chemical biocides are used to control microbial growth and plaque accumulation on denture surfaces. Those agents include sodium hypochlorite and various surfactants. As previously mentioned, this study assessed the antifungal activity of the surfactant AO. The hypotheses of this study are: (1) AO is fungistatic and fungicidal against *C. albicans*; (2) AO will prevent or reduce fungal biofilm formation on denture acrylic surfaces; and (3) biofilm grown *C. albicans* will stimulate human monocytes to secrete a greater amount of inflammatory cytokines than cells derived from fresh culture.

Results of this study indicate a significant difference in both viability and monocyte stimulatory ability following exposure to AO between both fresh and biofilm-cultured cells. The degree of AO activity was found to be time and concentration dependent as higher concentrations of AO and higher exposure times to AO were found more effective at killing fungal cells. Further, AO was found effective in altering some protective characteristic of biofilms. The presence of the surfactant during matrix formation rendered inhabitant cells more subject to AO's antifungal activity than cells cultured from biofilms allowed to develop outside the presence of AO.

These results suggest that AO is effective at reducing the number of viable *C. albicans* cells isolated from fresh and biofilm culture, as well as fungal cells located in a biofilm environment. Further, this series of experiments suggest that by reducing the number of viable fungal cells, AO is also capable of indirectly decreasing the activity of human monocytes to release the inflammatory cytokines IL-1 β and TNF- α . Future investigations should address the intraoral applications of AO, and its effectiveness in a clinical setting.

TABLE OF CONTENTS

CHAPTER 1. INTRODUCTION	1
CHAPTER 2. REVIEW OF LITERATURE	3
Polymethyl Methacrylate (PMMA)	3
The Complete Denture	3
<i>Candida albicans</i>	4
Biofilm	4
Denture-Related Stomatitis (DRS)	4
Amine Oxide	6
CHAPTER 3. RESEARCH OBJECTIVES	7
Specific Aim I	7
Specific Aim II	8
Specific Aim III	8
CHAPTER 4. METHODS AND MATERIALS	9
Amine Oxide	9
Fungal Strains	9
Denture Substrate Material	9
Preparation of Fungal Biofilms	9
Antifungal Activity of Amine Oxide	10
XTT Cell Proliferation Assay	10
THP-1 Cells	10
IL-1 β and TNF- α Cytokine Assay	11
Data Analysis	11
CHAPTER 5. RESULTS	12
Effect of pH on Antifungal Activity of AO	12
Effect of AO on Adherence of <i>C. albicans</i> to PMMA Discs	12
Influence of AO on Salivary Pellicle Formation on PMMA Discs	12
Effect of AO Exposure Time on Viability of <i>C. albicans</i> .	12
Effect of AO on Fresh and Biofilm-Cultured <i>C. albicans</i> .	17
Effect of AO on Biofilm Formation on PMMA Discs	17
Effect of AO on IL-1 β Secretion by THP-1 Cells.	17
Effect of AO on TNF- α Secretion by THP-1 Cells	21
CHAPTER 6. DISCUSSION	23
Effect of pH on Antifungal Activity of AO	23
Effect of AO on Adherence of <i>C. albicans</i> to PMMA Discs	23
Influence of AO on Salivary Pellicle Formation on PMMA Discs	23
Effect of AO Exposure Time on Viability of <i>C. albicans</i> .	24
Effect of AO on Fresh and Biofilm-Cultured <i>C. albicans</i> .	25

Effect of AO on Biofilm Formation on PMMA Discs	25
Effect of AO on IL-1 β Secretion by THP-1 Cells.	26
Effect of AO on TNF- α Secretion by THP-1 Cells	26
CHAPTER 7. CONCLUSION	27
LIST OF REFERENCES	28
VITA	32

LIST OF TABLES

Table 1	Effect of pH on Antifungal Activity of AO	13
Table 2	Effect of AO on Adherence of <i>C. albicans</i> to PMMA Discs . . .	14
Table 3	Influence of AO on Salivary Pellicle Formation on PMMA Discs. . .	15
Table 4	Effect of AO Exposure Time on Viability of <i>C. albicans</i>	16
Table 5	Effect of AO on Fresh and Biofilm-Cultured <i>C. albicans</i>	18
Table 6	Effect of AO on Biofilm Formation on PMMA Discs	19
Table 7	Effect of AO on IL-1 β Secretion by THP-1 Cells	20
Table 8	Effect of AO on TNF- α Secretion by THP-1 Cells. . . .	22

LIST OF FIGURES

Figure 1	Effect of pH on Antifungal Activity of AO	13
Figure 2	Effect of AO on Adherence of <i>C. albicans</i> to PMMA Discs	14
Figure 3	Influence of AO on Salivary Pellicle Formation on PMMA Discs	15
Figure 4	Effect of AO Exposure Time on Viability of <i>C. albicans</i>	16
Figure 5	Effect of AO on Fresh and Biofilm-Cultured <i>C. albicans</i>	18
Figure 6	Effect of AO on Biofilm Formation on PMMA Discs	19
Figure 7	Effect of AO on IL-1 β Secretion by THP-1 Cells.	20
Figure 8	Effect of AO on TNF- α Secretion by THP-1 Cells.	22

CHAPTER 1. INTRODUCTION

Dr. Poul Petersen perhaps said it best in a 2005 article when he stated, “A demographic revolution is underway” (1). According to studies conducted by the United Nations, the age group categorized as older persons (those over 60) is growing faster than any other age group. It is estimated that world-wide, over 600 million people are over the age of 60, and it is further figured that this number will double over the next 25 years and more than triple in the next 50 years, reaching the billions. Eighty-percent of those individuals will live in industrialized countries, with close to 100 million living in the U.S. by the year 2050 (1).

In America, 26% of individuals 60 and older are edentulous (2). Of this 26% of today’s population, many are wearing complete dentures that are not properly maintained. While the suggested life span of a complete denture is between 5-7 years (3), literature suggests that the average denture in America is approximately 17.6 years old (4)! When a denture is in service for more than 7 years, it is frequently necessary that it be lined to maintain adequate fit and retention. Sadly, few seek proper care for their ‘over-worked’ prosthesis, and as a result much soft and hard tissue irritation and remodeling may result, leading to what is commonly referred to as ‘denture-related stomatitis’ (DRS). DRS is an inflammatory process of the mucosa that has been found to occur in 45-60% of the denture wearing population (5).

In the absence of proper denture hygiene, an environment (or biofilm) favorable for the proliferation of microorganisms may develop. A biofilm is defined as a structured community of microorganisms surrounded by a self-produced polymeric matrix that is adherent to an inert or living surface (6). It consists of a thick layer of bacteria, yeast, and desquamated epithelial cells containing more than one-trillion organisms per gram in wet weight. Without mechanical and/or chemical removal of this biofilm, a network of fungal and bacterial microorganisms may form between the intaglio surface of a prosthesis, and the opposing soft tissue, likely resulting in DRS (7). *C. albicans* can usually be isolated from a biofilm on both the fitting surface of the denture and the denture-bearing mucosa of DRS patients (5).

While in most cases a candida infection can be controlled with antifungal medications (either locally or systemically), control is difficult in persons with immunodeficiencies (e.g., HIV/AIDS) (8). In fact, organisms may become resistant to standard therapy, so prevention is key.

Surfactants (*surface-active-agents*) are used in a variety of household products as emulsifying agents to trap dirt, oils, bacteria, and other debris located on skin, clothes, dishes, and even dentures. Surfactants are simple molecules consisting of a hydrophilic head (i.e., N = O) and hydrophobic tail (long carbon chain) (9). Amine Oxide (AO; C10-C16-alkyldimethyl N-oxides) is an example of a surfactant. AO is an amphoteric, cationic surfactant. These surfactants are not only very mild (making them useful for personal care and household cleaning products), but also have a disinfecting/sanitizing

property (imparted by the cationic nature of the molecule) (9) that has recently found an oral application. In a study by Fraud, *et al.*, AO was found to be an excellent biocide for eliminating *Streptococcus mutans* biofilms from hydroxyapatite discs (10,11). No research has been done on the efficacy of AO as a biocide to eliminate *C. albicans* from denture acrylic surfaces. If found effective at reducing biofilm formation and/or its ability to harbor viable fungal cells, AO could promise to be a mechanism of prevention of DRS that currently evades the profession.

CHAPTER 2. REVIEW OF LITERATURE

Polymethyl Methacrylate (PMMA)

The principal type of material utilized for the fabrication of oral and maxillofacial prostheses is heat-activated PMMA (12). It has remained a predictable and effective tool in dentistry for the past nearly seventy years namely because it is not only economical, but easy to use (13). Technology is no stranger to denture acrylic, as its advancements have consistently improved the properties of PMMA since its inception in 1937 (13). However, one property not likely to be eliminated in heat-cured PMMA is the issue of porosity. The polymerization of heat-processed denture acrylic is an exothermic reaction initiated by a heat-activated chemical element (14). While porosity is less of an issue at the surface of the acrylic, the body of the material remains at risk due to boiling monomer within the depth of the acrylic caused by poor conduction of heat by the material itself. The product of this reaction is a very stable and strong material that although seemingly completely dense remains essentially a porous structure.

