Adhesion and subsequent biofilm formation of Candida albicans on chemically different surfaces as investigated using confocal scanning laser microscopy
by Karen Emma Wesenberg

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemical Engineering
Montana State University

Abstract:
Candida albicans comprises part of the normal human flora whose growth is usually restricted by the normal flora bacteria and the host's immune system. C albicans is an opportunistic fungal pathogen which causes infections in immunocompromised individuals, mechanical trauma victims, and iatrogenic patients. C. albicans can ingress the human host by adhering to a plastic surface (i.e., prosthetic devices, catheters, artificial organs, etc.) and forming a protective biofilm which provides a continuous reservoir of yeast to be hematogeneously dispersed. In order to battle device-related infections, the mechanisms of adhesion and biofilm formation of C. albicans must be recognized. A well-defined culture surface allows the initial adhesion and biofilm development to be studied. There has been some skepticism as to whether the initial adhesion events have any relationship to subsequent biofilm formation. Thus, to better comprehend the relationship between the initial adhesion rates and the long term growth rate and mature biofilm formation, these events were studied on two different culture surfaces, native polystyrene and Pluronic F127-conditioned polystyrene. The adhesion studies determined that Pluronic F127 adsorption dramatically reduced the adhesion of two strains of C. albicans of different serotypes to polystyrene. The biofilm growth studies, analyzed by confocal scanning laser microscopy, revealed that Pluronic F127 decreased the biofilm surface coverage, cluster group size, thickness, and the presence of hyphal elements over the untreated polystyrene. These findings indicate that the effect of a material's surface chemistry on the initial adhesion process has a direct influence on subsequent biofilm formation.

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This dissertation has been read by each member of the dissertation 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

Candida albicans comprises part of the normal human flora whose growth is usually restricted by the normal flora bacteria and the host's immune system. *C. albicans* is an opportunistic fungal pathogen which causes infections in immunocompromised individuals, mechanical trauma victims, and iatrogenic patients. *C. albicans* can ingress the human host by adhering to a plastic surface (i.e., prosthetic devices, catheters, artificial organs, etc.) and forming a protective biofilm which provides a continuous reservoir of yeast to be hematogeneously dispersed. In order to battle device-related infections, the mechanisms of adhesion and biofilm formation of *C. albicans* must be recognized. A well-defined culture surface allows the initial adhesion and biofilm development to be studied. There has been some skepticism as to whether the initial adhesion events have any relationship to subsequent biofilm formation. Thus, to better comprehend the relationship between the initial adhesion rates and the long term growth rate and mature biofilm formation, these events were studied on two different culture surfaces, native polystyrene and Pluronic F127-conditioned polystyrene. The adhesion studies determined that Pluronic F127 adsorption dramatically reduced the adhesion of two strains of *C. albicans* of different serotypes to polystyrene. The biofilm growth studies, analyzed by confocal scanning laser microscopy, revealed that Pluronic F127 decreased the biofilm surface coverage, cluster group size, thickness, and the presence of hyphal elements over the untreated polystyrene. These findings indicate that the effect of a material's surface chemistry on the initial adhesion process has a direct influence on subsequent biofilm formation.

INTRODUCTION

Fungi and bacteria have been shown to adhere to biological and non-biological surfaces with the subsequent formation of biofilms. These biofilms provide a refuge from the effect of antimicrobial agents and molecules of the immune system while also serving as a source for seeding further biofilm development. Pluronics, poly(ethylene oxide) (PEO) containing triblock copolymers, have been shown to minimize adhesion, an initial stage of biofilm formation, of host proteins, bacteria, and fungi on inert surfaces. This introduction is meant to provide insight into the findings of previous investigators in the areas of

1) *Candida* ecology, epidemiology, virulence, morphology, and structure,
2) fungal and bacterial attachment, growth, and differentiation into mature, recalcitrant biofilms, and
3) surface modification with Pluronics or PEO polymers, generating highly hydrophilic surfaces.

