

Candida Mikroorganizmalarının Polimerik Yüzeyle Yapışmasının İncelenmesi*

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ÖZET

Bir malzeme yüzeyi üzerindeki mikroorganizma adezyonu biyomedikal cihazlar, implantlar, ilaç salım sistemleri, yapı iskeleleri v.b. uygulamalarda kritik önem taşır. Biyoaktif, biyo inert, biyolojik kirlenmeyi önleyici özelliklere sahip olması istenen yüzeyler, spesifik bileşiklerin bu yüzeyler üzerine fiziksel adsorpsiyonu ya da kimyasal modifikasyon ile hazırlanabilirler. Mikro/nano desenli yüzey üretimi yüzey modifikasyon yollarının en önemli olanlarından biridir. Çalışmalar ıslanabilirlik (hidrofilite/hidrofobite), yüzeyin özelliği, yüzey yükü, serbest yüzey enerjisi ve yüzey pürüzlülüğü gibi yüzey özelliklerinin malzeme yüzeylerine olan mikroorganizma adezyonunu etkilediğini ortaya koymaktadır.

Bu çalışmanın amacı polimerik malzemelerin yüzey özellikleri ile medikal alanda fırsatçı patojenler olarak da bilinen ve sistemik mantar enfeksiyonlarının başlıca sebebi olan *Candida* mikroorganizmalarının adezyonu arasındaki ilişkiyi incelemektir.

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Investigation of Candida Microorganisms Adherence to Polymeric Surfaces: A Review

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ABSTRACT

Microorganism adhesion on a material surface plays a crucial role on the application of biomedical devices, implants, drug delivery systems, scaffolds and so on. Desirable surfaces which have bioactive, bio inert or anti-biofoul property, can be prepared by physical adsorption of specific compounds on these surfaces or chemical modification. Micro/nano patterned surface fabrication is one of the most important ways of surface modification. Studies reveal that surface properties such as wettability (hydrophilicity/hydrophobicity) character of the surface, surface charge, surface free energy, and surface roughness effect the adhesion of the microorganisms on material surfaces.

The aim of this study is the investigation of relationship between surface properties of polymeric materials and adhesion of *Candida* microorganisms who known as opportunist pathogens in medical area and the main reason of the systemic fungal infections.

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1. INTRODUCTION (*GİRİŞ*)

Microorganism adhesion occurs on the top of a specific surface and is the reason for infections of the medical devices/tissues, dental decay, fouling process under the sea, waste water and sewage treatment. Surface properties (such as wettability, surface charge, surface free energy and surface roughness) of a biomaterial or a medical equipment highly effect microorganism growth on these surfaces and this adhesion is thought to be a reason for diseases that comes from applied material or infections associated with medical equipments. Since it is important to develop antimicrobial/antibacterial /anti-biofoul surfaces which serving this area, many studies have been made on the understanding of microorganism adhesion mechanism and also relationship between surface properties of the material and the microorganism. Investigating effect of surface properties of the biomaterial surfaces on the adhesion of microorganisms is still present among the current research topics and thought to be main factors which effects on the adhesion and proliferation of the microorganisms on these surfaces. So it is very important to synthesise and develop anti-biofoul surfaces which prevents microorganism adhesion [1-3].

2. CANDIDA BIOFILM FORMATION PROCESS (*CANDIDA BİYOFİLM OLUŞMA PROSESİ*)

Biofilms can be formed by microbial communities attached to living or non-living surfaces (human tissues and medical equipments) and composed of approximately 85% of extracellular matrix (contains polysaccharides, DNA and other components) and 15 % of the sessile cells which embedded in the matrix [4]. The density and width of the matrix vary not only between cellular and noncellular structures, but also between species of microorganisms [5]. A multi-layered heterogeneous structure of biofilm with water channels permit the transport of essential nutrients and oxygen to cells that survive in the matrix [6]. Microorganisms have a number of advantages by forming biofilms, such as resistance to environmental factors, storage of nutrients, removal of waste, protection from phagocytosis and antibiotics, acquisition of new genetic properties [7].