The Complete Denture

A common prosthesis fabricated using PMMA is the complete denture. The denture base derives its support through intimate contact with the underlying oral tissues (14), a result not easily achieved even at delivery of a new prosthesis. Conventionally heat-cured, compression molded PMMA has a volumetric shrinkage of 7% and linear shrinkage of less than 1% on average (14). The need of clinical and laboratory remounts along with adjustment of the intaglio surface of the new prosthesis evidence these minor, yet significant changes from the pre-processed state of the complete denture (15). Likened to a time capsule by some authors, a complete denture remains, over time, an accurate representation of the state of the denture bearing oral mucosa at the time of master impression in creation of the prosthesis.

The lifespan of a complete denture is not clearly defined in literature due in part to the subjective nature of that determination, however, anecdotally, 5-7 years is an accepted term of many (3). The need for a new prosthesis by the patient remains the true definer of the lifespan of a complete denture, and is usually a result of lack of fit, and a consequent decrease in function. While that finding is expected to occur in the 5-10 year range, the vast majority of denture wearers are more hopeful, with the average complete denture in America being 17.6 years old (4).

With an increase in age of an un-modified prosthesis comes a decrease in its stability as a result in the non-intimate contact of its intaglio surface to the underlying mucosa. What is created at that point is a space sufficient for the retention of food, saliva, denture adhesive, and native bacteria and fungi.

Candida albicans

Candida albicans is the most common fungus isolated from the human oral cavity, being found in over 65% of the population. Introduction into the oral cavity may occur via many routes including kissing others whose mouths are colonized by the fungus, to ingesting contaminated processed and unprocessed food (16). It is a polymorphic fungus consisting of a thick cell wall that preferentially colonizes mucosal surfaces of the GI tract. Components of the cell wall (adhesins) mediate the binding of *C. albicans* to other cells (host or microbial), inert polymers, or proteins (16). It is this adhesion that allows *C. albicans* to colonize the oral cavity. Without adhesion, the yeast cells would be washed out by saliva and swallowed (16). While *C. albicans* will grow more quickly and reach higher cell concentrations in moist environments (16), if the degree of contamination is high enough, cells can remain viable on dry surfaces for at least 24 hours (16,17).

Biofilm

A biofilm is a structured community of micro-organisms surrounded by a self-produced polymeric matrix, and is adherent to an inert or living surface (5,6). Biofilms may easily form on intraoral prostheses in the absence of good oral hygiene. As a result, a thick biofilm of bacteria, yeast, and desquamated epithelial cells containing more than one-trillion organisms per gram in wet weight may quickly form. The biofilm matrix is believed to alter the characteristics of its inhabitants through not only mechanical and chemical protection from surrounding agents, but also from intimate interaction between bacterial and fungal neighbors (18). In fact, certain mechanisms of phagocytosis of fungal cells are ineffective when the fungus is located in this biofilm environment (19). Biofilms have also been found effective at increasing the antifungal resistance of fungal inhabitants. Lamfon, *et al.* has shown that biofilms can increase the antifungal resistance of its fungal tenants up to one thousand times over that of planktonically grown cells (20). Without mechanical and/or chemical removal of this biofilm, this network of fungal and bacterial microorganisms may persist on the intaglio surface of a prosthesis likely resulting in the type of local tissue inflammation characteristic of DRS (7).

Denture-Related Stomatitis (DRS)

A disease characterized by a lesion that is almost invariably asymptomatic and usually affects the hard palate, DRS is defined as an inflammatory process of the mucosa underlying a removable, partial, or total dental prosthesis or appliance (7). *Candida albicans* has been associated with DRS since 1936 (21), and remains one of the most suspected major agents of this disease process. While the pathogenesis of DRS is not fully understood, it is known that it is not an allergic reaction to *C. albicans* (22) that leads to the characteristic inflammation associated with the disease. There are, however, many other theories and models of likely modes of infection. Whether due to invasive forms of the fungus (23), a compromised state of the immune system (24), or alteration of

the typical monocyte release of inflammatory cytokines and their mediators (25), denture stomatitis remains a persistent problem for dentists and their edentulous patients. What is consistent throughout literature is the consensus on the first step in the infection process of DRS: the adherence of *C. albicans* to oral epithelial cells. Without that mechanism, the fungus would be unable to overcome the normal flushing of saliva through the oral cavity, and would not be able to reach an infective number of colony forming units in its habitat (26). When invaded by *C. albicans*, oral epithelial cells are the frontline defense mechanism of the body, releasing large numbers of pro-inflammatory and immunoregulatory cytokines including IL-1 β and TNF- α (27). These cytokines are believed to target cell surface components of *C. albicans*. While this mechanism is effective, various studies have shown the ability of *C. albicans* to modify the characteristics of its cell surface based on not only the temperature of its environment, but also the pH and nutrient content (25,28) of the oral cavity. Other recently reported cell-mediated fungicidal mechanisms involve the hyaluronic acid that is bound to the epithelial cell wall and excreted by the cell itself (29). The mechanism of action of hyaluronic acid has not been identified.

The fungus maintains in its own defense not only the ability to modify its own cell surface characteristics (as earlier described), but also to fabricate a protective matrix or biofilm. Perhaps more effective in evading host defensive mechanisms, the biofilm environment provides not only a physical barrier to clearance mechanisms of the oral cavity and host cells, but also serves as a venue for phenotypic alteration of its inhabitants (18,20). Reports in literature attribute this physical barrier and eventual phenotypic alteration as significant contributors to DRS initiation and perpetuation as well as a platform to developed resistance to traditional antifungal therapies (18,20,30). Factoring in the belief that poorly fitting dentures predispose to a candida infection by breaking down the epithelial barrier (31) of the oral cavity, the pathogenicity and astonishing number of individuals that experience DRS becomes less of an enigma.

According to literature, 65% of current denture wearers will experience DRS at one point in their lifetimes, resulting in not only localized, chronic inflammation, but also modification of the denture bearing mucosa, thus making stability of the complete denture an exception, instead of a rule. PMMA used to fabricate complete dentures serves as a perfect environment for colonization by bacteria and fungi. Micro-porosity of the denture base combined with space present beneath an ill-fitting prosthesis accommodate the colonization of these surfaces by biofilms of fungi and bacteria (31). Large levels of colonization and tissue invasion create a situation not easily overcome by the traditional tissue conditioning and antifungal therapy. Some authors propose the eventual use of immunomodulatory drugs to treat denture stomatitis hoping to alter the host response to fungus invasion (32). However, as identified by Perezous, *et al.*, treatments aimed at only the intraoral condition of the infection may result in an immediate decrease in inflammation, but the effect is short lived if the fungus is not eliminated from the denture surface as well (24).

Studies of the effectiveness of surface charges on acrylic resin to inhibit fungus hyphae attachment are reported in recent literature (33,34,35). Commercially available

denture cleaning agents have also undergone the scrutiny of recent research (36). While both reports claim effective prevention of fungal cell attachment to acrylic surfaces, they do not address prevention or dissolution of biofilm attachment. Also, neither group of studies purports a fungicidal or fungistatic mechanism to their experimental material. As reported collectively in literature on DRS, there is a clear need for further investigation of factors that may moderate the adhesion of organisms and subsequent colonization of denture base materials *and* oral mucosa.

Amine Oxide

Surfactants (*surface-active-agents*) are used in a variety of household products as emulsifying agents to trap dirt, oils, bacteria, and other debris located on skin, clothes, dishes, and even dentures. Surfactants are simple molecules consisting of a hydrophilic head (i.e., N=O) and hydrophobic tail (long carbon chain) (9). Discovered and studied before 1900 (37), amine oxide (AO; C10-C16-alkyldimethyl N-oxides) is an example of a surfactant. AO is an amphoteric, cationic surfactant. These surfactants are not only very mild (making them useful for personal care and household cleaning products), but also have a disinfecting/sanitizing property (imparted by the cationic nature of the molecule) (9) that has recently found an oral application. In a study by Fraud, *et al.*, AO was found to be an excellent biocide for eliminating *Streptococcus mutans* biofilms from hydroxyapatite discs (10,11). However, efforts to apply AO as a surfactant to control *C. albicans* in complete denture patients with DRS have not been investigated.

CHAPTER 3. RESEARCH OBJECTIVES

DRS is an erythematous inflammatory reaction of the denture bearing mucosal layer of the oral cavity. *Candida albicans* (the most common fungal pathogen associated with DRS) has been shown to cause significant problems in both healthy and immunocompromised individuals (24). The ability of *C. albicans* to colonize denture material and readily form biofilms imparts to the fungus a resistance to antifungal agents (18,20). This may constitute a significant public health problem, particularly in immunocompromised individuals (24). Blocking fungal colonization and biofilm formation on denture base material in an effort to prevent *C. albicans*-mediated DRS has been identified as a 'clear need' in reviews on the topic (21).

Studies have shown that *C. albicans* and cell components elicit stimulation of inflammatory cytokines by human monocytes, *in vitro* (25,27,28). However, the interaction of biofilm grown *C. albicans* versus freshly cultured cells with human monocytes has not been studied. It is possible that biofilm-grown cells differ in either their ability to stimulate human monocyte release of different types of cytokines, different quantities of cytokines, or both.

