Interactions between the pathogenic yeast Candida albicans and poly(vinyl chloride)
by Kevin James Siedlecki

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemical Engineering
Montana State University

Abstract:
The use of biomedical implants is becoming increasingly widespread in the medical field. The most common cause of device failure is infection of the implant by microorganisms. The pathogenic yeast Candida albicans is the third leading cause of these infections trailing only S. epidermidis. and S. aureus. Interactions between these microorganisms and the implant surfaces are not well-defined, and insight into this area could lead to better material construction to minimize the effects of microbial colonization.

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Yeast cell adhesion was found to be drastically reduced as shear rate at the time of attachment was increased in the flow cell. Attachment of glucose-grown cells was more affected by shear rate than was attachment of galactose-grown cells. Carbohydrate source also had a significant effect on adhesion. Glucose-grown cells were more adherent than galactose-grown cells at the same shear rate. However, variation of carbohydrate concentration in the growth media had minimal effect on cell adhesion. Attached cells were tenaciously bound to the surface and unaffected by increased shear rates. This phenomena was true for both carbohydrate sources. Carbohydrate source also influenced cell size and the number of yeast cells per cluster.

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APPROVAL

of a thesis submitted by

Kevin J. Siedlecki

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

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CHAPTER 1

INTRODUCTION

The use of biomaterials for permanent or temporal implantation into the human body is becoming increasingly established.[1] The global market for devices is valued at $86 billion per annum, with a growth of 7% per year.[2] These devices include catheters, prosthetic heart valves, pacemakers, and joint replacements. While biomaterials are generally considered safe, problems are often encountered with these implants. The most commonly encountered problem is an infection associated with the material. The incidence of device-related infections ranges from 2.7% to 60%, depending upon the type of device, the underlying disease of the patient, and the criteria used for diagnosis of device-related infection.[3] In Canada alone, over 100,000 device-related infections occur each year, costing the health care system over $135 million.[4] These infections are usually very serious, and in most cases require removal of the infected device. The results are both costly, inconvenient, and in some cases, life-threatening to the patient.

These devices can easily become colonized by microorganisms which form a biofilm on the surface of the biomaterial.[5] The biofilm typically consists of the microorganisms as well as a matrix of extracellular polymeric material surrounding the organisms. The infection may then be caused by either the biofilm, detachment of the biofilm cells into the patient, or by planktonic cells. Investigations with pathogenic bacteria have shown that biofilms composed of bacterial pathogens in vitro, have a substantially reduced sensitivity to clinically-important antibiotics compared with cells of the same organism in dispersed form.[6] Infections typically result in removal and replacement of the infected device. While

this is often an effective treatment, it is costly, time consuming, and dangerous. The ideal solution would be to prevent the organisms from initially adhering to the surface. To accomplish this, a knowledge of the specific mechanism of microbial adhesion to the surface would be valuable. From this, a designer material could theoretically be constructed.

Much work has been accomplished in the area of adhesion of bacterial cells to biomaterials. Although the majority of implant infections are caused by gram-positive bacteria, notably staphylococci, infections due to gram-negative bacteria and fungi tend to be more serious.[7] Relatively little work has been done on yeast adhesion to surfaces. Among the yeasts, the most important human pathogens are those belonging to the genus *Candida*.[8] All are opportunistic pathogens causing disease when the host defenses are impaired. The attachment of *Candida albicans* to various biomaterials and host tissues has been deemed an important step in the initiation of both superficial and deep-seated candidiasis.[9] It is therefore important to elucidate the mechanism of yeast adhesion to biomaterial surfaces.

Previous work has focused primarily on two aspects of yeast cell adhesion to polymer surfaces. The first of these is characterization of environmental effects (such as pH, temperature, media, and cation concentration) on adhesion. Most of the studies have used static adhesion assays that do not accurately portray in vitro situations. The other major focus has been biochemical elucidation of the specific attachment mechanism used by the yeast cell. While these studies have yielded important results, the use of more highly advanced analytical techniques could prove to be a key in elucidating the specific compounds used in the cell-biomaterial interface. This thesis presents results obtained using a flow system which more closely approximates an in vitro situation. It also uses highly advanced

analytical methods to probe the cell-biomaterial interface.

CHAPTER 2

BACKGROUND RESEARCH

2.1 Candida albicans

2.1.1 Candida albicans Overview

Candida albicans is a pathogenic yeast which causes a variety of infections in people that are characterized by cutaneous, mucosal, or systemic invasion.[10] *C. albicans* is the major etiologic agent of candidiasis and studies show that at least 60% of the *Candida* isolated from sites of infection are of this species.[11] Because *C. albicans* is part of the normal human flora, it represents an opportunistic infection.

