

Original Article

Evaluation of *Candida Albicans* Biofilm Formation on Various Parts of Implant Material Surfaces

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INTRODUCTION

The oral cavity contains almost half of the commensal bacterial population present in the human body. An increase in the number of these microorganisms may result in systemic diseases and oral infections.^[1] *Candida albicans* is the prime fungus normally found in the oral cavity of 20-40% of healthy individuals^[2] and the major pathogen in oral and systemic candidosis.^[3] It can cause severe opportunistic infections in immunosuppressed patients and, to a large extent, in polytrauma patients or other patients with damaged barriers.^[4] The frequency of mucosal and cutaneous fungal infections has increased worldwide in recent years. *Candida* is now regarded as a major human pathogen in clinical settings. Candidiasis is

ABSTRACT

Aims: *Candida albicans* adhesion to any oral substrata is the first and essential stage in forming a pathogenic fungal biofilm. In general, yeast cells have remarkable potential to adhere to host surfaces, such as teeth or mucosa, and to artificial, non-biological surfaces, such as dental materials. *C. albicans* adhesion to denture materials is widely recognized as the main reason for the development of stomatitis. This study compared the susceptibility of different parts of the implant system with *C. albicans* adhesion. **Material and Methods:** Each material maintained contact with *C. albicans* suspension, and biofilm formations around the implant materials were evaluated. To evaluate the biofilm formation, the XTT technique and scanning electron microscopy (SEM) were used. **Results:** In general, a fine biofilm layer of *C. albicans* species was found on the surface of all examined materials. However, when examining the SEM images, candidal growth was significantly lower on the surfaces of the gingival former, abutment, and machined surface implant samples. According to the colorimetric assay (XTT), the gingival former samples revealed the lowest quantity of biofilms formed (median XTT value, 0.0891) ($P < 0.001$). The abutment and machined surface implant samples had low XTT values with similar values. The highest median colorimetric XTT values (0.1741), significantly higher than those of the other materials ($P < 0.001$), were for the bone level implant samples. **Conclusions:** This finding emphasizes implant treatment would be chosen complacency in patients who are prone to oral candidosis, medically compromised patients under immunosuppression, and patients with tumor who are being treated with chemotherapy or radiation.

KEYWORDS: Biofilm, *Candida albicans*, implant

the third or fourth leading cause of nosocomial infection in the United States, ranking even higher than some common bacterial infections.^[5] This is probably because of the increasing number of seriously ill patients and immunosuppressive therapies, as well as the increased use of antibiotics and more invasive therapeutic medical procedures.^[6]

Oral environmental stabilization procedures are commonly employed in dentistry. The aim of

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these procedures is the elimination of pathogenic microorganisms, preventing the progression of oral diseases, and creating conditions for the improvement of oral health. The ability of *C. albicans* to form biofilms on dental materials is a key attribute that enhances its ability to cause disease in humans. However, with the increase in *C. albicans* infections because of the interaction between virulence factors of *C. albicans* and host defense mechanisms, resistance against commonly used antifungal agents has been observed.^[7,8] Targeted chemoprophylaxis with effective antifungal agents is the most effective prevention strategy against candidiasis.^[8] However, because of the development of antifungal resistance, its indications should be considered carefully. Alternative strategies would be advantageous, in particular, for the long-term prevention of candidiasis.

Since adhesion is an essential prerequisite in colonization and infection, the role of adhesion in the pathogenesis of several diseases caused by *C. albicans* is widely acknowledged. Several studies have suggested that the initial stage of various microbial diseases involves microorganisms adhering to the target tissue.^[9,10] Generally, it is important to obtain information on how biofilm may be influenced by implant materials because microorganisms that adhere to implant materials can colonize other oral surfaces and eventually cause oral infections in predisposed individuals. Thus, studies concerning the adhesion of *C. albicans* to biomaterials have focused on the denture base and denture relining materials,^[11-13] although fungi effectively adhere to all kinds of resin, glass, and even metal surfaces.^[14]

The adhesion of *C. albicans* to dental implant materials in the human oral cavity may promote the occurrence of oral candidosis. Considering this background, we undertook a study that aims was compared the susceptibility of various parts of implant materials [tissue level implant, bone level implant, abutment, gingival former, cover screw (from Straumann Inc. AG, Basel, Switzerland) and machined surface implant (from Implanse AGS Medical, Istanbul, Turkey)] to *C. albicans* adhesion.