Studies have demonstrated that a biofilm environment evokes changes in microorganisms, whereby biofilm-associated organisms behave differently than freshly cultured cells (18,20). Such changes have been further demonstrated by various investigators by studying the differences in *C. albicans* sensitivity to commonly used antifungal agents. Determining how these changes may alter a host response to *C. albicans* may prove useful. Thus, a comparison of the interaction of freshly-grown *C. albicans* with human monocytes to biofilm-grown *C. albicans* with human monocytes was accomplished in this project.

The bacteriostatic and bactericidal activity of AO on several microorganisms has recently been reported (10,11). AO has been shown to possess anti-bacterial function, and to reduce oral streptococcal plaque and biofilm formation. This prompted us to further investigate the antifungal activity of AO, and examine AO's ability to prevent the formation and/or dissipation of biofilm formed on denture acrylic surfaces by *C. albicans*. The following specific aims and hypotheses were formulated to focus this study.

Specific Aim I

Study the fungicidal and fungistatic properties of AO on freshly cultured and biofilm-grown *C. albicans*. It is hypothesized that AO is fungistatic and fungicidal against *C. albicans*.

Specific Aim II

Investigate the effect of AO on (a) the formation of *C. albicans* biofilm formation on PMMA discs and (b) Study the effect of AO on the preformed biofilm on PMMA discs. It is hypothesized that AO will prevent or reduce fungal biofilm formation on denture acrylic surfaces.

Specific Aim III

Investigate the interaction of freshly cultured and biofilm-grown *C. albicans* extracts with cultured monocytic THP-1 cells to secrete cytokines, IL-1 β and TNF- α . It is hypothesized that biofilm-grown *C. albicans* will be a better stimulant of monocyte secreted inflammatory cytokines than the freshly cultured cells.

The results of this study may help clarify the pathogenic role of *C. albicans* in and out of a biofilm environment. Results may also identify AO as a valuable agent in controlling DRS mediated by *C. albicans*.

CHAPTER 4. METHODS AND MATERIALS

Amine Oxide

The AO, N,N-dimethyldodecylamine N-oxide solution (C₁₄H₃₁NO), was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). AO was diluted to a concentration of 0.1% (stock) in saline and then two-fold dilutions of the AO were prepared (up to 0.001%).

Fungal Strains

C. albicans (ATCC 44505) maintained in the UTHSC Dental Research Center was grown for 24 hours at 37 °C in Sabouraud-dextrose broth (SDB; Difco Laboratories) in an orbital water bath shaker at 60 rpm to obtain stationary-phase yeast cells. Cells were harvested by centrifugation at 4 °C and washed twice with phosphate-buffered saline (PBS; pH 7.2). Suspension of *C. albicans* were prepared by measuring the absorbance of the cells at 600 nm and counted under a light microscope to determine the number of yeast cells per milliliter. The suspension was adjusted to a concentration of 1 x 10⁵ colony-forming units (CFU) per milliliter, and used in experiments as a standard cell suspension. The cells were examined microscopically to verify non-budding yeast phase, and used immediately.

Denture Substrate Material

Two-millimeter thick sheets of conventionally heat-activated polymethyl methacrylate (PMMA) were processed according to manufacturer's specifications (Densply Trubyte, Densply International). Sheets were cut into circular discs of 7mm in diameter. The discs were stored in purified water for seven days to remove any residual monomer. The discs were disinfected by dipping them in 70% alcohol for one minute, and washed with sterile, distilled water before experimental use. The discs were routinely tested for sterility.

Preparation of Fungal Biofilms

Paraffin-stimulated whole human saliva was collected from 20 healthy volunteers in 50 mL sterile tubes, and was kept in a beaker of ice. The saliva was clarified by centrifugation (10,000 x g; 20 minutes; 4 °C). The samples were kept on ice during collection in order to avoid any enzymatic degradation. PMMA discs were incubated with clarified whole human saliva for 8 hours at 37 °C. Discs were removed and washed with PBS and then used in *C. albicans* adhesion assays and biofilm formation.

Saliva-coated PMMA discs were incubated with 500 μ l of standardized cell suspension of *C. albicans* (10^5 cells/mL) and incubated for 3 hours at 37 °C on a rocker. The discs were gently washed with PBS, agitated to ensure the removal of non-adherent cells and then transferred to a new plate. After this adhesion phase the discs were immersed in SDB medium with 50mM of glucose and incubated on a rocker table for 72 hours for biofilm formation. The number of adherent fungal cells were determined by XTT (sodium 3*-[1-(phenylamino-carbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate; Roche Laboratories) assay.

Antifungal Activity of Amine Oxide

Freshly cultured and biofilm grown *C. albicans* were suspended to a stock concentration of 1×10^5 cells/ml. Ten-fold dilutions of the stock fungal cells were then made in saline. A two-fold dilution of AO was prepared (0.1% to 0.001%) in saline. Equal amounts (0.5 mL) of AO and *C. albicans* (diluted as described above) were mixed and incubated for 30 min at 37 °C. The viability of fungal cells after exposure to AO was assayed by the XTT cell proliferation method.

XTT Cell Proliferation Assay

The biofilm formation on PMMA discs was also measured by the metabolic reduction of XTT (sodium 3*-[1-(phenylamino-carbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate; Roche Laboratories) to a soluble formazan product by yeast cells. The discs with biofilms were transferred to microtiter plates that contained 100 μ l PBS per well to which 50 μ l of XTT reagent (final concentration of XTT 0.3mg/ml) was added. Plates were incubated at 37 °C for two hours. The absorbance of the supernatant was measured at 492nm using an ELISA reader (Bio-Rad Laboratories). A standard curve with known number of fungal cells was generated which enabled the determination of viable number of fungal cells. Fungal cells were then collected by scraping the PMMA discs, washed with saline and adjusted to a concentration of 1×10^5 cells/mL in preparation for assays.

THP-1 Cells

THP-1 cells (ATCC TIB 202) were grown in suspension in 75 cm² plastic tissue culture flasks (Corning Glass Works, Corning, N.Y.) in RPMI 1640 (GIBCO, Gaithersburg, Md.) complete medium (CM) with addition of the antibiotics penicillin G sodium (100 U/ml) and streptomycin sulfate (100 μ g/mL) and supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum, and amino acids. Cells were incubated at 37 °C in a humidified atmosphere consisting of 5% CO₂.

Suspensions of freshly grown and biofilm grown fungal cells (10^5 cells) were exposed to varying concentrations of AO. The fungal cells were then subjected to mild

sonication, keeping the cells on ice for 60 seconds, and then centrifuged. The cell supernatants were collected, their protein concentration adjusted and then used in cytokine assays. A suspension of THP-1 cells (1×10^5) was incubated with extracts of fresh and biofilm *C. albicans* for 24 h at 37 °C. The culture supernatants were collected, centrifuged and assayed for levels of cytokines.

IL-1 β and TNF- α Cytokine Assay

THP-1 cells were incubated with extracts of gently sonicated fresh and biofilm-cultured *C. albicans* cells (1×10^5) for 24 hours. Supernatant fluids of untreated THP-1 cells and those treated with extracts of freshly grown and biofilm grown *C. albicans* (standardized to contain same amount of protein, determined by BioRad protein assay) were stored at -80 °C until used for measurement of IL-1 β and TNF- α secretion. Cytokines released by THP-1 cells (when stimulated with fresh and biofilm grown *C. albicans*) were measured with commercial enzyme-linked immunosorbent assay (ELISA) kits (R & D Systems, Minneapolis, Minn.). The average absorbance values for each triplicate set of standards, controls, and samples were calculated by using a standard curve. Results were expressed as picograms of IL-1 β or TNF- α per milliliter of supernatant fluid.

Data Analysis

Each experiment was performed a minimum of three times, with triplicate determinations at each point. Results were evaluated for statistical significance by ANOVA followed by Scheffe's f-test. Differences in results with a *p*-value less than 0.05 were considered significant.

CHAPTER 5. RESULTS

Effect of pH on Antifungal Activity of AO

C. albicans (1×10^5 CFU/mL) from fresh and biofilm culture was treated with 0.001% AO at various pH values (4.5 to 7.0) for 10 minutes, and then assayed for cell viability using the XTT method previously described. As demonstrated in **Table 1** and **Figure 1**, AO demonstrated antifungal activity within culture groups at all pH values. However, AO's antifungal activity differed between culture groups, affecting fungal cells cultured from fresh environments more than cells cultured from biofilm environments. AO activity was not statistically different within culture groups, but was found statistically different between culture groups at $p < 0.05$.

Effect of AO on Adherence of *C. albicans* to PMMA Discs

Fresh *C. albicans* (1×10^5 CFU/mL) was combined with saliva-coated PMMA and varying concentrations of AO (0.005%, 0.001%, 0.01%), and was allowed to incubate for 3 hours at 37 °C. Fungal cells adhering to the discs following the incubation period were assayed using the XTT method previously described. As demonstrated in **Table 2** and **Figure 2**, at increasing concentration of AO, the number of viable *C. albicans* cells decreased. Cell viability was statistically different between all AO concentration groups at $p < 0.05$.