Candida albicans

Candida is a normal commensal of the human gastrointestinal and genitourinary tracts and mucosa.(36,43) Candidiasis is an infection caused by species of *Candida* with *C. albicans* being the major etiologic agent. Since *Candida* is a part of the normal human flora, this is an opportunistic fungal infection. Generally the growth of *Candida* is kept in check by the body's immune defenses and normal flora bacteria, however, overgrowth can

occur in immunocompromised individuals (e.g., diabetes, lymphoma, leukemia, DiGeorge Syndrome, AIDS), mechanical trauma victims (e.g., burn patients), and as a result of iatrogenic factors including drugs that affect the normal human flora or immune system (e.g., broad spectrum antibiotics, corticosteroids, antitumor chemotherapy), catheters and other medical devices, and surgical procedures (e.g., abdominal, heart, and transplant surgery).(8,43)

Proposed virulence factors of *C. albicans* include hyphal formation, adhesion properties, toxin production, and the dynamic cell surface. *C. albicans* can exist in one of three different morphological states, namely as blastoconidia, hyphae, or pseudohyphae. The form of the yeast depends on environmental conditions such as pH, incubation temperature, inoculum size, and composition of the growth medium.(8,43,49,81) The budding phenotype is typically observed at low pH or low temperature whereas the hyphal phenotype is generally associated with high pH and high temperature. Stationary phase yeast cells inoculated into medium at 37°C and a pH of 4.5 grow solely in the yeast form. If stationary phase yeast cells are introduced to medium at 37°C and pH of 6.7, the yeast will grow solely as hyphae as long as the pH remains above 6.0. At a pH between 5.5 and 6.5, the stationary phase yeast cells will initially form elongated daughter cells and later revert to the budding form and intermediate phenotypes.(81) The change from yeast to hyphal form is accompanied by changes in the chemical and structural makeup of the cell wall components.(86) The morphological changes may occur as a result of modifications in gene regulation in response to contact with a surface.(32)

S. cerevisiae has been proposed as a model system for *C. albicans*. However, there are important differences in the regulation of genes responsible for hyphal formation. Thus, although *S. cerevisiae* gene homologs in *C. albicans* influence morphology, they still fail to provide a clue as to the relationship between hyphal formation and virulence.(49)

The cell wall of *C. albicans* plays a role in adhesion to host tissues and plastics, in procuring nutrients from its environment, resisting drugs and products of the immune system, and in eliciting an immune response.(37) The cell wall of blastoconidia is composed of chitin, glucan, mannan, and lipids. The total glucan and mannoprotein content remains fairly constant in the transition from the yeast to filamentous form, whereas the chitin and lipid levels increase. The cell wall of *C. albicans* has been described in terms of regions of enrichment.(14,61,78) The outermost fibrillar layer consists primarily of mannoproteins. The yeast to hyphal transition is associated with an increase in adhesion and cell surface hydrophobicity (CSH). The increase in CSH is due to changes in the outer fibrillar layer mannoproteins which are thought to be the primary adhesins.(19,24,38)

The adhesion of stationary phase yeast cells is greater than that of log-phase cells to tissues, but adhere primarily to splenic tissue. (Note: humans are naturally exposed to endogenous stationary phase yeast cells.) Adhesion to host tissues, however, is not in and of itself responsible for the onset of disease since *S. cerevisiae*, a nonpathogenic yeast, binds to host tissues. In otherwise immunologically normal mice, *C. albicans* binds to macrophage-rich regions of lymph nodes and spleen and kidney tissue. The kidney is the

only organ that consistently supports fungal growth, thereby contributing to disease.(9,19) Hydrophilic yeast cells bind to the macrophage rich regions of lymph nodes and spleen; whereas hydrophobic yeast cells adhere to all tissues and in regions that are void (or nearly so) of macrophages. Hydrophobic yeast cells are more virulent than hydrophilic yeast cells. Hydrophobic yeast cells seed kidney tissues, germinate at a faster rate than hydrophilic cells, and display decreased killing by phagocytoses.(36)