MATERIAL AND METHODS

In the present setup, six implant materials (tissue level implant, bone level implant, abutment, gingival former, cover screw, and machined surface implant) were assessed. Five samples were used for each test material.

Fungal growth conditions

The *C. albicans* clinical strain SC5314 was used. Cells were grown for 24 h at 37°C in the yeast nitrogen base (YNB; Sigma, St Louis, Missouri, USA) supplemented with 50 mM dextrose. After the incubation period, the cells were harvested, washed with phosphate-

buffered saline (PBS; Cellgro, Media-tech, Herndon, Virginia, USA), and standardized to 1×10^7 cells/mL spectrophotometrically at 492 nm for the biofilm formation experiments.

Quantitative measurement of *C. albicans* biofilms

Metabolic activity of *C. albicans* biofilms was assessed using a colorimetric assay (XTT). To evaluate biofilm formation by *Candida* isolates, the samples were washed with PBS, placed in 24-well culture plates with 2 mL standardized cell suspension (1×10^7 cells/mL), and incubated for 72 h at 37°C on a rocker. Biofilms were quantified using a tetrazolium XTT [2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide] as described previously.^[15] After this period, the number of colony-forming units (expressed in values of logarithms of colony-forming units) per milliliter (log CFU/mL) was obtained. Values of mean, standard deviation, and median of log CFU/mL were calculated for each experimental group. Formula for calculating the % cell viability was as follows; % cell viability = (absorbance of test well/absorbance of control well) \times 100.

Scanning electron microscopy

Each material was used for scanning electron microscopy (SEM) investigation. The samples with the adhering fungi were rinsed in PBS, fixed with ethanol, and air-dried. The test samples were then mounted on aluminum stubs and sputter-coated with gold. Samples were examined with a SEM (magnification \times 50 and \times 1.00 K; EVO LS 10; Carl Zeiss Microscopy, LLC, New York, USA).

Statistical analysis

A multiple significance test with the Duncan correction was used to compare the XTT and biofilm vitality values of *C. albicans* biofilm formation. Data are presented as the mean \pm standard deviation (SD), and the level of statistical significance was set at 5% for all analyses. The statistical analyses were performed using a computerized statistical software program SPSS 11.5 (SPSS, Chicago, Illinois, USA) for Windows.

RESULTS

In general, the *C. albicans* biofilms adhered firmly to the implant materials. In addition, no differences were observed in the gross morphology and adhesion of biofilms formed by this pathogen on various implant materials.

During the SEM examination, a fine biofilm layer of *C. albicans* species was found on the surfaces of all examined materials [Figure 1-Figure 4]. However, the quantity of adhering microorganisms varied among the materials. The amount of candidal growth was significantly lower on the surfaces of the gingival

Table 1: *Candida albicans* biofilm XTT (OD492) and vitality values (%) on implant materials

| Materials (n) | OD value (mean±SD) | Vitality (%) (mean±SD) | F |
|------------------------------|---------------------------|---------------------------|---------|
| Tissue level implant (5) | 0.1271±0.030 ^a | 55.0±4.05 ^a | p<0.001 |
| Bone level implant (5) | 0.1741±0.014 ^b | 75.1±2.58 ^a | |
| Abutment (5) | 0.0912±0.036 ^c | 45.4±3.61 ^b | |
| Gingival former (5) | 0.0891±0.015 ^d | 38.2±4.17 ^d | |
| Cover screw (5) | 0.1149±0.016 ^a | 49.1±6.13 ^c | |
| Machined surface implant (5) | 0.0956±0.032 ^c | 47.0±5.85 ^c | |

