MONTANA STATE UNIVERSITY

An exploration of the utility of TOF-SIMS as a new method for studying the cell surface of Candida albicans

by Hong Shi

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

Abstract:
Biomedical implants are becoming increasingly used in the medical field. The most common cause of failure for medical devices is infection, and the pathogenic yeast Candida albicans is the third leading cause of these infections. Adhesion is the first step in establishing an infection, the initial adhesion of microorganisms to synthetic polymer surfaces involves physicochemical interactions between molecules present at the polymer surface and those present on the cell surface. These interactions are not well defined, and insight into this area could lead to better material construction to effectively control the infection.

The objective of this thesis is to study the initial adhesion event of C. albicans grown in glucose-based medium and galactose-based medium to Fluorinated Ethylene Propylene(FEP) and use Secondary Ion Mass Spectrometry (SIMS) to study the surface structural differences between these two cell surfaces with an attempt to correlate surface functionalities to the adhesion results.

Adhesion experiments were conducted with a laminar flow cell. Yeast cell adhesion was found to be significantly reduced as shear rate at the time of attachment was increased in the flow cell. Glucose-grown cells were more adherent than galactose-grown cells at the same shear rate. Attachment of glucose-grown cells was more affected by shear rate than attachment of galactose-grown cells. Attached cells were tenaciously bound to the surface and unaffected by increased shear rates. This phenomenon was true for both carbohydrate sources.

A freeze-drier with ultimate vacuum less than 1.00x10-9 torr was constructed “in house” to freeze-dry cells for SIMS analysis. A filtration method was used to prepare a smooth layer of cells to be freeze-dried. Scanning Electron Microscopy showed that the freeze-dried cells appeared intact. High resolution SIMS spectra were obtained from these freeze-dried cell surfaces and Linear Discriminant Function Analysis combined with Principal Components Analysis were used to analyze the SfMS spectra. The results showed that the surfaces of glucose-grown cells contained more hydrophobic amino acid residues relative to those of galactose-grown cells. These hydrophobic amino acid residues probably promoted the adherence of glucose-grown cells to FEP surface. The results obtained in this study suggest that hydrophobic interactions are important in the initial attachment of C albicans to FEP surface. The SIMS spectra presented in this study were the first report of the freeze-dried C. albicans.

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Chemical Engineering

MONTANA STATE UNIVERSITY-BOZEMAN
Bozeman, Montana

June 1998

APPROVAL

of a thesis submitted by

Hong Shi

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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ACKNOWLEDGMENTS

I wish to express my deepest gratitude to my advisor Dr. Bonnie Tyler for her encouragement, advice and assistance through all the course of this study and thesis preparation. I would also like to thank Dr. Diane Brawner and Dr. Phil Stewart for their kind guidance and serving on my committee.

I would also like to thank Dr. John Sears and the staff of the Chemical Engineering Department at Montana State University for their help and friendship.

Thanks are extended to James Anderson and Nancy Equall of the Imaging and Chemical Analysis Laboratory at Montana State University for their assistance with XPS and SEM work, and Dr. Recep Avci for his help during the study.

Thanks are also extended to Wayne Marchwick and Angela Keefe for all their generous help around the lab. Ace Baty is acknowledged for his valuable suggestion of the freeze-drying operation and help with freeze-drying experiment using commercial freeze-drier. Kris Battleson is also acknowledged for her statistics knowledge and help with XPS work.

This work has been funded by NSF Grant #BES-9457217.

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ABSTRACT

Biomedical implants are becoming increasingly used in the medical field. The most common cause of failure for medical devices is infection, and the pathogenic yeast *Candida albicans* is the third leading cause of these infections. Adhesion is the first step in establishing an infection, the initial adhesion of microorganisms to synthetic polymer surfaces involves physicochemical interactions between molecules present at the polymer surface and those present on the cell surface. These interactions are not well defined, and insight into this area could lead to better material construction to effectively control the infection.

The objective of this thesis is to study the initial adhesion event of *C. albicans* grown in glucose-based medium and galactose-based medium to Fluorinated Ethylene Propylene(FEP) and use Secondary Ion Mass Spectrometry (SIMS) to study the surface structural differences between these two cell surfaces with an attempt to correlate surface functionalities to the adhesion results.