The hydrophobicity/hydrophilicity of *C. albicans* depends on the growth form and environmental conditions. Excessive and limited carbohydrate levels in the growth medium result in increased cell surface hydrophobicity (CSH). Yeast cells grown on solid or liquid culture exhibit increased CSH over those grown within a liquid.(32,37) Although temperature alone does not dictate CSH, stationary phase yeast cells grown at 28°C are typically more hydrophobic than cells grown at 37°C. Actively growing yeast cells display modest levels of CSH, pseudohyphae display variable amounts of CSH, and hyphae are highly hydrophobic.(19,24,36,37) Due to the dynamic nature of the cell surface, *C. albicans* can quickly change from hydrophilic to hydrophobic. Within 60 minutes of exposure of yeast cells to tissue culture medium or fresh growth medium, *C. albicans* can change from hydrophilic to hydrophobic. Low concentrations (10^8 cells/ml) of yeast in suspension on ice can convert rapidly from hydrophilic to hydrophobic. If pelleted on ice, however, the cell surface hydrophilicity can be maintained for four hours.(36,37)

The fibrils of hydrophobic cells are blunt and aggregated whereas those of the hydrophilic cells are long, distinct, thin, and tightly packed. The fibrils of hydrophilic cells

consist of high molecular mass mannoproteins. Hydrophobic and hydrophilic cells have similar hydrophobic proteins. The hydrophobic proteins are small, poorly glycosylated, and are tightly associated with the cell wall. In hydrophilic cells, the hydrophilic proteins mask the hydrophobic proteins. The hydrophilic proteins are large and loosely associated with the cell wall and are synthesized and shed throughout cell growth. Hydrophobic proteins are present during the various stages of growth regardless of temperature or medium composition. Exposure of the hydrophobic proteins, however, depends on the growth phase, growth form, and temperature. Adhesion of *C. albicans* to low surface charge substrata involve nonspecific hydrophobic interactions, whereas adhesion to host tissues requires specific hydrophobic adhesins.(37,38,39)

The adhesion of *C. albicans* to host tissues and plastics is influenced by the type and quantity of sugars. Adhesion to polystyrene (PS) is blocked by amino sugars and enhanced by increasing glucose concentrations up to 50mM. Above this concentration, however, adhesion to PS is prevented. Adhesion of *C. albicans* strain 51 was shown to be augmented by galactose, glucose, and divalent ions (e.g., Ca^{2+}, Mg^{2+}) and hindered by Fe^{3+}. At 37°C, adhesion of *C. albicans* strain 51 was greater than at 20° and 25°C and attained a maximum at 40°C. Binding to host tissues is precipitated by the interaction between host proteins and *C. albicans* surface molecules. *C. albicans* has cell surface receptors specific for host proteins including fibrinogen, fibronectin, laminin, and type I and type IV collagen which promote adhesion to conditioning films that form on surfaces exposed to host fluids.(19)

C. albicans can exist as either serotype A or serotype B, which differ in the composition of the phosphomannan (PM) complex. The PM complex of serotype A is similar to that of serotype B with the exception of the presence of some non-phosphodiester-linked β-(1,2)-oligomannosyl side chains in the phosphomannan acid stable portion (PM-AS). The monoclonal antibodies (MAb) B6.1 and B6 are of the IgM isotype with the former conferring increased resistance against experimentally disseminated candidiasis. Both MAb B6.1 and B6 are specific for yeast cell wall mannan. MAb B6.1 epitope is part of the cell wall PM complex, namely fraction III and fraction IV of phosphomannan acid labile (PM-AL) portion. Fraction III comprises the greatest portion of the B6.1 epitope and consists of isomers of mannotriose. The B6.1 epitope is a β-(1,2)-mannotriose. Fraction IV is composed of mannotriose and mannotetraose. MAb B6 epitope is part of the PM-AS portion and is mannan in nature.(29)

Mannan adhesins can be extracted from the fungal cell wall with 2-mercaptoethanol (2-ME). This extract inhibits the adhesion of *C. albicans* to lymph node and spleen tissue, independent of strain or serotype. Fraction IIa and fraction IIb, obtained from fractionation of the 2-ME extract, displayed greater adhesion than the other three fractions. Fraction IIa prohibited adhesion to lymph node and spleen and is comprised primarily of mannose (98-99%). The mannose portion of mannoproteins is responsible for the adhesion of hydrophilic yeast cells to tissue such as spleen and lymph node. Adhesion of hydrophobic yeast cells to tissue is mediated through the cell wall proteins.(45)