Biomaterials and medical devices such as catheters, stents, shunts, prostheses, contact lenses etc. are commonly contact with biofilms which are the most important reason for microbial biofilm related infections and variety of diseases [8-12]. By

the time a medical equipment keeps in touch with body fluids, a conditioning film will coat top of the surface and this surface accelerates adhesion of the microorganisms. As a consequence, a biofilm layer forms [4,5,8]. A biofilm formation process consists of several key steps such as attaching microorganisms to the material surface (first colonies), irreversible attachment, microcolony formation, biofilm maturation and dispersion [13-16]. Microorganisms such as *Candida* species, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* are the most important ones that were responsible for the formation of biofilms [16-18].

Candida related infections are generally associated with biofilm formation where occurs on implantable medical devices which promote biofilm formation and also the main reason for a significant part of medical *candidiasis* cases. [19,20].

Candida species are generally as normal flora of cutaneous and mucocutaneous surfaces and may cause in a wide range of infections, from mucosal colonization to multiorgan involvement [21,22]. Virulence factors of *Candida* species has been studied in some of the studies to clarify the pathogenesis of *Candida* infections [23]. Formation of biofilm is a kind of virulence factor and makes difficult the treatment against *Candida* infections. Microorganism colonization, resistance to antimicrobial agents and immune system occur with this biofilm formation which hinder microorganism detachment from the material surfaces.

The most important *Candida* species are *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida dubliniensis* and *Candida lusitanae* [24,25]. In all of these microorganisms, *Candida albicans* (*C. albicans*) is the most common significant opportunistic pathogen in medical area and the main reason of the systemic fungal infections. So many parameters effect biofilm structure and formation of *C. albicans* such as material surface [26,27], growth medium [28,29], incubation conditions [30,31], effect of secretions, liquid flow rate and extracellular polysaccharide [5,32,33].

C. albicans biofilm formation process are reported as three major phases by researchers. These phases are early phase (0-11 hours), intermediate/developmental phase (12-30 hours) and maturation phase (38-72 hours) [34-36]. Early phase is the

adhesion of free floating *Candida* microorganisms on a material surface. Hydrophobic interactions and electrostatic forces occur among *C. albicans* cells (blastospores) and material surface (first 1 to 2 hours). In the next step, more stronger adhesion is performed by glycoproteins. Microcolonies become visible at 3 to 4 hours. *C. albicans* aggregates exist on irregular regions of the surface after 11 hours. In the intermediate phase (12-30 hours) a bilayer becomes visible that composed of young hyphae, germ tubes and yeasts with a matrix of extracellular polymeric substances. Finally, in the maturation phase (38-72 hours), *C. albicans* yeasts, pseudohyphae and hyphae are entirely embedded into the extracellular matrix since matrix material expands with the increase of incubation time [5,34,37-39].

All of these scenarios called as microorganism adhesion and growth, highly influence from surface material characteristics. So it is important to change physicochemical characteristics of a surface to reduce or prevent microorganism/surface interactions and to restrain biofilm formation [40].

3. EFFECT OF SURFACE PROPERTIES ON CANDIDA ADHESION (YÜZEY ÖZELLİKLERİNİN CANDIDA ADEZYONU ÜZERİNE ETKİSİ)

Physicochemical parameters of a surface that effect cell-biomaterial surface interactions can be summarized as wettability (hydrophilic/hydrophobic structure), surface free energy and its parameters, surface charge, functional groups on the surface, surface morphology, surface roughness, etc. [2,41]. Contact angle and also surface free energy measurements are useful and highly preferred from the researchers to evaluate the *Candida* adhesion on different type of material surfaces. Surface free energies of the material surfaces can be calculated from contact angle measurements of the test liquids (e.g. ethylene glycol, formamide, methylene iodide, etc.) and can be used to analyze wetting and adhesion characteristics of material surfaces. There is an inverse correlation between wetting characteristic and contact angle of the surface. Hydrophobic surfaces which have contact angles higher than 90° can be classified as non-wettable. But hydrophilic surfaces that have contact angles lower than 90° can be categorized as wettable. Surfaces that have higher contact angles (hydrophobic substrates) show lower wettability. And hydrophobic surfaces generally have lower surface free energies. Not only surface free energy of the substrate but also surface free energy of

the microorganism and surface tension of the suspending medium are very important to calculate how these values are close to each other for explaining surface free energy of adhesion [2,41].