Adhesion experiments were conducted with a laminar flow cell. Yeast cell adhesion was found to be significantly reduced as shear rate at the time of attachment was increased in the flow cell. Glucose-grown cells were more adherent than galactose-grown cells at the same shear rate. Attachment of glucose-grown cells was more affected by shear rate than attachment of galactose-grown cells. Attached cells were tenaciously bound to the surface and unaffected by increased shear rates. This phenomenon was true for both carbohydrate sources.

A freeze-drier with ultimate vacuum less than 1.00×10^{-9}torr was constructed "in house" to freeze-dry cells for SIMS analysis. A filtration method was used to prepare a smooth layer of cells to be freeze-dried. Scanning Electron Microscopy showed that the freeze-dried cells appeared intact. High resolution SIMS spectra were obtained from these freeze-dried cell surfaces and Linear Discriminant Function Analysis combined with Principal Components Analysis were used to analyze the SIMS spectra. The results showed that the surfaces of glucose-grown cells contained more hydrophobic amino acid residues relative to those of galactose-grown cells. These hydrophobic amino acid residues probably promoted the adherence of glucose-grown cells to FEP surface. The results obtained in this study suggest that hydrophobic interactions are important in the initial attachment of *C. albicans* to FEP surface. The SIMS spectra presented in this study were the first report of the freeze-dried *C. albicans*.

CHAPTER 1

INTRODUCTION

In recent years, the use of medical implants such as catheters, pacemakers, prosthetic heart valves, and joint replacements has increases dramatically [1]. The global market for these devices is valued at $86 billion per annum, with a growth of 7% per year [2]. Infections constitute one serious complication of the use of these biomaterials, especially with skin penetrating devices [3]. These devices are easily colonized by microorganisms which form a biofilm consisting of a mono- or multilayer of cells embedded within a matrix of extracellular polymeric material. Release of microorganisms from the biofilm may initiate an acute disseminated infection. Implant-associated infections are difficult to resolve, because biofilm microorganisms are resistant both to host defense mechanisms and antibiotic therapy [1]. Frequently, the implant must be removed. The results are both costly, inconvenient, and in some cases, life-threatening to the patient.

Although the majority of implant infections are caused by gram-positive bacteria, infections due to gram-negative bacteria and fungi tend to be more serious [4]. Fungal infections are most commonly caused by the pathogenic *Candida sp.*. More than 850,000 catheter-related infections occur annually in the U.S. [5], of which 22% are caused by *Candida sp.*, predominantly *Candida albicans* [6]. The attachment of *C. albicans* to various biomaterials and host tissues has been deemed an important step in the initiation of both superficial and deep-seated candidiasis [7].

Because adhesion is the first step in establishing infection, the ideal solution is to prevent the organisms from initially adhering to the surface. To accomplish this, a knowledge of the mechanisms of microbial adhesion to the surface would be valuable. From this knowledge, design strategies for implant devices or well

engineered novel materials used for the construction of these devices can be developed to effectively control the infection.

In the literature, there are only a handful of publications focused on fungal adhesion to biomaterial surfaces, and most of them were focused on *C. albicans* because of its relevance to modern medicine. Previous work on fungal adhesion to polymer surfaces was primarily focused on three aspects. The first of these was to characterize the adhesion differences between different stains, serotype, phenotype or clinical origin of *C. albicans* to polymer surfaces or to characterize the effects of environmental factors such as media, pH, temperature and cation concentration on cell adhesion. The second was to use biochemical methods to characterize the nature of the adhesins. The third was to characterize the contributions of electrostatic and electrodynamic forces to the adhesion, which are theoretically involved in the 'nonspecific' interactions between cells and artificial surfaces. Almost all this work used static adhesion assays which can not accurately portray the adhesion event in vivo situations because cells are usually subjected to the flowing fluids in the human body. Also it is generally impractical to study adhesion kinetics with common static adhesion assays [8].