An antigen (10G Ag) was located within *C. albicans* cell wall surface and plasmalemma. The 10G Ag epitope was determined to be a β-(1,2)-linked tetramannose and is thought to be part of the acid labile region of the phosphomannan complex. Both the 10G Ag and the 10G Ag epitope bind to mouse spleen marginal zone macrophages and block binding of *C. albicans*.(70)

Prevention of candidiasis precipitated through the application of catheters and other medical devices requires the generation of a non-fouling surface. Production of a non-fouling surface necessitates not only the blocking of *Candida* adhesion but also that of host proteins. Upon exposure of surfaces to host fluids, proteins and other components adhere to the surface, forming a conditioning film. *C. albicans* has cell surface receptors specific for host proteins, enabling it to bind to surfaces coated with a conditioning film. The problem then is how to generate such a non-fouling surface.(19) Generation of a surface that completely precludes binding of yeast and host proteins may prove difficult, but it should be possible to produce a surface that limits adhesion. By limiting adhesion, we minimize one of the virulence factors associated with *C. albicans.* By reducing the presence of the hyphal form of *C. albicans*, another proposed virulence factor can be controlled. Pseudohyphae and hyphae are associated with higher levels of CSH than the yeast form of *C. albicans*.(19,24,36,37) This, along with the observation that *C. albicans* adheres to low surface charge substrata through hydrophobic interactions (37,38,39), leads to the question of whether a highly hydrophilic surface could limit both the interaction of *C. albicans* with the surface and the presence of the hyphal phenotype.

Fungal Adhesion and Biofilm Formation

C. albicans can adhere to a variety of biomedical implants including contact lenses, prosthetic devices, pacemakers, artificial joints, and urinary, central venous, and peripheral catheters. Following adhesion to a surface, yeast can develop microcolonies sheathed within a polymeric matrix, forming a biofilm which acts as a protective barrier. A biofilm provides a setting where the yeast can proliferate and release cells into the surrounding fluids and tissues, contributing to the onset of acute disseminated infections. Although slow to develop, the infections are relentless as the microorganisms are unreachable by host defenses and antibiotics. Thus, suppressing the infection usually requires removal of the implant.(32,92)

The preponderance of manifestations of candidiasis are associated with the formation of biofilms on inert or biological surfaces and in mucosal and systemic sites.(66) Enhanced adhesion of *Candida* species in an oral environment is influenced by sucrose- and glucose-rich diets, acidic pH, cell surface hydrophobicity and cell surface mannoproteins. *C. albicans* and *C. dubliniensis* have been acquired simultaneously from the mouths of immunocompromised individuals. Under planktonic conditions in Sabouraud liquid broth modified antibiotic medium 13 (SDB), *C. albicans* prevails over *C. dubliniensis*. When exposed to consistent conditions of pH, temperature, nutrient levels, and waste removal, the phenotype or organism with the enhanced growth rate will predominate. *C. albicans* also prevails over *C. dubliniensis* under sessile conditions in

SDB, however, not to as great an extent. Population differences due to variations in growth rates are not as notable in biofilms as they are in suspension cultures.(48)

The ability of *S. cerevisiae* to demonstrate the initial stages of biofilm formation has led to its indicated use in ascertaining the role of *C. albicans* cell surface proteins in pathogenesis and in evaluating the capacity of compounds to block fungal adhesion. Flo11p is a *S. cerevisiae* cell surface protein which is required for cell-cell adhesion and cell-surface adhesion. The ortholog of Flo11p in *C. albicans* is a proposed virulence factor since expression in *S. cerevisiae* led to adhesion to mammalian cells.(71) However, as indicated earlier, adhesion does not by itself account for the onset of disease.(9)

The emergence of candidiasis is attributed in large part to the formation of a biofilm (66), and therefore prevention requires circumventing biofilm formation. The question is then whether limiting the initial adhesion event can lead to diminished growth and thwart the appearance of a mature differentiated biofilm.