As an earlier study, Minagi et al. investigated substrate hydrophobicity on microbial adhesion carried by *C. albicans* and *C. tropicalis* on 21 denture base resin material surfaces and observed that *C. albicans* adherence increased and *C. tropicalis* adherence decreased with the increase of surface free energy of resin plates. In this study surface free energy of *C. albicans* was higher than that of all materials while surface free energy of *C. tropicalis* was lower than all of them. They calculated free energy changes between substrate and microorganism and concluded that higher adherence probability could be said when these were closer to each other [42]. Inert polymeric surfaces; poly(tetrafluorethylene), poly(ethyleneterephthalate), poly(methylmerthacrylate) and bacteriological grade polystyrene petri dishes were examined by Klotz et al. for the adhesion of *Candida* species and reported that more hydrophobic substrates showed an increased *Candida* adhesion [43]. PTFE was the most hydrophobic one showed the maximum adhesion per unit area on its surface. Nearly a linear relationship was obtained between contact angle of the polymeric surfaces and the number of the cells adhered per unit area [43]. Sometimes surface free energy components (for example polar component) can be a determinant factor for the proliferation of the microorganisms. So it is very important a fully characterization of a material surface to solve uncertainties. One of the studies on this area was performed by Koch et al [44]. They used different chemistries of denture base materials to investigate adherence and proliferation of *C. albicans* on their surfaces. An importantly more proliferation was determined on the surface of the materials which showed the highest contribution as polar component to surface free energy and emphasized that a correlation might be exist between surface free energy polar component of the material and *C. albicans* proliferation [44]. On the other hand there are some studies where no significant correlation have been found between *C. albicans* adherence and surface free energy of the material [45-47].

Since maxillofacial prostheses are clinically significance, investigation of the attachment and colonization of *C. albicans* on these material surfaces has a great importance. Promotion of a significant fungal colonization on the thermal cycling materials

was demonstrated by Nikawa et al [48]. In this concept, they analyzed a single isolate *C. albicans* colonization on commercial products (thermo cycled 15 commercial maxillofacial materials) and found a correlation between exponential of the fungal colonization on 1000- and 10 000- thermocycled materials and contact angles of them. Although a negative correlation had been reported by Minagi et al. between number of adherent cells and hydrophobicity of denture materials [42], similar correlativity was observed between the exponential of the colonized *C. albicans* and the hydrophobicity of the material surfaces by Nikawa et al [48]. Since thermocycling enhanced the *Candida* colonization, necessity of the more appropriate controls emphasized by the authors for long-term usage of the maxillofacial materials [48]. An another study on maxillofacial materials was performed by Atay et al [49]. They examined effect of surface wettability determined from contact angle measurements on the evaluation of *C. albicans* adherence on maxillofacial silicone materials since adherence of *Candida* is one of the most important risks for maxillofacial prosthesis wearer. They emphasized a relationship between surface wettability and the attachment quantity by showing decrease in contact angle values and increase in *Candida* adhesion quantity. In this study, they said that many kind of factors that comes from material properties may influence the complex nature of the adhesion process of *Candida* [49]. Also they reported some conflicting findings from the literature. For example, more hydrophobic surfaces show less cell adhesion according to Minagi et al [42]. But an increase of *Candida* adhesion with more hydrophobic materials reported by Klotz et al [43].

Poly(methylmethacrylate) (PMMA) is a common material that was used for denture based material and *Candida* behavior as the adhesion on PMMA surfaces has been extensively studied from the researchers for the purpose of having an ideal denture material with a reduced *Candida* adhesion. A systematic study on the reduction of *C. albicans* on medically used resin surfaces introduced by Park et al [50]. They proposed surface charge modification of PMMA surfaces for the reduction of *C. albicans* adhesion. An exceptionally correlation was found between methacrylic acid component which was incorporated into the resin at varying ratios: 0% (control), 5%, 10%, and 20% and the attachment of *C. albicans*. They obtained that with the increase of methacrylic acid component, surface area of adhered *C. albicans* decreased. A significant decrease was observed especially when the content of methacrylic

acid was 10% of the PMMA. Also with the help of the contact angle measurements they revealed that surface free energy of the resins increased with the increase of methacrylic acid content [50]. This study reveals that if we optimize physicochemical properties of resin surfaces, we can develop medically valuable surfaces which have non-biofoul properties. Kang et al. selected four type denture lining material (tissue conditioners, acrylic and silicone soft liners and hard reline materials) to see the surface characteristic effect on the adhesion of *C. albicans*. They calculated and measured surface energy parameters and surface roughness of the materials and found that acrylic soft liners showed more hydrophilicity than other materials and acrylic soft liners and tissue conditioners displayed greater *Candida* adhesion than silicone soft liners and hard reline materials [51].