C. albicans is a dimorphic yeast that grows as both a budding yeast form and a mycelial form. It may exist as either of two phenotypes; opaque or white. Two serotypes have been defined depending on the surface glycoproteins present. One unique aspect of the yeast is its anthropomorphic ability to rapidly change its cell surface in response to new environmental conditions. It has been shown that yeasts grown in media promoting hydrophilicity can change to a hydrophobic cell surface within sixty minutes of a change of media.[12] It has also been shown in laboratory tests that expression of cell surface hydrophobicity results in an increased level of virulence.[13]

The yeast cell can exist in either the budding yeast form, the hyphal form, or the pseudo-hyphal form. The hyphal form is indicated by germ-tube formation from the mother yeast cell. The budding yeast form is round to ovular in nature with a diameter of 3-5 microns. The yeasts reach stationary phase within approximately 18-24 hours of growth in most media.

2.1.2 Candida albicans Cell Wall

C. albicans possesses a cell wall consisting of five to eight distinct layers.[14] The total thickness of the cell wall has been estimated at 200-300 nm depending upon growth conditions.[15] The cell wall serves two major purposes; it maintains cell shape and is the point of contact between the cell and its environment. The cell wall is a complex structure composed of mannan, glucan, mannoproteins, chitin, proteins, and a small amount of lipid. Glucan, mannan and mannoproteins constitute at least 80-85% of the cell wall, with the remaining percentage being distributed between proteins (5-15%), lipids (2%), and chitin (0.9-9%).[16] The physical structure of the cell wall is shown in Figure 1.[17]

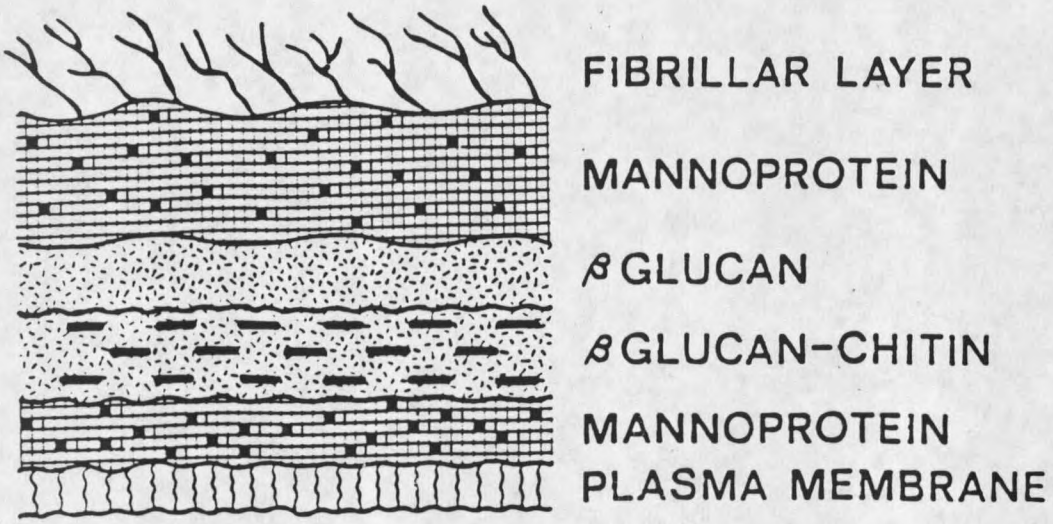

Figure 1: Diagram of *Candida albicans* cell wall structure.

Proteins of the cell wall represent an extraordinary array, the number depending on the growth conditions.[18] Conflicting reports have indicated that proteins either compose a

fibrillar layer projecting from the cell surface, or are equally distributed throughout the cell wall. It is agreed upon, however, that a fibrillar layer on the outermost region of the cell wall appears to be composed primarily of mannoproteins. Since these mannoproteins represent the outermost region of the cell, they may play a key role in the surface-mediated activities of the yeast such as adhesion. It has been shown that growth in a medium with a high sugar concentration will promote the growth of this fibrillar layer.[19]

2.1.3 Media Effects

Generally, the pH and chemical composition of the growth medium, as well as inoculum size and incubation temperature, determine the growth form. Hyphal formation is generally enhanced in a medium with a pH higher than 6.5 and a non-fermentable carbon source when grown at high temperatures.[20] Growth in an acidic medium using a fermentable carbon source typically promotes blastospore (budding yeasts) production at low incubation temperatures.[21]

2.2 Cellular Attachment to Surfaces

2.2.1 Cellular Attachment:Physical Requirements

Many different environmental and biological factors have been proposed as influencing the adhesion of microbial cells to surfaces. Two main stages of biological adhesion have been recognized; a primary physical attraction stage followed by a secondary biological adhesion stabilization.[22] For a cell to become adhered tenaciously to a surface, both of these stages must occur successfully.