Bacterial Adhesion and Biofilm Formation

Biofilms are found in association with ship hulls, oil drilling pipes, food fermentors, and dental plaque. Biofilms can be a contributing factor in upper respiratory infections, kidney stones, prostate infections, urogenital infections, periodontal disease, Legionnaire's disease, peritonitis, and middle ear infections. Approximately 10 million infections that occur in the U.S. each year are precipitated by biofilm formation on permanent medical implants. These surfaces which are not adequately protected by the host immune system provide binding sites for microorganisms. In North America, more than 100 million

urethral catheters and urinary stents are employed each year. In the absence of antibiotic treatment, up to 28% of urethral stents and up to 100% of catheters are prone to infection which can lead to death. Upon exposure to body fluids (e.g., saliva, blood, urine) surfaces are coated with a conditioning film consisting of host proteins and other substances. Host proteins including serum albumin, fibrinogen, fibronectin, and collagen can serve as sites for bacterial adhesion. The existence of a conditioning film is thought to be an initiating stage for infectious biofilm development. An effort to compromise or eliminate the link between the conditioning film and the biofilm, such as through fluctuating shear forces or surfactants, could hinder biofilm formation or allow for removal through sloughing.(68)

The recalcitrance of bacterial biofilms is thought to be due to a multicellular endeavor. For example, degradation of hydrogen peroxide by catalase produced by bacterial cells, including nonviable cells, requires a concerted effort by a group of cells. A single cell could not produce enough catalase to overcome the debilitating effects of hydrogen peroxide. Similarly the activity of some antibiotics requires oxygen. The cells on the perimeter of the biofilm consume the oxygen and thereby protect their deeper neighbors. Again, a single cell could not deplete enough oxygen from its surroundings to prevent the antibiotic's activity. The presence of various metabolic states within a biofilm also provides for protection from chemical and physical assaults. The majority of cells are in an active growing state which leaves them prone to the effects of antimicrobial agents. However, some of the biofilm cells are in a static, spore-like state and are protected, allowing them to reseed the biofilm. Thus, the symptoms of an infection may subside only to flare up later.(59,82)

The presence of various metabolic states represents a physiologically based mechanism of resistance. Another potentially contributing mechanism to bacterial biofilm recalcitrance could be a transport-based mechanism. The reduced susceptibility of biofilm bacteria can not be explained by reversibly sorbing, nonreacting solutes or by stoichiometrically reacting solutes. In contrast, irreversibly sorbing, nonreacting solutes or catalytically reacting, nonsorbing solutes could account for the increased resistance of biofilm bacteria provided the reaction is fast enough in the latter instance. However, there is no indication of extensive irreversible sorption of antibiotics to biofilms, and the vast majority of antibiotics do not react rapidly enough to account for the increased tolerance.(83)

Bacterial biofilms consist of microcolonies shrouded in extracellular polysaccharide (EPS) matrix or glycocalyx and demarcated by water channels. These sessile communities are physiologically and morphologically distinct from their planktonic counterparts. The exterior cells of microcolonies receive sufficient nutrients and waste removal. The aerobic conditions allow for the growth and activity of bacteria that require oxygen for these functions. These bacteria produce toxins and other substances that produce deleterious effects on the host. However, this active state also contributes to their destruction by drugs such as penicillin which act on replicating cells. The interior cells of microcolonies experience limited nutrient levels and waste removal and depend on the diffusion of these molecules. These cells are exposed to reduced oxygen levels which leads to an inactive state. Thus, these cells pose little threat to the host. This state also provides resistance to

drugs such as penicillin, leaving these cells to consume those cells that expire and to restore the biofilm.(6,10)