Not only acrylic based polymers but also silicone elastomers, polyolefins can be used to test antifouling properties of the material surfaces against microorganisms by considering the material surface's roughnesses, wettabilities and surface free energies. Parylene coatings effect on denture bases resin and silicone elastomers was studied by Zhou et al. to test *C. albicans* adhesion. Parylene coating caused a reduction on *C. albicans* adhesion and aggregation on silicone elastomer and resin surfaces. Parylene coating also enhanced silicone elastomer wettability [52]. Singh et al. investigated *C. albicans* biofilm formation on polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC) and silicone rubber (SR) surfaces. They found that biofilm formation was maximum with PVC, followed by PS, PP and SR with the decrease in hydrophobicity which was measured by contact angle measurements. It was concluded that PVC was the most suitable material for the attachment of *C. albicans* since roughness and hydrophobicity promotes adhesion and biofilm production of *C. albicans* [53].

Modifications highly influence and change wetting behavior of the surfaces by making them sometimes hydrophobic and sometimes hydrophilic. Plasma treatment is a general method to obtain hydrophilic substrates and can be used to obtain wettable materials to analyze anti-biofoul properties and also as an economical and effective method for the modification of material surfaces. Yıldırım et al. studied on the effect of glow-discharge plasma modification of acrylic denture base polymers on the adhesion of *C. albicans*. Glow-discharge modification enhanced the wettability of the acrylic

surfaces and with the increase of surface wettability, adhered *Candida* cells showed an increase [54]. Surface of heat-polymerized acrylic resin was modified by atmospheric-pressure cold plasma to explore early attachment of *C. albicans* by Pan et al. [55]. Plasma treat modified acrylic resin surfaces showed a significant decrease in their contact angle values and diminution in the early attachment of *C. albicans*. This conclusion showed that cold plasma seemed promising for dental applications in the early attachment of *C. albicans* [55]. Nanometer or micrometer scale surface topographies are also important on the adhesion of microorganisms on material surfaces. For this purpose nanoparticles can behave as a surface modifier. If a topography can be fabricated smaller than the attached microorganism size, a reduction may be obtained on the number of the attached cells. Effect of the modification of surfaces by nanoparticulates for the attachment of *C. albicans* was examined by Cousins et al [40]. *C. albicans* attachment and growth was reduced with the modification of the surfaces by different sizes of silica nanoparticles. Cell coverage decreased with the decrease of diameter of the silica nanoparticles [40].

Roughness of a surface is another important surface property effects the microorganism adhesion. Especially for the fabrication of denture prosthetics, PMMA is a commonly used material and its surface roughness highly influences microorganism adhesion since smooth surfaces reduce the initial adhesion and accumulation of microorganisms [43,50]. Voids, imperfections, scratches, cracks etc. as favorable sites for colonization on a material surfaces are used as a protection area by microorganisms and results accumulation of the attached microorganisms on roughened surfaces than smooth ones.

A significant number of researchers studied roughness effect on *Candida* adhesion especially on the denture base resin materials, acrylics and silicone rubbers [56-60]. Adhesion of oral microorganisms to variety of surface textures of denture resins was examined by Yamauchi et al. and it was concluded that *Streptococci* and *Bacteroides* showed relatively more adhesion on rougher surfaces than *C. albicans*. They also reported that a greater attachment was obtained from various oral microorganisms than *Candida*. This is important to detect the initial colonizer microorganisms if there is a selective binding on the different surface textures [56]. Verran et al. reported that low adherence existed on smooth surface acrylics with comparing *C. albicans* cell numbers on rough and smooth acrylic surfaces.