There have been few studies employing modern methods of surface analysis to study adhesion in the literature. Because the initial adhesion of micro-organisms to synthetic polymer surfaces involves physico-chemical interactions between molecules present on the polymer surfaces and those present on the cell surfaces, highly advanced surface analysis methods including XPS (X-ray Photoelecton Spectroscopy), also known as ESCA (Electron Spectroscopy for Chemical Analysis), and static SIMS (Static Secondary Ion Mass Spectrometry) should be very powerful in this regard. In the outermost 10nm of a surface, XPS can provide: identification of all elements (except H and He) present at concentrations greater than 0.1%; semiquantitative determination of the approximate elemental surface

composition (error<±10%); information about the molecular environment (oxidation state, bonding atoms, etc.) [9]. However, XPS is limited in its ability to provide detailed molecular information. This is particularly true when atoms are in a wide variety of chemical states, as exist in a protein. Also the achievable lateral resolution of XPS is limited to a few micrometers. Hence, it can not analyze smaller spots on the sample surface. Static SIMS is both more surface sensitive (sample depth is within 20Å of the sample surface) and more chemically selective than XPS. It allows the identification and quantification of all elements, isotopes, and molecular species [10]. Compared with XPS and other widely applied surface analytical techniques, such as Auger spectroscopy, static SIMS offers some unique features: isotope sensitivity; hydrogen sensitivity; direct compound detection by molecular secondary ion emission; extremely high sensitivity for many elements and compounds, very often in the ppm range. As in any MS technique, the quality and reproducibility of the data require that several criteria be met: controlled desorption of atoms and molecular species, efficient ionization of these desorbed particles, and unambiguous identification of the generated ions by their charge/mass ratios. A considerable fraction of molecular surface species should survive these processes without fragmentation . It has been shown that static SIMS meets these criteria and is well suited for elemental and molecular applications [11]. In static SIMS, the primary ion current density is kept very low so that no sample damage will occur during analysis. Nonconductive samples are readily analyzed with charge compensation.

As a surface analytical method, static SIMS is unrivaled in its molecular selectivity, because of its basis in mass spectrometry. Static SIMS has been used to analyze a wide variety of "real" surfaces, ranging from semiconductor materials to complex copolymers. The spectra produced reflect the surface chemistry of the material but, in general, do not contain large (m/z>=500) molecular ions or

fragments. However, useful information is readily extracted from the fragment ions in this lower mass range. The ability of static SIMS to produce a surface-sensitive mass spectrum gives it great potential as a probe of proteins adsorbed on surfaces [12]. The application of static SIMS for the study of the interaction of polymers with biological molecules and systems has been demonstrated by Mantus, D.S. and co-workers [12]. In their work, a spectral interpretation protocol was established by examining homopolymers of 16 amino acids. This protocol allowed for the assignment of peaks unique to the various amino acids. Consequently, it was used to study proteins adsorbed on polymer surfaces. They also demonstrated that the intensities of peaks in a static SIMS spectrum might be sensitive to the conformation of a macromolecule on a surface. This is a unique characteristics of static SIMS which makes it unrivaled in the study of adhesion between microorganisms and biomaterials, because conformation is very important for the function of a macromolecule such as protein or glycoprotein on a cell surface, and this function may be closely related to the adhesion event.

Freeze-drying is a technique by which biological specimens are frozen, then dehydrated by sublimation, with complete retention of physical detail and form. Sublimation is the process by which the water in cells passes from a frozen to a gaseous state without going through liquid state [13]. No chemical agents are required and freeze-dried cells don't shrink or otherwise become distorted as those dried from non-frozen state. Physical and chemical changes within the cells are reduced to the minimum degree. It is proposed in this study that freeze-drying is the most suitable method for preparing dried samples for surface structure analysis using static SIMS when a cryostage for SIMS is not available.

In this study, a laminar flow cell which has a well-defined hydrodynamic regime was used in the adhesion study of *C. albicans* to a polymer FEP (Fluorinated Ethylene Propylene) which is commonly used in intravenous catheters. Process

and growth parameters were varied to examine their effects on the adhesion kinetics. Subsequently *C. albicans* were freeze-dried using a freeze-drier built "in house" and static TOF-SIMS was used to analyze the surface structure differences between the freeze-died cells cultivated from different media with an attempt to correlate specific functional groups on the cell surfaces to the adhesion results.

CHAPTER 2

BACKGROUND RESEARCH

2.1 *Candida albicans*

2.1.1 A Brief Overview of *Candida albicans*

C. albicans is a dimorphic fungus which may be present in humans either as a commensal or as an agent of infection in the compromised host [14].