Biofilm bacteria generate signal transduction proteins which gather information from the environment and relay it to chromosomal elements. This form of communication allows for a group virulence response.(68) *P. aeruginosa* has two known cell-to-cell signaling processes including the *lasR-lasI* and *rhlR-rhlI.* The *lasR-lasI* and *rhlR-rhlI* gene products are homoserine lactones which attain, with sufficient population sizes, concentration levels necessary for gene activation. This type of gene regulation is termed "quorum sensing and response". Both the wild-type (PAO1) and the *lasI-rhlI* double mutant biofilms attained steady-state within two weeks. The *lasI-rhlI* double mutant formed thin, dense biofilms and failed to produce quorum sensing signals. The *rhlI* mutant generated biofilms with similar thickness and cell packing as the wild-type. The *lasI* mutant developed biofilms that had similar thickness and cell packing to the double mutant and unlike the wild-type was sensitive to sodium dodecyl-sulfate (SDS). In the presence of synthetic signal molecules, however, the *lasI* mutant formed biofilms which appeared normal. Thus, the quorum sensing signal $3OC_{12}$-HSL, the gene product of *lasR-lasI*, is necessary for normal biofilm differentiation. The initial stages of biofilm development, adhesion, and growth are normal for the *lasI* mutant, but it fails to form a mature differentiated biofilm. The wild-type and planktonic counterparts generate similar amounts of EPS but differ in the glycocalyx appearance which is "compressed and incomplete" in planktonic systems. This may explain the close packing observed in the

mutant biofilms. Thus, an ability to block cell-to-cell signaling provides a means of preventing mature biofilm formation.(10)

A chemical being developed to target bacterial communication was spurred by findings that the red marine alga *D. pulchra* does not contain biofilms on its fronds. The chemicals responsible for this antibacterial behavior are substituted furanones which act by binding to locations on bacteria that are normally occupied by signal molecules. Thus, the bacteria do not receive a signal to amass and create a biofilm. Substituted furanones have been shown to not only prevent biofilm formation but to disperse existing biofilms. They are also nontoxic and fairly stable in the human body and bacteria have not developed a tolerance over the years of exposure to them in the oceans.(6,59)

Another potential way to limit biofilm formation is to employ drugs directed against the production of extracellular matrix and against adhesion to surfaces, preventing subsequent biofilm formation. Upon adhering to a surface, the production of many proteins not found in planktonic cultures is initiated. For example, *Pseudomonas aeruginosa* expresses the algC gene which is required for the production of alginate, a principle component of the extracellular matrix.(6)

In nature, biofilms are a multispecies conglomeration.(48) Dental plaque biofilms have over 500 species. Gram-positive bacteria, primarily streptococci, are the first to appear. They are followed by gram-negative anaerobic bacteria such as *P. gingivalis* which represents a transition from a commensal to a pathogenic entity. *P. gingivalis* adheres to oral surfaces through fimbriae. The *fimA* gene product, FimA, is a primary protein subunit of fimbriae. Expression of the *fimA* gene is influenced by environmental

factors and signaling molecules. *S. cristatus* produces a signal molecule which reduces *fimA* gene expression and thereby prevents *P. gingivalis* biofilm formation. *S. gordonii*, in contrast, provides a binding site for *P. gingivalis* through an adhesin-receptor interaction with FimA and *S. gordonii* surface molecules.(89)

Host proteins that makeup the conditioning film that forms on surfaces exposed to body fluids provide sites for the adhesion of bacterial cells. The presence of a conditioning film is considered one of the initiating stages for infectious bacterial biofilm development.(68) Thus, generating a surface that repels the adhesion of host proteins would eliminate not only one element of *Candida* adhesion, but also bacterial adhesion. The recalcitrance of bacterial biofilms is described as being a multicellular endeavor (59, 82), studies indicate that this is also true for *Candida* biofilms (3,5,33). Therefore, the ability to produce a surface which limits adhesion and obstructs the appearance of multicellular structures would also increase the sensitivity of those cells that attach to the surface. Thus, the cells that adhere to the surface would be subject to the effects of antimicrobial agents and antiseptics. The thin, dense biofilms produced by *P. aeruginosa lasI* mutants were sensitive to SDS and failed to form mature differentiated biofilms, in contrast to the wild-type.