Microorganisms seem to prefer to cluster around defects, voids, scratches, etc. to find a protection themselves from shear forces [57]. Heat-cured hard and soft denture-base materials had been analyzed by Radford et al. to see the surface roughness effect on the adhesion of *C. albicans* and it was concluded that significantly greater adhesion was seen on rougher surfaces than smooth ones [58]. *C. parapsilosis* was studied by Gallardo-Moreno et al. to see its detachment from glass and silicone rubber which represents smooth and rough surfaces. Adhered cells were easily eliminated from glass which is a hydrophilic, low roughness surface whether hydrophobic and high roughness silicone rubber surface was able to maintain the adhered *C. parapsilosis* longer. They revealed importance of the substrate hydrophobicity on the detachment of the adhered cells. They also studied on the cell shape effect on the detachment of microorganisms from the substrates and concluded that much more detachment was seen for the elongated and irregular cells from both substrates than the ovoid-like shape ones [59]. Al Bakri et al. studied on PMMA surfaces modified by fluoridated glass fillers to test bacterial and fungal adhesion (the adherence ability of *Streptococcus mutans* and *C. albicans*). For this purpose, they added 1%, 2.5%, 5% and 10% concentrations (by weight) of fluoridated glass fillers to PMMA. Surface roughness was increased with the increase loading capacity of fillers but no drastic difference was seen on contact angle results. On the other hand, at 10% fluoridated glass fillers concentration, a significant decrease on the adhesion of *Streptococcus mutans* and *C. albicans* was obtained when compared to others. Microbial adhesion significantly decreased with saliva coating [60]. Al Bakri et al. also emphasized on there may many factors on the attachment of microorganisms to PMMA surfaces such as microorganism surface charge, surface roughness, surface morphology, surface topology, surface free energy and presence and non presence of saliva coating [60].

Table 1 shows numerical data of *Candida* attachment extracted from the literature. Researchers uses different presentation terms to highlight this topic. Some of them are % surface coverage values, yeast/mm², CFU/ml, CFU/mm², µg/cm² etc. (CFU: Colony Forming Unit). Researchers found contradictory results when they investigated relationship between surface free energy of the material and the attachment of *Candida*. Some of them reported that attachment of *C. albicans* increased with the increase of surface free energy of

enture base materials [42].

Table 1. Numerical data of *Candida* attachment obtained from the literature (*Candida* tutunmasına dair literatürden elde edilen nümerik veriler)

Microorganism	Substrate	Method	Numeric Results	Reference
<i>C. albicans</i>	silica treated polystyrene	XTT	approx. 93% mean cell coverage for control approx. 11% mean cell coverage for 4 nm silica approx. 31% mean cell coverage for 21 nm silica	[40]
<i>C. albicans</i> <i>C. tropicalis</i>	denture base resin materials	Microscope	253±75 <i>C. albicans</i> and 1707±437 <i>C. tropicalis</i> for the most hydrophobic surface 1056±276 <i>C. albicans</i> and 617 ±281 <i>C. tropicalis</i> for the most hydrophilic surface	[42]
<i>C. albicans</i>	PTFE, PS,PET, PMMA, Pyrex Glass	Photomicrographs	approx. 900 yeast/mm ² for PTFE approx. 50 yeast/mm ² for glass	[43]
<i>C. albicans</i>	pure and modified PMMA (95:5, 90:10, and 80:20 MMA)	Microscope	approx. 77% surface area for pure PMMA and approx. 68%, 27% and 8% surface area for 5%, 10% and 20% methacrylic acid (MMA) content respectively.	[50]
<i>C. albicans</i>	glow-discharge modified acrylic denture base polymers	Microscope	7.48 ± (1.76) cells/mm ² for unmodified surface 24.62 ± (1.74) and 28.45 ± (2.51) cells/mm ² for plasma treated groups	[54]
<i>C. albicans</i>	Parylene coating effect on silicon elastomers and denture bases resin	Microscope (haemocytometer)	2.18 x 10 ⁷ cfu/ml for silicon elastomer surface 0.48 x 10 ⁷ cfu/ml for parylene coated silicone elastomer surface. 0.94 x 10 ⁷ cfu/ml on resin surface 0.41 x 10 ⁷ cfu/ml on parylene coated resin surface	[52]
<i>C. albicans</i>	Polypropylene (PP) Polystyrene (PS) Polyvinyl chloride(PVC) Silicone rubber (SR)	Acetone precipitation technique, XTT	Exopolysaccharides (EPS) production during biofilm formation: 11.45, 9.41, 8.65 and 6.95 µg/cm ² for PVC, PS, PP and SR respectively. From XTT analysis , the biofilm formation: 64.19, 50.31 and 45.09% for PS, PP, SR, respectively in comparison to PVC, used as a control.	[53]
<i>C. albicans</i>	Denture lining materials	Colony Forming Unit (CFU) assay	approx. 15 CFU/mm ² for the most hydrophobic denture lining material approx. 260 CFU/mm ² for the most hydrophilic denture lining material	[51]
<i>C. albicans</i>	Plasma treat modified acrylic resin surfaces	Colony Forming Unit (CFU) assay	approx. 6.5 CFU for the control surface approx. 6.2 CFU for plasma treated acrylic resin	[55]
<i>C. albicans</i>	PMMA surfaces modified by fluoridated glass fillers	Microscope	% Surface coverage (as % pixels): 0.023±0.008 and 0.008±0.003 before saliva coating and 0.019±0.004 and 0.002±0.001 after saliva coating for control and 10% glass filler addition respectively.	[60]