The dimorphic yeast *C. albicans* exists as either one of several phenotypes. First, *Candida* cells are capable of growing as a budding yeast form, the hyphal form, or the pseudo-hyphal form. In the budding yeast form, a mature yeast cell forms an evagination which expands into a mature daughter cell. In this growth form, cells are round to ellipsoidal with a diameter of 3-5 microns. In the hyphal form, cells grow as elongated, compartmentalized tubes. Budding cells can be induced to form hyphae and hyphae can be induced to form buds. Hyphae are believed to have evolved primarily as a mechanism for tissue penetration[15]. Another phenotypic variability is white and opaque and *C. albicans* is also able to switch between these two. Two serotypes, serotype A and B, have been defined by the variation in the mannan moiety of surface mannoproteins of *C. albicans*.

C. albicans is also capable of expressing surface hydrophobicity (CSH) when grown in either the yeast or hyphal form. Associated with expression of surface hydrophobicity is enhanced virulence[16]. Several groups of investigators have demonstrated that hydrophobic yeast and hyphal cells attach more than hydrophilic cells to various types of plastics used in prostheses and catheters [17,18,19,20]. Various observations suggest that fungal surface hydrophobicity expression is pivotal to the success of parasitizing *C. albicans* in causing serious infection [16]. The hydrophobic properties of the cell surface of *C. albicans*

appear to exhibit significant variation depending on the strain as well as the growth medium and conditions [21]. *C. albicans* isolates generally appear more hydrophobic when grown to stationary phase at room temperature (23-25^{0}C) than at 37^{0}C [21]. The expression of surface hydrophobicity in vivo may be transient. Hydrophilic cells obtained by growth at 37^{0}C in vitro, exposed hydrophobic surface macromolecules for only a short period when subcultured into fresh medium [22]. The results suggest that antigenic variation occurring during pathogenesis of candidiasis could involve surface hydrophobic molecules in addition to mannoprotein antigens [23,24,25].

One unique aspect of the yeast is its 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 [22]. *C. albicans* takes an active role in pathogenesis by producing several virulence factors that may promote the successful colonization or invasion of tissues [26]. Cell wall hydrophobicity and switching of expressed phenotypes have been proposed as attributes contributing to the virulence of this yeast [27,28]. The adhesins of *C. albicans* appear to be diverse, reflecting the ability of the organism to colonize and invade a variety of host cells and tissues [29,30]. Phenotypic and dimorphic states [31,32,33,34, 35,36] as well as environmental factors [37] appear to regulate the expression of *C.* adhesins and it is very likely that multiple adhesins are employed by *C. albicans* in adhering to host cell surfaces [38,39].

For in vitro studies, 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. Growth in an acidic medium using a fermentable carbon source

typically promotes blastospore (budding yeasts) production at low incubation temperature. *C. albicans* reaches stationary phase within approximately 18-24 hours of growth in most media [40].

2.1.2 *Candida albicans* Cell Wall

The cell wall of the organism is essential to its success as a pathogen, since it is required for growth, provides rigidity and protection against osmotic insult, and is the site of contact between the organism and its environment. The outermost layer of the *C. albicans* cell wall plays an important role in pathogenesis, mainly because it possesses macromolecules that adhere to host tissues [41]. Although the synthesis of the cell wall components is dynamically influenced by growth conditions and metabolic states, the literature contains fairly consistent data regarding the chemical composition of the cell wall [42]. The cell wall of *C. albicans* makes up approximately 30% of the dry weight of the cell [43]. A number of studies have shown that the cell wall is composed of mannoproteins (20%-30%), β-1, 3-D-glucans (25%-35%), β-1, 6-D-glucans (35%-45%), a small quantity of chitin (0.6%-2.7%), protein (5%-15%) and lipid (2%-5%) [44]. Observations by electron microscopy reveal at least six layers in the cell wall of *C. albicans* [45]. The architecture of the cell wall includes a fibrillar layer followed, in order, by zones rich in mannoprotein, glucan, glucan-chitin, and mannoprotein, as shown in Figure 1 [42].