Fungal and Bacterial Biofilm Formation

Biofilm development is influenced by nutrient supply, hydrodynamic flow, cell movement, and interactions between organisms. Interactions between bacteria and fungi can either promote or hinder adhesion. The presence of bacteria decreased the

susceptibility of fungi to antimicrobials. The initial adhesion event is a random process which depends on the surface free energy and proximity to the surface. Following adhesion, cells multiply and differentiate into a heterogeneous multilayer community that allows for the presence of various metabolic states. The biofilm presents a diffusion barrier which cannot necessarily be attributed to the EPS.(19,42)

Poly(ethylene oxide) and Pluronic

Highly hydrophilic surfaces are non-thrombogenic due to a low blood-material interfacial tension which results in a low driving force for adsorption. The grafting of hydrophilic polymers such as poly(ethylene oxide) (PEO) onto polymer surfaces produces a hydrophilic polymer. The propinquity of protein molecules to the surface is limited as a consequence of excluded volume effects and decreasing configurational entropy of mobile PEO chains. The adsorption of amphiphilic PEO block copolymers onto glass, polystyrene, and polyethylene diminished adhesion of albumin, fibrinogen, and blood platelets as compared to the unmodified surfaces.(21)

Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) copolymers are surfactants which are commercially available as Pluronics (produced by BASF) or Poloxamers (produced by ICI). Pluronics are utilized by the pharmaceutical industry as drug solubilizers, in controlled release systems, and as a burn covering. The conjugation of Pluronic copolymer micelles containing drugs to a vector allows for efficient transport of the drugs into tissues, including the brain. In bioprocessing, Pluronics serve to guard microorganisms against mechanical and chemical stress.(1)

The maximum adsorption of Pluronic to surfaces occurs at a concentration greater than the apparent critical micelle concentration (CMC). The PPO segment makes contact with surfaces such as polystyrene while the PEO segments extend into the bulk fluid (Figure 1). The PEO segments prevent fibrinogen and platelet adhesion through steric repulsion.(1) PEO chains grafted to particle surfaces prevents binding of blood components through steric stabilization. The thickness of the barrier increases with increasing PEO chain length. The steric stabilization is the result of osmotic and elastic contributions on overlap of polymer chains and an elastic contribution due to loss of configurational entropy between neighboring polymer chains.(30)

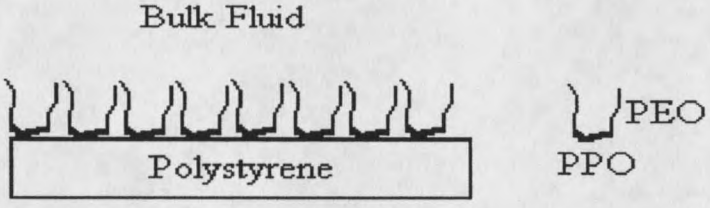

Figure 1. Schematic representation of adsorbed Pluronic F127 surfactant on PS.

The protection conferred by Pluronic F68 to insect and animals cells against shear stress is thought to be the result of decreased membrane fluidity. Pluronic F68 has been found to alter membrane permeability. The presence of Pluronic F68 extended the lag phase of *Saccharomyces cerevisiae* without significantly affecting the biomass concentration. A 1% Pluronic F68 solution did not affect the growth or flocculation of *Saccharomyces cerevisiae*.(20,41) In adhesion studies of human epithelial cells (HepG2) to hydrophobic surfaces, the presence of Pluronic F68 prevented collagen adsorption and

thereby HepG2 binding to the more hydrophobic BGPS but not to the less hydrophobic TCPS.(13)

Highly hydrophilic surfaces which are non-thrombogenic can be produced by adsorption of PEO-PPO-PEO triblock copolymers. The use of Poloxamers has also been shown to significantly limit the adhesion of *S. epidermidis* to polystyrene (4) and the adhesion of *P. aeruginosa* to hydrophilic contact lenses (64). Thus, the question for this thesis to consider is whether Pluronics are also capable of significantly inhibiting the adhesion of *C. albicans*.

Studies were performed to examine the following questions:

1) Does adsorption of Pluronic F127 onto the PS surface significantly limit the adhesion of *C. albicans*?

2) Does the ability to limit adhesion translate into limited biofilm formation of *C. albicans*?

3) Is the effect of Pluronic F127 on *C. albicans* adhesion and biofilm formation strain or serotype dependent?