Influence of AO on Salivary Pellicle Formation on PMMA Discs

Fresh *C. albicans* (1×10^5 CFU/mL) was combined with saliva and varying concentrations of AO coated PMMA (0.0005% to 0.01%) that had been incubated for 8 hours at 37 °C. Following addition of the fungal cells, the mixture was allowed to incubate further for 3 hours at 37 °C. As demonstrated in **Table 3** and **Figure 3**, at increasing concentration of AO, the number of viable *C. albicans* cells decreased. Cell viability was statistically different between all AO concentration groups at $p < 0.05$.

Effect of AO Exposure Time on Viability of *C. albicans*

C. albicans (1×10^5 CFU/mL) from fresh and biofilm cultures was treated with 0.001% AO for varying periods of time (5 minutes to 60 minutes), and then assayed at each time period for cell viability using the XTT method previously described. As demonstrated in **Table 4** and **Figure 4**, at increasing exposure time to AO, the number of viable *C. albicans* cells decreased in both fresh and biofilm groups. However, a greater number of viable cells from biofilm culture persisted in exposure times (20 minutes to 30 minutes) in which fresh cells could not survive. Cell viability was statistically different

Table 1. Effect of pH on Antifungal Activity of AO.

Source	pH 4.5	pH 5.0	pH 5.5	pH 6.0	pH 7.0
Fresh	360 ± 40	470 ± 20	540 ± 60	490 ± 40	410 ± 30
Biofilm	870 ± 30	1120 ± 60	1070 ± 30	1210 ± 20	980 ± 40

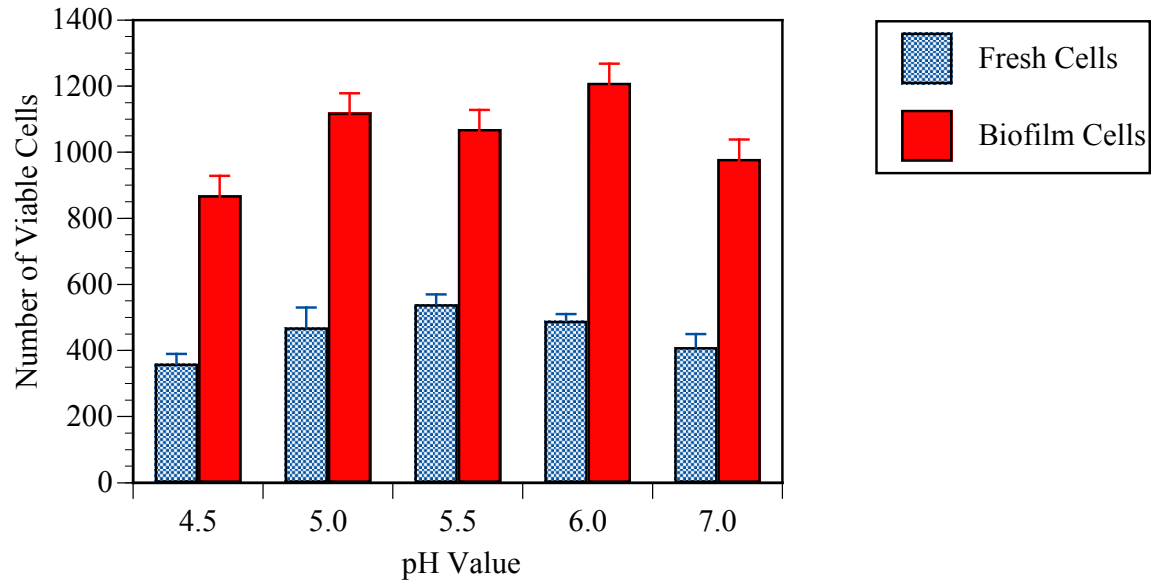


Figure 1. Effect of pH on Antifungal Activity of AO.

Table 2. Effect of AO on Adherence of *C. albicans* to PMMA Discs.

Concentration of AO	# of Cells
0	97500 ± 3200
0.0005%	3150 ± 280
0.001%	960 ± 80
0.01%	0

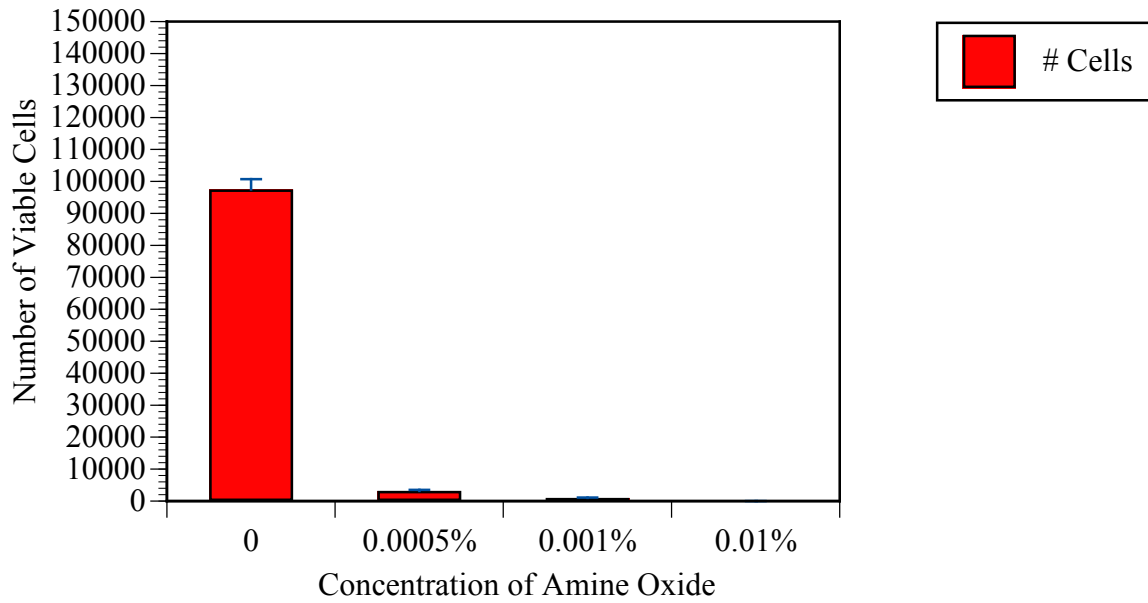


Figure 2. Effect of AO on Adherence of *C. albicans* to PMMA Discs.

Table 3. Influence of AO on Salivary Pellicle Formation on PMMA Discs.

Concentration of AO	# of Cells
0	95000 ± 3250
0.0005%	7800 ± 350
0.001%	600 ± 300
0.01%	150 ± 40

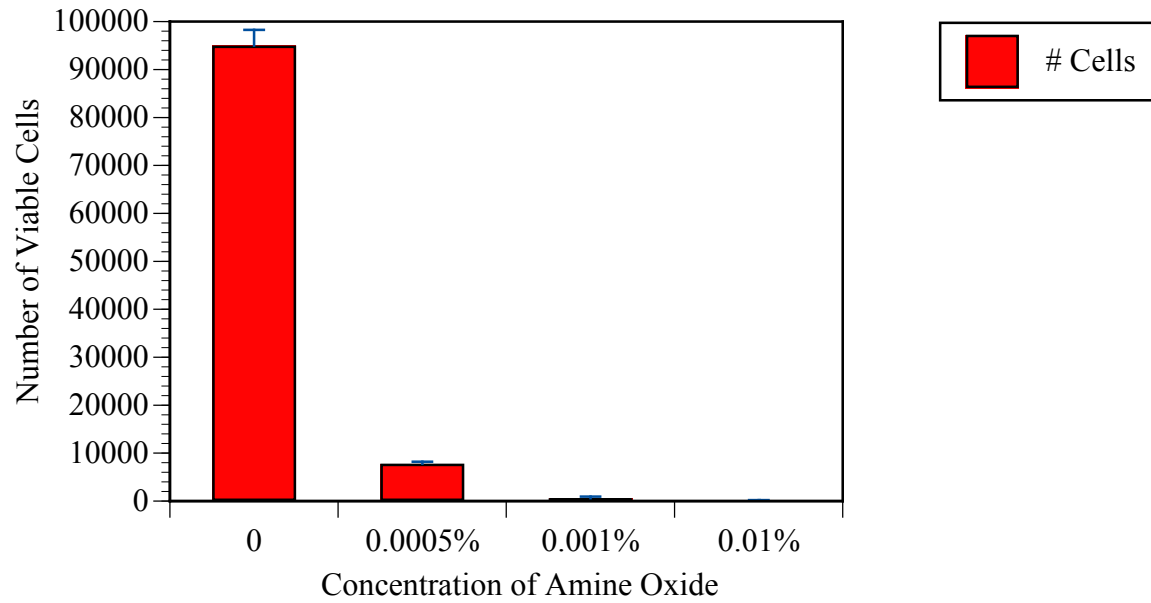


Figure 3. Influence of AO on Salivary Pellicle Formation on PMMA Discs.

Table 4. Effect of AO Exposure Time on Viability of *C. albicans*.