Although the appearance of the cell wall suggests a discrete packaging of the various macro-molecules, the layering is more likely a result of differences in the proportions of the components in each layer rather than absolute differences in chemical composition [46].

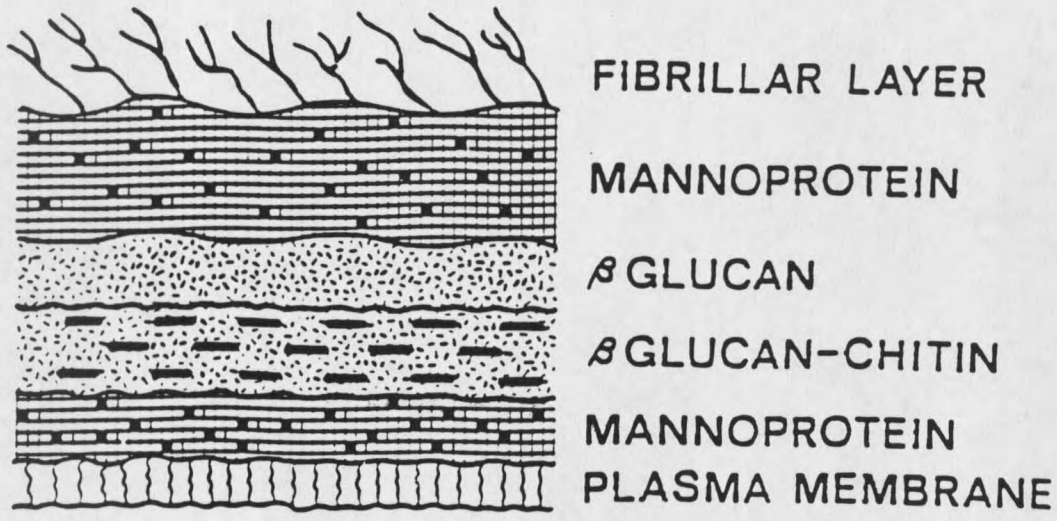

Figure 1. Schematic diagram of the cell wall of *Candida albicans*

The fibrillar layer on the outermost region of the cell wall appears to be composed primarily of mannoproteins [41,42]. The amount of fibrillar material produced depends on the growth medium, is proportional to the concentration of sugar, and increases with the age of the culture [41]. For example, incorporation of galactose or sucrose instead of glucose in the culture medium results in a greater production of the fibrillar outer layer [47,48]. Recently, Yu et al [49] reported that the major structural subunit of the fibrous structure is a glycoprotein which consists of 80%~85% carbohydrate (consisting primarily of D-mannose) and 10%~15% protein. Since this fibrillar outer layer represents the outermost region of the cell, it may play a role in the adhesion of the yeast to host cell or plastic surfaces. Both the roles of protein moiety and oligosaccharides of the mannan moiety in adhesion ability cannot be ruled out [50,51,52,53].

The two outermost and the innermost electron-dense layers that are present on each side of an intermediate electron-lucent layer have been shown to be composed of mannoprotein [45], and are thus called the mannoprotein layer. There

have been various types of mannoprotein consisted of protein and mannan or mannooligosaccharide in different proportions [41]. One of the surface structure mannoproteins of *C. albicans* has been reported to be a 260kDa polymer composed of 1.5% protein and 98.4% hexose [54]. In addition to structural mannoproteins mentioned above, several mannoproteins with distinct adhesion activity which range from 50 to 165kDa have been reported [55,56,57,58], although the mode of the molecular organization of such mannoproteins has not been elucidated. The evidence reported suggests that there are different mannoproteins which contain different protein/peptide groups and possess different functions. Moreover, the wall contains a number of enzymes including N-acetylglucosaminidase, acid phosphatase, proteinase, glucanase and chitinase [45].

2.2 Cellular Attachment to Surfaces

2.2.1 Molecular Mechanisms of Cellular Attachment

Cellular attachment to surfaces is a very complex process, the physico-chemical properties of the organism surface and the substratum surface as well as environmental factors are all important in this process. Two main stages of biological adhesion have been recognized. A reversible initial attachment or primary physical attraction stage followed by a secondary irreversible adhesion stabilization [59]. Initial attachment depends on the general long-range physical characteristics of the organism, the fluid interface, and the substratum [60]. The second stage may be facilitated by a metabolic process or just result from the formation of multiple contacts once the cell is held on the surface. For a cell to become adhered tenaciously to a surface, both of these stages must occur successfully.