F: Duncan test frequency; the common letters on the columns are not statistically significant. ^aP < 0.05

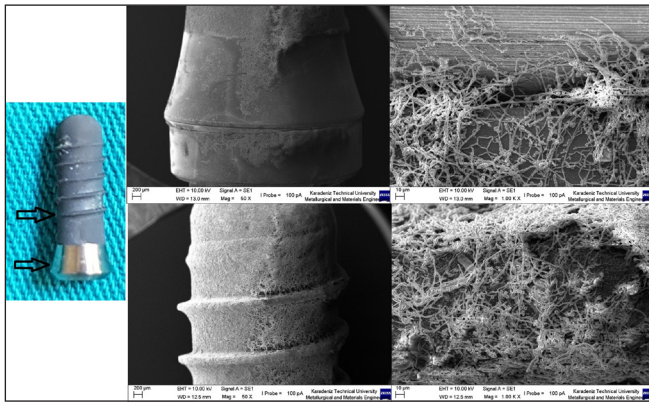


Figure 1: Scanning electron micrographs of *C. albicans* biofilm layers on the tissue level implant.

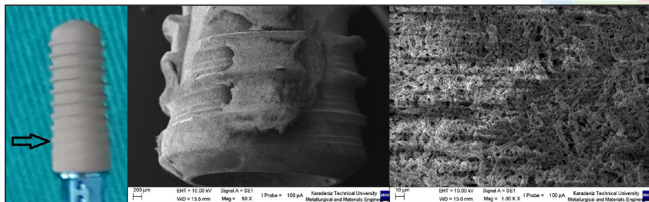


Figure 2: Scanning electron micrographs of *C. albicans* biofilm layers on the bone level implant.

former, abutment, and machined surface implant samples (Figure 3 and Figure 4). The tissue level implant, bone level implant, and cover screw samples showed more candidal adhesion, and on these samples, dense oval colonies and round blastospore colonies dominated the fungal biofilm [Figure 1, Figure 2, Figure 4]. The biofilm formation appeared to be thicker on the body surfaces of the tissue level and bone level implants than on the other samples. *C. albicans* biofilm XTT (OD492) and vitality values (%) on implant materials are shown in [Table 1]. When the adhering fungi were investigated, the gingival former samples revealed the lowest quantity of biofilms formed (median XTT value, 0.0891) of all the materials with statistically lower XTT values than the other materials ($P < 0.001$). The abutment and machined surface implant samples had low XTT values with similar values. The highest median XTT values (0.1741),

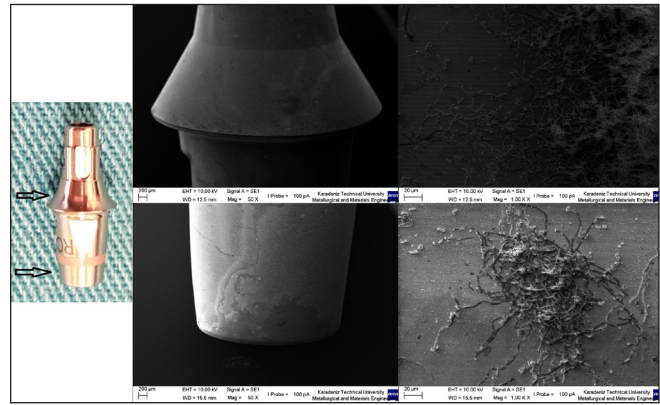


Figure 3: Scanning electron micrographs of *C. albicans* biofilm layers on the abutment.