Exposure Time	# Cells—Fresh	# Cells—Biofilm
0 minutes	96500	93000 ± 2600
5 minutes	980	12500 ± 1150
10 minutes	460	1050 ± 90
15 minutes	120	820 ± 60
20 minutes	0	440 ± 50
30 minutes	0	160 ± 30
45 minutes	0	0
60 minutes	0	0

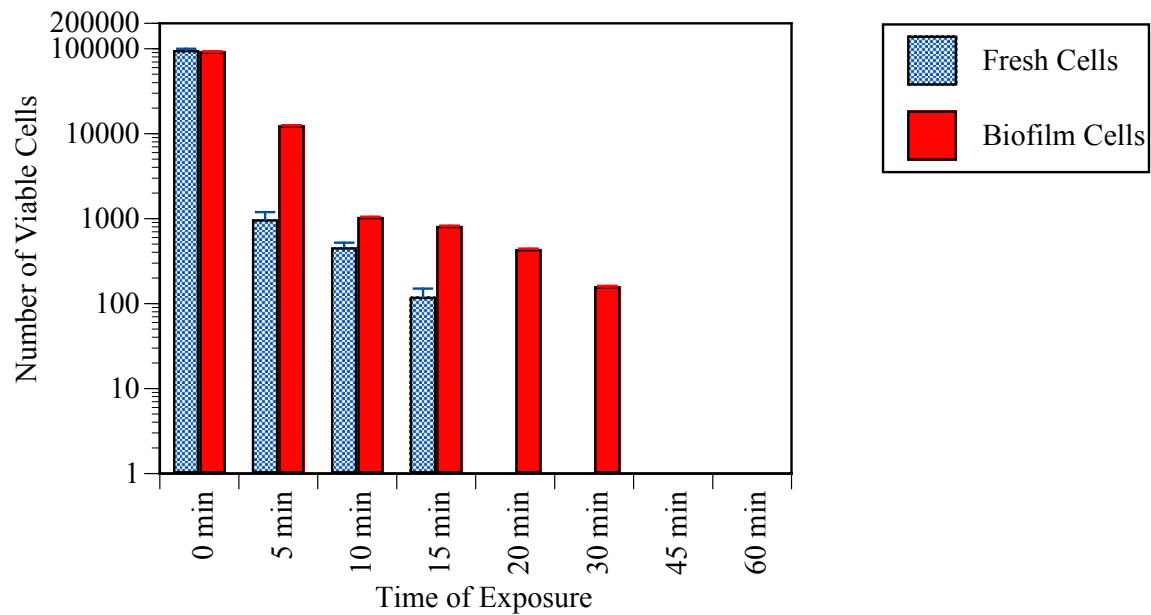


Figure 4. Effect of AO Exposure Time on Viability of *C. albicans*.

between culture groups at all exposure times, and was also statistically different within culture groups up to 20 minutes and 45 minutes for fresh and biofilm culture groups, respectively ($p < 0.05$).

Effect of AO on Fresh and Biofilm-Cultured *C. albicans*

C. albicans (1×10^5 CFU/mL) from fresh and biofilm cultures was treated with varying concentrations of AO (0.0001% to 0.01%) for 10 minutes, and then assayed for cell viability using the XTT method previously described. As demonstrated in **Table 5** and **Figure 5**, at increasing concentration of AO, the number of viable *C. albicans* cells/mL decreased in both fresh and biofilm groups. However, a greater number of viable cells from biofilm culture persisted in levels of AO concentration (0.005% to 0.1%) in which fresh cells could not survive. At AO concentrations of 0.0001% and higher, cell viability was statistically different between and within both culture groups at $p < 0.05$.

Effect of AO on Biofilm Formation on PMMA Discs

Fresh *C. albicans* (1×10^5 CFU/mL) was combined with saliva-coated PMMA and allowed to incubate for 3 hours at 37 °C. Varying concentrations of AO (0.0001% to 0.005%) were then added to this environment for 10 minutes. Cells located in the biofilm were sampled and assayed for cell viability using the XTT method previously described. As demonstrated in **Table 6** and **Figure 6**, at increasing concentration of AO, the number of viable cells/mL decreased. Cell viability was statistically different between all AO concentration groups at $p < 0.05$.

Effect of AO on IL-1 β Secretion by THP-1 Cells

C. albicans (1×10^5 CFU/mL) from fresh and biofilm culture was exposed to increasing concentrations of AO (0.0001% to 0.005%) for 10 minutes and then separately pelleted and gently sonicated to obtain cell extracts. The cell extract (100 μ L of mg/mL stock) was used in the assay to stimulate THP-1 cells. The level of IL-1 β secretion was assayed using the ELISA method previously described. As demonstrated in **Table 7** and **Figure 7**, at increasing concentration of AO, the level of IL-1 β stimulation decreased. Further, a lower level of cytokine stimulation resulted from exposure to fresh culture cells than from biofilm culture cells. While the level of IL-1 β stimulation at different AO concentrations was statistically different within each culture group ($p < 0.05$), the level of stimulation was only statistically different between culture groups at AO concentration of 0.0001% ($p < 0.05$).

Table 5. Effect of AO on Fresh and Biofilm-Cultured *C. albicans*.

Concentration of AO	# Cells—Fresh	# Cells—Biofilm
0	95000 ± 3500	93500 ± 1200
0.0001%	31000 ± 1250	75000 ± 3500
0.0005%	1650 ± 200	4200 ± 450
0.001%	480 ± 50	950 ± 40
0.005%	0	400 ± 80
0.01%	0	120 ± 60
0.1%	0	0

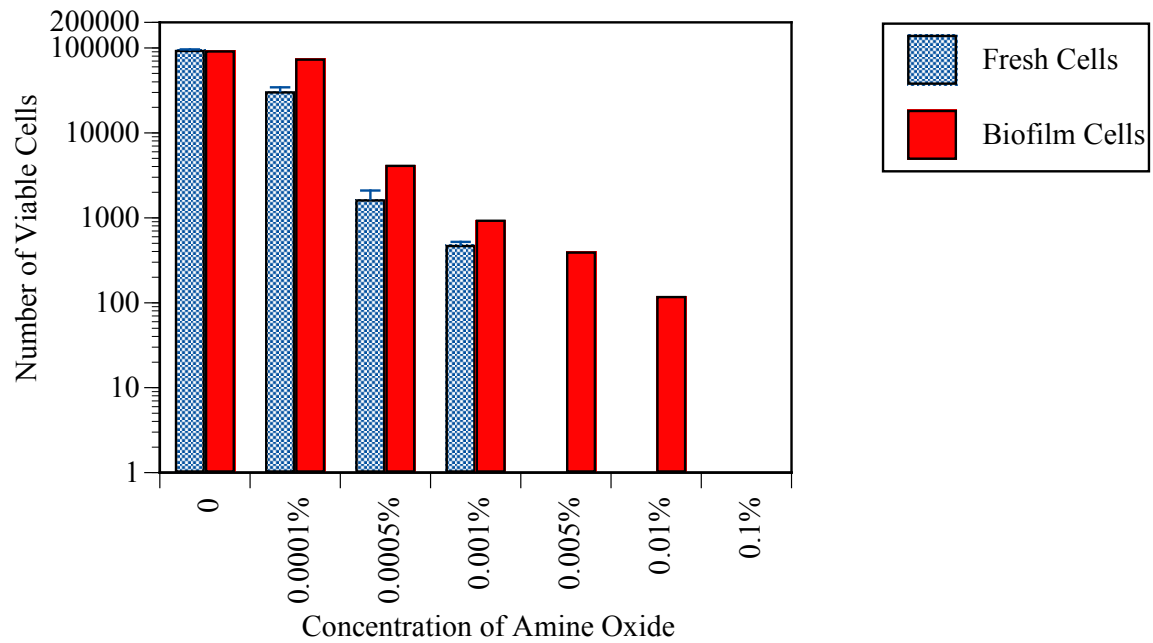


Figure 5. Effect of AO on Fresh and Biofilm-Cultured *C. albicans*.

Table 6. Effect of AO on Biofilm Formation on PMMA Discs.