Surface free energies must dominate any explanation of the adhesion between different phases which are not mechanically linked [61]. The surface of a substance or phase requires an extra term in the description of its energy which is due to its location at a boundary where there is a sharp change in concentration or properties of the substance. The energy associated with this location is the surface free energy and is described by the energy required to form a new unit area of surface (Eq.1).

$$\frac{dG}{dA} = \gamma \qquad (1)$$

where: G= Gibbs free energy

A= area

γ = Surface free energy per unit area

Surface free energy is equivalent to surface tension (the force required to part a unit length of surface) for liquid which is very easy to measure experimentally. But for solid, this is not necessary true. To calculate solid surface energies, Eq.2 is the mostly used empirical equation in the literature.

$$\cos\theta = \frac{(0.015\gamma^{SV} - 2.00)(\gamma^{SV}\gamma^{LV})^{1/2} + \gamma^{LV}}{\gamma^{LV}[0.015(\gamma^{SV}\gamma^{LV})^{1/2} - 1]} \qquad (2)$$

Where:

θ = measured contact angle of a sensing liquid on the solid surface.

γ^{SV}= solid vapor interfacial free energy.

γ^{LV} = liquid vapor interfacial free energy (liquid surface tension).

The interfacial free energy between the solid surface and the liquid (r^{SL}) is given by Eq.3

$$\gamma^{SV} = \frac{[(\lambda^{SV})^{1/2} - (\gamma^{LV})^{1/2}]^2}{1 - 0.015(\gamma^{SV}\gamma^{LV})^{1/2}} \qquad (3)$$

For adhesion between a fungus and a polymeric surface, the surface free energy of the fungus (r^{FV}) and the interfacial free energy between the fungus and the liquid (r^{FL}) can be obtained from Eq.2 and Eq.3, respectively, by replacing r^{SV} with r^{FV} and r^{SL} with r^{FL}. The change in interfacial free energy which corresponds to the process of adhesion (Δ Ga) is given by Eq.4:

$$\Delta Ga = \gamma^{SF} - \gamma^{SL} - \gamma^{FL} \qquad (4)$$

where: r^{SF}= interfacial free energy between the solid surface and the fungus.

From thermodynamic point of view, adhesion will occur spontaneously when Δ Ga is negative.

From Antonov's equation, r^{SF} is obtained by Eq.5

$$\gamma^{SF} = | \gamma^{SV} - \gamma^{FV} | \qquad (5)$$

Hence, by measuring contact angle of a sensing liquid on the polymeric surface and the contact angle of the fungus layer, Δ Ga is readily calculated from combination of Eq.2, 3, 4 and 5. These equations are commonly used in the

literature to calculate ΔG_a and surface free energies and hence to explain the adhesion phenomena from thermodynamic point of view.

It should be noted that cells of different species and even cells of different strains of the same species may have widely different surface free energies, and thus the surface free energy of the cells should be measured under conditions close to those which will exist at the time attachment is attempted [61]. Polymeric materials also have a wide range of surface energies. So explaining adhesion events through the surface free energy driving force is case-dependent. It depends on the molecular properties of both the cell and the biomaterial surface.

Surface free energies describe adhesion phenomena once molecular contact has been achieved between the adhering phases. That means surface free energy effects predominate at small separation distances (primary minimum) between the cell and the polymer surface [61]. For a cell to come from a distance into molecular contact with a surface requires consideration of long-range forces which influence approach. These factors include:

1) Van der Waals forces
2) Hydrophobic forces (or attractive London Van der Waals forces)
3) Electrostatic interactions
4) Fine surface projections

These factors are expected to play key roles during the initial attachment of a cell to a polymer surface.

The surface of ionizable substances attract ions of opposite charge. The net surface charge of a particle is known as the zeta potential. Clearly, cells will be attracted to surfaces of opposite zeta potential. If cells have the same zeta potential as a surface, attachment is still possible provided the electrostatic barrier can be penetrated by small surface projections [61], or other attractive forces which favor adhesion.