But an another group of researchers (more recent studies) couldn't find this correlation in their experimental results [45-47]. Irregularities on a biomaterial surface serves as a protection area for the microorganisms from shear forces [47,61,62] so that surface roughness is an effective parameter on *Candida* attachment [47]. Even though it was generally reported that an increase in surface roughness results an increase in *Candida* adhesion, some researchers didn't observe this relationship [58,63]. Saliva medium effect on the *Candida* attachment also gives contradictory results. For example, while some of the studies reported that there were no effect of saliva on the adhesion of

fungi to material surfaces [63,64], some of them defended that an increase in *C. albicans* adhesion was obtained in the presence of saliva coating [65]. Also a reduction in the level of adherence was obtained by some of them [46,47,66,67] in the presence of saliva coating.

4. CONCLUSION (SONUÇ)

We can clearly say that there are still too many questions need to be answered. Contradictions in the obtained results clearly shows us that we need to do much more research in this area. This adhesion phenomenon that controlled by more than one

parameter not only requires a domination on the biofilm formation and development stages of microorganisms but also requires a very good analysis and understanding of the physicochemical properties (surface topology/morphology/roughness, surface free energy, surface wettability, surface charge, etc.) of the surfaces that microorganisms will interact with. Innovative materials and specific surfaces that will be developed and synthesized in this area can only achieve their purposes if we understand and correlate these parameters each other. Since the first interaction is mainly important to inhibit initial adhesion, strategies should be developed effectively at beginning of the process.

When we look at this subject from the material frame, these surfaces can be obtained from synthesis of biomaterial surfaces by functional groups, surface morphology/surface roughness modifications, micro/nano patterning, and so on. Since nontoxic materials need to be use, antimicrobial/antifungal coating agents, biosurfactants can be used from naturally obtained compounds since they can be better tolerated by human body. Also their concentration on the surface are needed to increase. Especially for dental researches, both artificial and natural saliva mediums should be tried for the materials to clinically describe the system. And also blood interactions should be taken into consideration to overcome blood-material compatibility handicaps.

Both contact angle measurements and surface free energy calculations are useful characterization techniques and can be used for the characterization of biomaterial surfaces at the top layer. Surface free energy and contact angle measurements are necessary to highlight this important subject. Not only wettability of the substrate but also hydrophobicity/hydrophilicity of the microorganism has to evaluate in these studies. Closeness between surface free energies of substrate and the organism should be considered to decide and fabricate how kind of surface would be effective with having anti-biofoul properties. Also surface tension of the suspending medium should be taken into consideration.

If we know the mechanism of microorganism-surface interactions, we can suggest to design desirable biomaterial surfaces to prevent infections. Anti-biofoul surfaces should be fabricated not only against monotype microorganisms such as *Candida* species but also against lots of the pathogens. So it is very important to analyze surface characteristics of a

biomaterial surface and microorganism to fabricate anti-biofoul surfaces. It is also medically valuable to determine surface characteristics which inhibit attachment of microorganism cells on biomaterial surfaces and reduce device-related and other chronic infections.

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