Concentration of AO	# of Cells
0	94300 ± 2900
0.0001%	37700 ± 800
0.0005%	2650 ± 380
0.001%	670 ± 40
0.005%	0

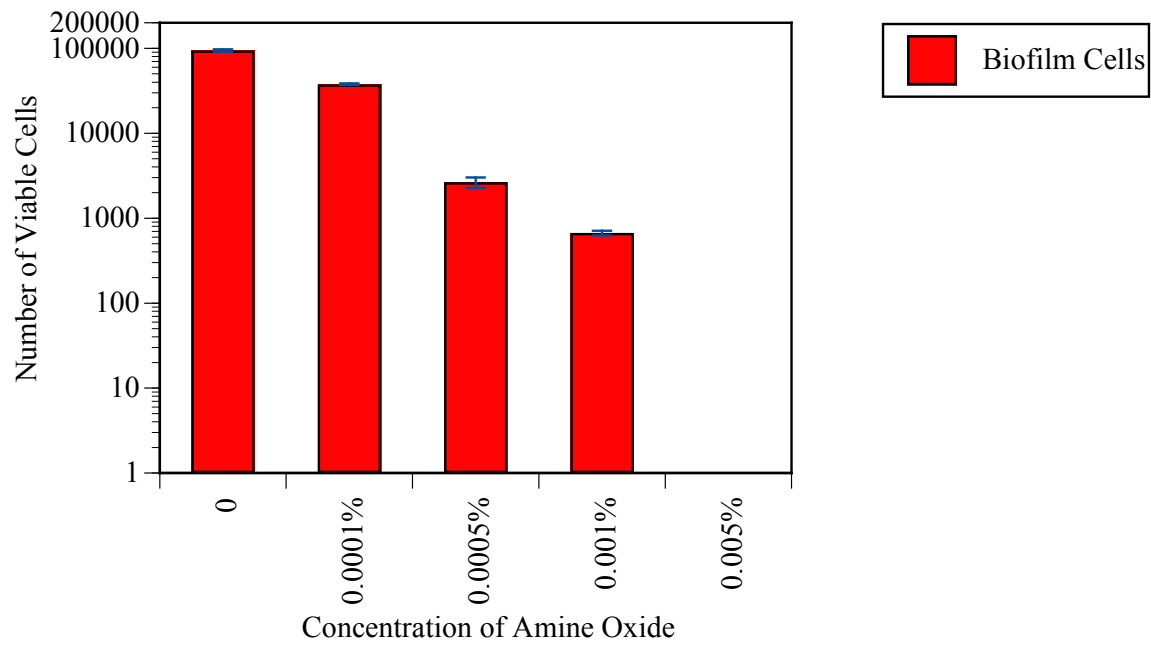


Figure 6. Effect of AO on Biofilm Formation on PMMA Discs.

Table 7. Effect of AO on IL-1 β Secretion by THP-1 Cells.

Concentration of AO	IL-1 β Stimulated by Fresh Cells (pg/mL)	IL-1 β Stimulated by Biofilm Cells (pg/mL)
0	270 \pm 24	346 \pm 21
0.0001%	126 \pm 18	178 \pm 23
0.0005%	74 \pm 9	89 \pm 14
0.001%	23 \pm 5	36 \pm 9
0.005%	12 \pm 3	19 \pm 6

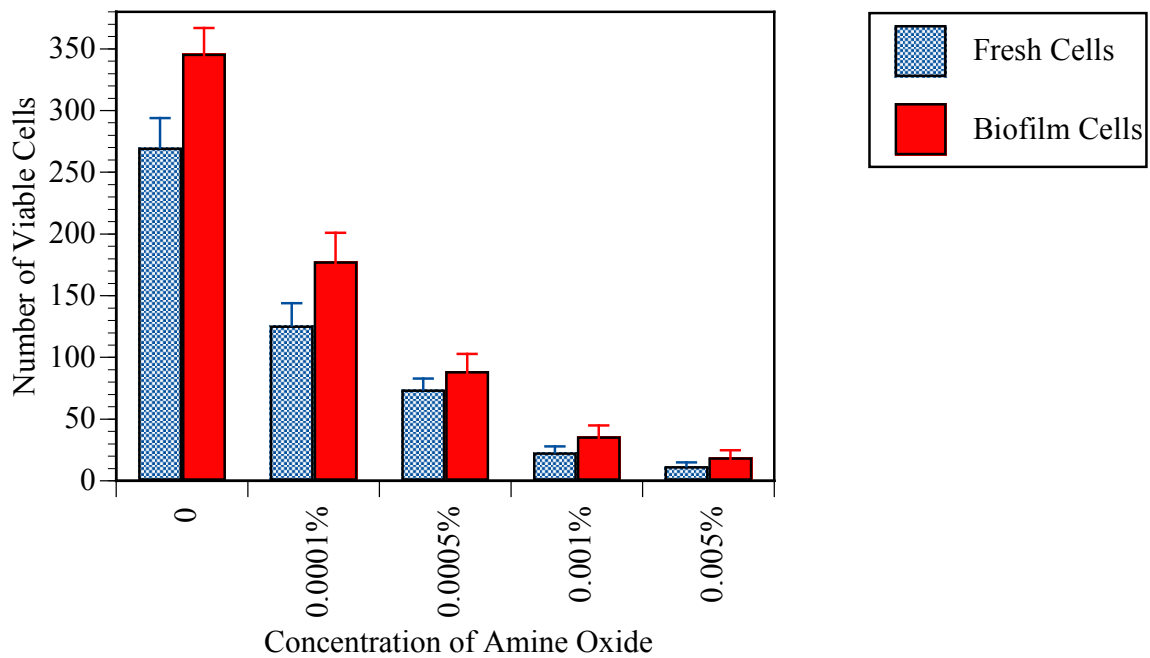


Figure 7. Effect of AO on IL-1 β Secretion by THP-1 Cells.

Effect of AO on TNF- α Secretion by THP-1 Cells

C. albicans (1×10^5 CFU/mL) from fresh and biofilm cultures was exposed to varying concentrations of AO (0.0001% to 0.005%) for 10 minutes and then separately pelleted and gently sonicated to obtain cell extracts. The cell extract (100 μ L of mg/mL stock) was used in the assay to stimulate THP-1 cells. The level of TNF- α secretion was assayed using the ELISA method previously described. As demonstrated in **Table 8** and **Figure 8**, at increasing concentration of AO, the level of TNF- α stimulation decreased. Further, the level of cytokine stimulation was lower for fresh culture cells than for biofilm culture cells. TNF- α activity was statistically different between and within culture groups at each AO concentration level at $p < 0.05$.

Table 8. Effect of AO on TNF- α Secretion by THP-1 Cells.

Concentration of AO	TNF- α Stimulated by Fresh Cells (pg/mL)	TNF- α Stimulated by Biofilm Cells (pg/mL)
0	390 \pm 41	546 \pm 38
0.0001%	286 \pm 22	338 \pm 44
0.0005%	114 \pm 9	159 \pm 21
0.001%	43 \pm 8	76 \pm 12
0.005%	19 \pm 5	29 \pm 6

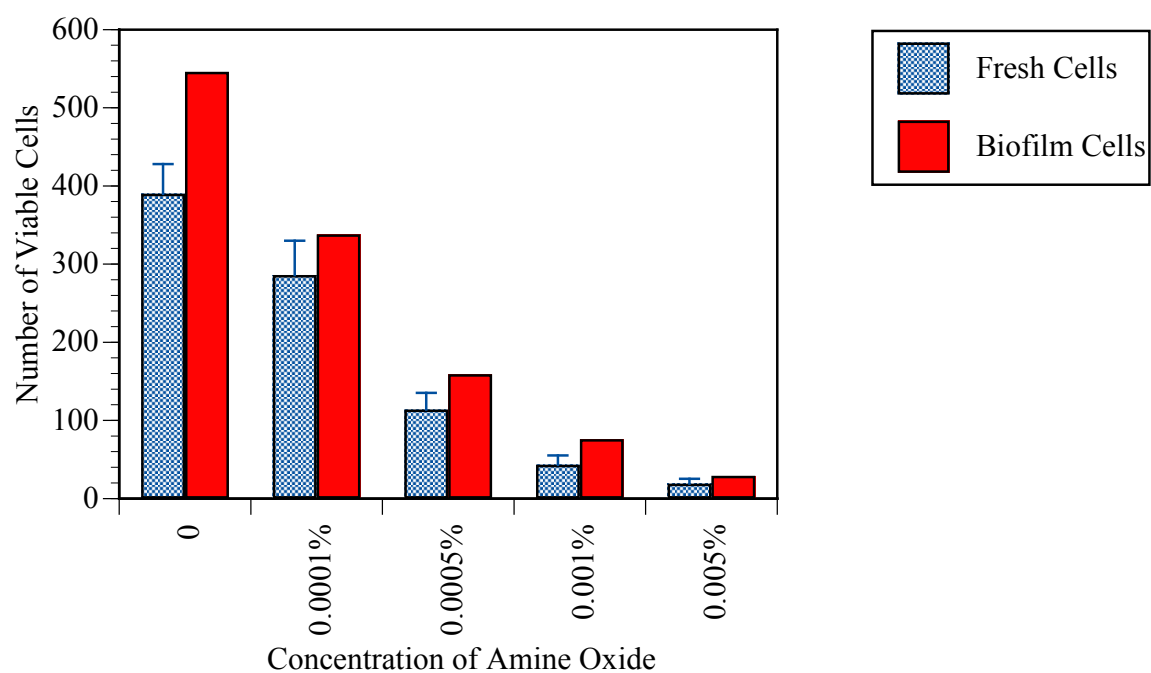


Figure 8. Effect of AO on TNF- α Secretion by THP-1 Cells.

CHAPTER 6. DISCUSSION

Effect of pH on Antifungal Activity of AO

The pH of human saliva may vary in several circumstances, including time of day, proximity to meals, quantity and quality of saliva, type of diet, and state of oral health (38). Of particular interest in this study is how a change in oral health evident in DRS (which is known to lower the pH of the oral cavity) could effect the anti-fungal activity of AO. Saliva collected from 20 healthy volunteers was pooled, and used throughout the experimental procedure, and was found to have a pH of 6.5. Modification of the pH of saliva samples separated from the pooled volume was accomplished by acid/base additions. Results of this study show that a 0.0001% AO concentration when exposed to fresh and biofilm-cultured *C. albicans* cells for a period of 10 minutes is not significantly affected in its antifungal ability. Therefore, pH does not appear to alter the effect that AO has on fungal cells derived from within and outside of a biofilm environment.