Electrodynamic forces including Van der Waals forces and hydrophobic forces should be considered key elements in adhesion process also. Studies indicate that hydrophobic forces are exerted at distances as great as 15nm, and at 8 to 10nm are 10 to 100 times as great as Van der Waals forces [62]. Van der Waals forces at the secondary minimum (approximately 10nm) can effectively position a particle near the surfaces. Within 1nm or less, it is conceivable that short-range chemical interactions (ionic, hydrogen, and covalent bonding) occur with extracellular moieties [60].

The process of cellular adhesion is a very complex and not a well-defined phenomenon, it consists of the balancing of many different physical and chemical forces [63]. An understanding of the critical aspects of this process would help elucidate the specific interactions between a cell and a surface.

2.2.2 *Candida albicans* Attachment to Surfaces

Of the relatively small amount of data published on yeast adherence to surfaces, the majority has focused on *C. albicans* because of its high relevance to medicine. The surface used for these studies include acrylics, poly (vinyl chloride), polystyrene, teflon, poly (ethyleneterephthalate), poly (methylmerthacrylate) and silicone rubber. Some researchers focused on the effects of environment conditions such as culture media and cations on adherence [64,65]. Others focused on adhesion mechanisms by characterizing contributions of molecular forces such as electrostatic and hydrophobic forces in adhesion process [66,67,68,69,70]. Almost all these researchers used static attachment conditions in which cell suspensions were incubated with synthetic polymers for a period of time and then the adhered cells on the polymer surfaces were counted as adhered cells per unit area. However, in order to closely portray in vivo situations, researchers must use both static assays and dynamic assays, since static fluid

rarely exists in the human body, and yeasts adhesion is usually subjected to circulating fluid in the human body. Also it is generally impractical to study adhesion kinetics with common static assays [8]. Yeast form cells instead of mycelial form cells were used in the majority of these works. That is because the adherence of mycelial form cells is much more complex than that of yeast form cells. Studies using mycelial form cells will be undertaken after an understanding of a simpler system using yeast cells is accomplished.

Douglas, et al. have studied the influence of culture variables on the adherence of *C. albicans* to acrylic surfaces [64]. In their experiments, the adherence of *C. albicans* to acrylic was measured in vitro after growth of the yeast to stationary phase in defined medium containing glucose, sucrose, galactose, fructose, or maltose as the carbon source. They found that incubation of *C. albicans* with high concentrations of sugar enhanced its adherence to acrylic surfaces. Cells grown in 500mM of fructose, glucose, maltose, sucrose or galactose were more adherent then cells grown in 50mM glucose but to a different extent. Cells grown in 500mM galactose promoted adherence to the greatest extent which were eleven times greater than cells grown in 50mM glucose. They also found that addition of divalent cations such as Ca^{2+}, Mg^{2+} or Fe^{2+} promoted adherence of *C. albicans* to acrylic surfaces. Electron microscopy of thin sections of yeasts stained with ruthenium red or polycationic ferritin showed the presence of an outer fibrillar layer on cell surfaces harvested from 500mM sucrose and 500mM galactose, but it was absent or mush less evident on yeasts grown in low concentration of glucose (50mM). This structural difference appeared to contribute to the enhanced adherence. They also suggested that this fibrillar component consisted largely of mannoprotein [65]. Also, the most conspicuous change in yeast cell surface composition resulting from growth in medium with a high sugar content was the production of this outer fibrillar-floccular layer which was

visualized with ruthenium red or polycationic ferritin suggesting that it might be rich in acidic polysaccharide [64].

The adherence of yeasts to inert polymeric surfaces is unlikely to be mediated by a receptor-adhesion interaction [66], as that of adherence to mammalian cell surfaces or biological macromolecules , because the nonbiological surfaces cannot be equipped with specific binding sites [67]. They are more likely to rely on nonspecific interactions. Previous studies have indicated that a number of physical forces potentially involved in the "nonspecific" interactions between fungal surfaces and artificial surfaces include electrostatic and electrodynamic forces which include the Van der Waals interactions, and the hydrophobic effect [67] as already discussed in this thesis. Several publications in this regard have focused on characterization of the roles of these molecular forces in the adherence of *C. albicans* to synthetic polymer surfaces [66,67,68,69,70].