Effect of AO on Adherence of *C. albicans* to PMMA Discs

An objective of tissue conditioning in DRS patients is prevention of further disease concomitant with resolution of existing infection. Typical clinical procedures involve thorough cleaning of the denture base material utilizing a 2.6% sodium hypochlorite solution following removal of lining materials and preceding their replacement (39). The cidal activity of this procedure is directly aimed at the fungal and bacterial components of denture bases of DRS patients' prostheses. While caustic to fungal and bacterial pathogens, sodium hypochlorite is also very abusive to intraoral tissues and may affect denture base chromaticity when not rinsed completely following application. Fraud, *et al.* has stated in previous projects that low concentrations of AO are safe for intraoral tissues, and even safe for intraoral use and application (10,11).

In this experimental procedure, the attempt to form a biofilm on PMMA in the presence of varying amounts of AO yielded decreasing numbers of viable cells scraped from the PMMA discs as AO concentration was increased. This result suggests that AO is perhaps capable of either attacking and destroying fungal cells before they are able to form the biofilm matrix, or more likely (given the presence of a biofilm on the PMMA discs even at high AO concentrations) that AO is somehow thwarting the protective characteristics of biofilms as they are formed by merely being incorporated into the matrix as it develops. Further study into this mechanism of action is warranted.

Influence of AO on Salivary Pellicle Formation on PMMA Discs

DRS patients are instructed to follow a strict and time consuming protocol for home care of their prosthesis in an effort to maintain the level of cleanliness between

clinical visits. Even the most meticulous home care may not address and prevent the recurrence of fungal, bacterial, and resultant biofilm reformation in hard to reach areas of the prosthesis (39). Impossible to reach by any method of home care are the porous portions of the denture (the base and overlying tissue conditioner lining material). Because of the presence and physical characteristics of the lining material, home use of sodium hypochlorite is not recommended, as incomplete removal of the hypochlorite solution following application could exacerbate the soft tissue inflammation the clinician and patient are striving to control.

In this experimental procedure, varying levels of AO were allowed to remain in contact with PMMA discs in the presence of saliva for an 8 hour period of time prior to introduction of fungal cells. As AO concentration increased, the number of viable cells remaining in biofilm culture following a three hour incubation period decreased. These results suggest that AO (when contact with PMMA is maintained for 8 hours) decreases the number of viable *C. albicans* cells able to attach to PMMA. AO may function by decreasing the number of cells capable of producing the biofilm, by weakening the matrix itself as it is incorporated into the structure during development and maturation, or by decreasing the adherence of saliva molecules to the PMMA which indirectly prevents biofilm attachment. In any case, the results are interesting, and even the suggestion that AO could modify the protective characteristic of biofilm environments warrants further investigation into the matter.

Effect of AO Exposure Time on Viability of *C. albicans*

A concern of any treatment modality, regardless of the condition being addressed, is patient compliance. The year 2009 has brought many advancements in technology that have 'streamlined' human existence. Anecdotally, clinicians find that patient patience and devotion to their prescribed care can sometimes fall short of expectation usually due to the lack of devoted time. Treatment of DRS is often extremely frustrating for clinicians as resolution of infection may take weeks to months of costly and frequent clinical visits. While prescription of antifungal creams, troches, and systemic medications have hastened the process of DRS treatment, it can remain painfully slow in severe cases of the condition, particularly in non-compliant patients. The need for an effective, easy, and efficient method to address DRS is greater than ever, particularly as populations of the denture wearing community increase annually.

In this experimental procedure, a 0.001% AO concentration was exposed to *C. albicans* cells derived from fresh culture and derived from biofilm culture for a varying period of time. As exposure time increased, the number of viable cells remaining in each culture source decreased, with biofilm-cultured *C. albicans* being more resistant to AO than those from fresh culture, requiring 45 minutes for complete destruction of all remaining cells. These results suggest that even at very low concentration of AO, increasing the exposure time to the different cultures will eventually result in complete destruction of any and all *C. albicans* cells derived from within and outside of a biofilm environment. These results further suggest that AO possesses an effective and efficient

mechanism of action, and that with a small increase in time, even low concentrations of the surfactant maintains its fungicidal effect.

Effect of AO on Fresh and Biofilm-Cultured *C. albicans*

It is known that biofilm environments can evoke changes in the cells that inhabit portions of its matrix—possibly by mechanical protection from host and antifungal agents or even phenotypic alteration of the fungal cell itself (18,20). Regardless of the mechanism, there is no doubt that a difference exists between cells before they become encased in a biofilm and after they become encased in a biofilm. The question at this point for researchers and clinicians alike is how can one circumvent the protective features of that self-formed polymeric matrix, the biofilm.

In this experimental procedure, *C. albicans* cultured from both fresh and biofilm environments were exposed to increasing concentrations of AO for 10 minutes, and were then cultured for cell viability using the XTT method. As the concentration of AO was increased, the number of viable cells in both groups decreased, with the biofilm-cultured cells being more resistant to the fungicidal effect of AO. These results suggest that although the protective properties of the once present biofilm persist in the biofilm-cultured cells when exposed to AO, AO becomes effective against biofilm cells at higher concentrations; the highest concentration tested in this case being 0.05% less than the concentration safe for intraoral use (0.06%) identified by Fraud, *et al.* (10,11). This series of experiments again suggest that AO possesses an effective and efficient mechanism of action that, with an increase in concentration, can produce a fungicidal effect to both fresh and biofilm-cultured fungal cells.

Effect of AO on Biofilm Formation on PMMA Discs

Perhaps the most clinically relevant procedure in this series of experiments investigated the effect that AO has on biofilm cells embedded in the protective matrix located on PMMA surfaces. This approach addressed the clinical manifestation and challenges encountered in private practices and institutional dental settings worldwide. To this point, AO has been introduced at either critical periods of biofilm development (namely initiation) or following physical removal of cells from a biofilm environment. The former attempting to discover AO's effect on formation of the polymeric matrix and subsequent resistance of fungal cells to AO's established antifungal activity, and the latter attempting to elucidate the change in response to AO between a freshly cultured cell and a cell allowed to live and mature in a biofilm environment.

In this experimental procedure, PMMA discs were combined with fungal cells, saliva, and supportive medium, and allowed to develop a biofilm matrix, undisturbed for a 3 hour period of time. Following that period of incubation, varying concentrations of AO were added to this medium for 10 minutes. Following *that* exposure time, the PMMA discs were scraped, and fungal cells were isolated from the biofilm environment

in a method previously described, and were assayed for cell viability by the XTT method also previously described.

These results suggest that AO is effective at killing cells as they are located within a biofilm environment, particularly at higher concentrations of AO. Perhaps AO possesses the ability to bypass the protective characteristics of the biofilm matrix.

Effect of AO on IL-1 β Secretion by THP-1 Cells

The clinical manifestation of DRS is reliant upon inflammation mediated by human monocyte release of inflammatory cytokines. The established effectiveness of AO at decreasing the number of viable fresh and biofilm-cultured *C. albicans* while they are located in and after they are separated from biofilm environments, means little if the agents of the inflammatory process are not affected by AO's activity.

In this experimental procedure, *C. albicans* cells cultured from fresh and biofilm environments were exposed to varying concentrations of AO for 10 minutes, and then exposed to human monocytes in a process and method previously described. The amount of IL-1 β (expressed in pg/mL) was assayed using the ELISA method previously described. As the concentration of AO was increased, the amount of IL-1 β released by exposed THP-1 cells decreased. These results suggest that AO effectively reduces the number of viable *C. albicans* cells to the point that it indirectly decreases THP-1 cell activity in terms of IL-1 β release.

Effect of AO on TNF- α Secretion by THP-1 Cells

In this experimental procedure, *C. albicans* cells cultured from fresh and biofilm environments were exposed to varying concentrations of AO for 10 minutes, and then exposed to human monocytes in a process and method previously described. The amount of TNF- α (expressed in pg/mL) was assayed using the ELISA method previously described. As the concentration of AO was increased, the amount of TNF- α released by exposed THP-1 cells decreased. These results suggest that AO effectively reduces the number of viable *C. albicans* cells to the point that it indirectly decreases THP-1 cell activity in terms of TNF- α release.

CHAPTER 7. CONCLUSION

Within the limitations of this study, results suggest that AO is effective at reducing the number of viable *C. albicans* cells isolated from fresh and biofilm culture, as well as fungal cells as they are located in a biofilm environment. Further, this series of experiments also suggest that by reducing the number of viable fungal cells, AO is also capable of indirectly decreasing the activity of human monocytes to release the inflammatory cytokines IL-1 β and TNF- α . Future investigations should address the intraoral applications of AO, and its effectiveness in a clinical setting.