The net surface charge of a particle is known as the zeta potential. The zeta potential of a cell surface will be influenced by the pH and ionic strength of the suspending fluid. For example, a protein molecule will possess a negative zeta potential if the pH of the suspending fluid is higher than its isoelectric point. Hence, it will move to the positive electrode if it is subjected to electrophoresis, and vice versa. The zeta potential of a cell depends on the surface - located ionizable moieties and the pH and ionic strength of the suspending fluid. Jones, et al. have studied the role of electrostatic nature of the cell surface of *C. albicans* in adhesion [67]. They suspended *C. albicans* in a series of electrolyte solutions with different electrolyte cation valences, concentrations and pH. These cell suspensions were subjected particle electrophoresis to determine the effects of electrolyte valence, concentration and pH on the electrostatic potential of *C. albicans* cells. The pH of the electrolyte solution was varied between 3.0 and 9.0. Cation valence was varied between +1 and +3. Electrolyte concentration was

varied between 10^{-7} and 10^{-1}M. Under all the conditions studied, the electrostatic potential of the fungal cell surface was found to be negative, but the magnitude of the corresponding electrostatic potentials was found to vary to a large extent over a large range of experimental media. In order to determine whether electrostatic interactions have any bearing on fungal adhesion, in addition to the electrostatic characterization of the fungi, similar determinations of the electrostatic potential were performed on the high index barium titanate glass and White-house Scientific Glass. Under all the conditions studied, the glass surfaces were also electronegative. Subsequently, *C. albicans* were allowed to adhere to the glass surfaces in the same series of media in which the electrostatic potentials of both the yeast and the glass were measured. They used a dynamic assay in which *C. albicans* were subjected to hydrodynamic shear forces during adhesion process. The extent of adhesion was found to be affected by the magnitude of the coulombic repulsion between electronegative fungal surfaces and electronegative glass surfaces. But significant adhesion still occurred, however, even when the coulombic repulsion was a maximum. This was taken to indicate that the fungal surfaces possessed other nonelectrostatic forces which were attractive. So the result indicated that both the electrostatic repulsive and the nonelectrostatic attractive forces were considered to play a role in the adhesion of the fungi to artificial surfaces such as glass. They suggested that this attractive forces were likely to be the so-called electrodynamic attractive forces which were likely to result from the hydrophobic effect. Following the publication of these experiment results, this group published another paper in which they employed another method using fluorescent probe 9-aminoacridine (9AA) to measure the electrostatic properties of the cell surface of *C. albicans* in the similar experiment set-up [68]. The results confirmed their previous results that under all the conditions studied the yeast form of *C. albicans* possessed a net electronegative

surface potential and appeared to behave like simple colloidal particles. They also used neuraminidase to treat the fungi surface. Neuraminidase is known to cleave selectively, the negatively charged moiety, sialic acid. The result was that the neuraminidase-treated fungi possessed a smaller electronegative surface potential, most probably due to the removal of negative charges by the enzymic treatment. This result indicated that sialic acid was present on the surface of *C. albicans* which must account in part for the electronegativity of the *C. albicans* surface [68].

It is of interest to identify the nature of the groups contributing to the surface potential of *C. albicans*. It is likely that the major contributor to this property will be the mannoproteins and charged polysaccharides known to be resident on the fungal surface [68]. A comprehensive study of the contribution made by such proteins has not been reported .

Hydrophobic forces appeared to be very important for the adhesion process of *C. albicans* to plastic surfaces. Klotz, et al. found that there was a nearly linear relationship between the cell number adhering per unit area and the contact angle of the substrate determined with distilled water, i.e., the more hydrophobic the surface, the greater the cell adherence per unit area [66]. To test the supposition that electrostatic interactions contributed to adherence, this group modified the net surface charges of the yeast. The control or untreated yeasts possessed a net negative surface charge in common with all living cells (including yeasts). They found the more positively charged yeasts, i.e., those treated with carbodimide, were considerably more adherent to polystyrene than more negatively charged formalin-treated yeasts. Plastic surfaces possess various degrees of negative net surface charge [71]. Plastic surfaces and yeast cells tended to repel each other since they were both electronegative. But electrical forces were minor to the hydrophobic forces since adherence to a considerable extent still occurred. These