LIST OF REFERENCES

1. Peterson PE, Yamamoto T. Improving the Oral Health of Older People: The Approach of the WHO Global Oral Health Programme. *Community Dent Oral Epidemiol* 2005; 33: 81-92.
2. World Health Organization. WHO Oral Health Country/Area Profile. Available from: <http://www.whocollab.od.mah.se/index.html> accessed February 12, 2007.
3. Boucher CO. Complete Denture Prosthodontics—The State of the Art. *J Pros Dent* 2004; 92(4): 309-315.
4. Melton AB. Current Trends in Removable Prosthodontics. *JADA* 2000; 131: 52S-56S.
5. Lamb DJ, Martin MV. Frequency of *Candida albicans* Serotypes in Patients with Denture-induced Stomatitis and in Normal Denture Wearers. *J Clin Pathol* 1983; 35: 888-891.
6. Andrucoli MCD, deMacedo LD, Lara EHG, Paranhos HDO. Comparison of Two Cleansing Pastes for the Removal of Biofilm from Dentures and Palatal Lesions in Patients with Atrophic Chronic Candidiasis. *Braz Dent J* 2004; 15 (3): 220-4.
7. Azul A, Branco FM, Figueiral MH, Fonesca PA, Scully C. Denture-Related Stomatitis: Identification of Aetiological and Predisposing Factors—A Large Cohort. *J Oral Rehab* 2007; 34: 448-455.
8. U.S. Department of Health and Human Services. Oral Health in America: A Report of the Surgeon General. Rockville, MD: U.S. Department of Health and Human Services, National Institute of Dental and Craniofacial Research, National Institutes of Health, 2000.
9. Proctor and Gamble. Science in the Box. Available from: http://www.scienceinthebox.com/en_UK/glossary/surfactants_en.html accessed February 12, 2007.
10. Fraud S, Maillard, JY, Denyer SP, Kaminski MA, Hanlon GW. A Simulated Oral Hygiene Model to Determine the Efficacy of Repeated Exposure of Amine Oxide on the Viability of *Streptococcus mutans* Biofilms. *Eur J Oral Sci* 2007; 115: 71-76.
11. Fraud S, Maillard JY, Kaminski MA, Hanlon GW. Activity of Amine Oxide Against Biofilms of *Streptococcus mutans*: A Potential Biocide for Oral Care Formulations. *J Antimicrob Chemo* 2005; 56: 672-677.

12. Void JD. Dental Laboratory Technology: Removable Prosthodontics. AF Pamphlet 1991 (2) 87: 162-166.
13. Braden M. Some Aspects of the Chemistry and Physics of Dental Resins. *Adv Dent Res* 1988; 2(1): 93-97.
14. Anusavice KJ. Phillips Science of Dental Materials. Chicago: Elsevier, Tenth Edition 1996; 254.
15. Zarb KE, Bolender MO. Prosthodontic Treatment for Edentulous Patients, Complete Dentures, and Implant Supported Protheses. Chicago: Mosby Eighth Edition 1997; 404.
16. Cannon RD, Chaffin WL. Oral Colonization by *Candida albicans*. *Crit Rev Oral Biol Med* 1999; 10 (3): 359-383.
17. Rangel-Frausto MS, Houston AK, Bale MJ, Fu C, Wenzel RP. An Experimental Model for Study of Candida Survival and Transmission in Human Volunteers. *Eur J Clin Microbiol Infect Dis* 1994; 13(7): 590-595.
18. Douglas LJ. Medical Importance of Biofilms in Candida Infections. *Rev Iberoam Micol* 2002; 19: 139-143.
19. Chandra J, McCormick TS, Imamura Y, Mukherjee PK, Ghannoum MA. Interaction of *Candida albicans* with Adherent Human Peripheral Blood Mononuclear Cells Increases *C.albicans* Biofilm Formation and Results in Differential Expression of Pro- and Anti-Inflammatory Cytokines. *Infect Immun* 2007; 75(5): 2612-2620.
20. Lamfon H, Porter SR, McCullough M, Pratten J. Susceptibility of *Candida albicans* Biofilms Grown in a Constant Depth Film Fermentor to Chlorhexidine, Fluconazole, and Miconazole: a Longitudinal Study. *J Antimicrob Chemo* 2004; 53: 383-385.
21. Radford DR, Challacombe SJ, Walter JD. Denture Plaque and Adherence of Candida Albicans to Denture-Base Materials *In Vivo* and *In Vitro*. *Crit Rev Oral Biol Med* 1999; 10(1): 99-116.
22. Davenport JC, Wilton JMA. Incidence of Immediate and Delayed Hypersensitivity to *Candida albicans* in Denture Stomatitis. *J Dent Res* 1971; 50(4): 892-895.
23. Villar CC, Kashleva H, Mitchell AP, Dongari-Batzoglou A. Invasive Phenotype of *Candida albicans* Affects the Host Pro-inflammatory Response to Infection. *Infect Immun* 2005; 73(8): 4588-4595.

24. Perezous LF, Flaitz CM, Goldschmidt ME, Engelmeier RL. Colonization of *Candida* Species in Denture Wearers with Emphasis on HIV Infection: A Literature Review. *J Prosthet Dent* 2005; 93: 288-293.
25. Bikandi J, Moragues MD, Quindos G, Polonelli L, Ponton J. Influence of Environmental pH on the Reactivity of *Candida albicans* with Salivary IgA. *J Dent Res* 2000; 79(6): 1439-1442.
26. Olivera MAM, Carvalho LP, Gomes MS, Bacellar O, Barros TF, Carvalho EM. Microbiological and Immunological Features of Oral Candidiasis. *Microbiol Immunol* 2007; 51(8): 713-719.
27. Dongari Bagtzoglou A, Fidel Jr. PL. The Host Cytokine Responses and Protective Immunity in Oraopharyngeal Candidiasis. *J Dent Res* 2005; 84(11): 966-977.
28. McCourtie J, Douglas LJ. Relationship Between Cell Surface Composition of *Candida albicans* and Adherence to Acrylic After Growth on Different Carbon Sources. *Infect Immun* 1981; 32(3): 1234-1241.
29. Sakai A, Akifusa S, Itano N, Kimatas K, Kawamura T, Koseki T, Takehara T, Nishihara T. Potential Role of High Molecular Weight Hyaluronan in the Anti-*Candida* Activity of Human Oral Epithelial Cells. *Med Mycol* 2007; 45:73-79.
30. Cowen LE, Sanglard D, Calabrese D, Sirjusingh C, Anderson JB, Kohn LM. Evolution of Drug Resistance in Experimental Populations of *Candida albicans*. *J Bacteriol* 2000; 182 (6): 1515-1522.
31. Akpan A, Morgan R. Oral Candidiasis. *Postgrad Med J* 2002; 78: 455-459.
32. Rodrigues-Archilla A, Urquia M, Cutando A, Asencio R. Denture Stomatitis: Quantification of Interleukin-2 Production by Mononuclear Blood Cells Cultered with *Candida albicans*. *J Prosthet Dent* 1996; 75: 426-31
33. Chandra J, Patel JD, Li J, Zhou G, Mukherjee PK, McCormick TS, Anderson JM, Ghannoum MA. Modification of Surface Properties of Biomaterials Influences the Ability of *Candida albicans* to Form Biofilms. *Appl Environ Microbiol* 2005; 71(12): 8795-8801.
34. Dhir G, Berzins DW, Dhuru VB, Periathamby AR, Dentino A. Physical Properties of Denture Base Resins Potentially Resistant to *Candida* Adhesion. *J Prosthodont* 2007; 16(6): 465-472.
35. Puri G, Berzins DW, Dhuru VB, Raj PA, Rambhia SK, Dhir G, Dentino AR. Effect of Phosphate Group Addition on the Properties of Denture Base Resins. *J Prosthet Dent* 2008; 100: 302-308.

36. Nalbant AD, Kalkanci A, Filiz B, Kustimur S. Effectiveness of Different Cleaning Agents Against the Colonization of *Candida* spp and the *In Vitro* Detection of the Adherence of These Yeast Cells to Denture Acrylic Surfaces. *Yonsei Med J* 2008; 49(4): 647-654.
37. Singh SK, Bajpai M, Tyagi VK. Amine Oxides: A Review. *J Oleo Sci* 2006; 55(3): 99-119.
38. Turner MD, Ship JA. Dry Mouth and Its Effects on the Oral Health of Elderly People. *JADA* 2007; 138(95): 15s-20s.
39. Barabe W, Neto TDM, Pimenta FC, Pegoraro LF, Scolaro JM. Efficacy of Sodium Hypochlorite and Coconut Soap Used as Disinfecting Agents in the Reduction of Denture Stomatitis, *Streptococcus mutans*, and *Candida albicans*. *J Oral Rehab* 2004; 31:453-459.

VITA

Dr. Jonathan Michael Hart was born on September 4, 1979. He received his D.D.S. degree from the University of Tennessee Health Science Center, College of Dentistry in 2005. He then completed a general practice residency at the Veterans Administration Hospital in Memphis. He was accepted into the University of Tennessee Health Science Center Advanced Prosthodontic program while a general practice resident at the VA. Dr. Hart is currently a third year resident, specializing in fixed and removable prosthodontics at UTHSC. He is also working toward his Master of Dental Science degree from the University of Tennessee. He is a current member of the American College of Prosthodontists, American Dental Association, and the Arkansas State Dental Association.