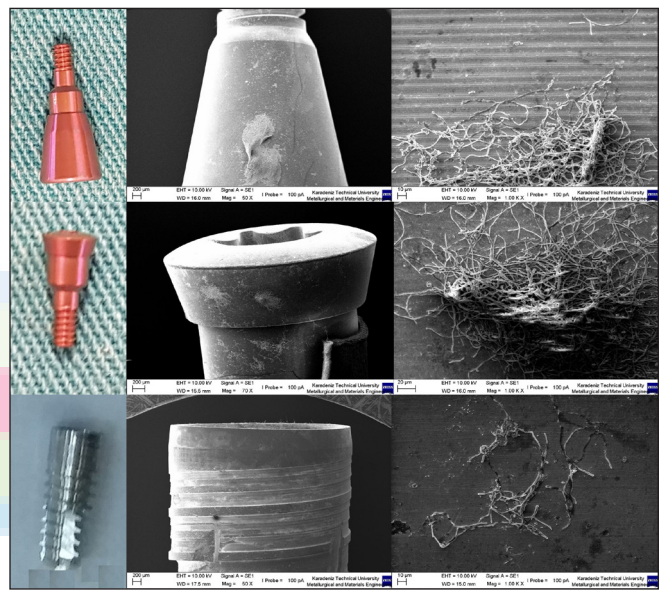


Figure 4: Scanning electron micrographs of *C. albicans* biofilm layers on the gingival former, cover screw, machined surface implant.

significantly higher than those of the other materials ($P < 0.001$), were for the bone level implant samples. Metabolic activity assays revealed that the *Candida* isolate tested formed significantly more vital biofilms on bone level implant samples. Taken together, these results demonstrate that *Candida* can form biofilms on implant materials and that this ability is influenced by the type and roughness of material.

DISCUSSION

This study compared the susceptibility of six implant materials with *C. albicans* adhesion. The human mouth presents various surfaces to which microorganisms of the oral microbiota can adhere.^[16] Therefore, evaluating dental biofilms grown on typical dental materials plays an important role in achieving long-term success of oral healing and protection from oral diseases. Although other species of the genus *Candida* are known to be involved,

C. albicans is the major microbiological factor in oral candidosis.^[3] Different fungal species have different adhesion potential for dental material surfaces.^[17] However, since we preferred to provide as simple a test model as possible, we used only one type of culture strain of *C. albicans*.

Studies concerning the adhesion properties of *C. albicans* have focused on the denture base and relining materials.^[11,13,17] Although implant materials may be a potential source of fungal infections, no investigations have been carried out on these materials. To our knowledge, the current work is the first to compare differences in *C. albicans* adhesion to implant materials.

Various material characteristics such as surface roughness, surface hydrophobicity, electrostatic forces, composition of the material, type of matrix, size of fillers, and configuration of fillers are relevant factors affecting attachment of organisms to the surface to form a biofilm.^[6,18-22] Surface roughness is well-documented as having a crucial influence on microbial adhesion.^[23] This property affects the attachment of biofilm bacteria because more or less “surface” is available for bacterial attachment, and more or less protection is provided for colonizing bacteria.^[24] Higher numbers of *C. albicans* are found on rough surfaces than on polished, smooth surfaces.^[25] Theoretically, and as a consequence, dental materials should be polished *in situ*, so that a surface as plane as possible is provided. Therefore, in the present study, the tested materials were examined from the different parts and regions. However, the correlation between surface roughness and candidal adhesion was not evaluated in the present study. Therefore, the results cannot be related to specific surface characteristics.

Currently, dental implants are often preferred for oral prosthetic treatments. The microbial adhesion on the surfaces of implants or their prosthetic parts can cause serious clinical conditions, such as mucositis, peri-implantitis, or stomatitis. Also, *C. albicans* can easily colonize on dental materials and cause serious infections. There is a direct relationship between the amount of adhesion, which creates colonies, and the formation of diseases.^[26] In the present study, the colorimetric assay (XTT) method and SEM were used for examining the metabolic activity and adherence of *C. albicans* on the implant surfaces. According to the results, the XTT values of the gingival former samples revealed the lowest number of biofilms formed of all the materials examined. The abutment and the machined surface implant showed low XTT values with similar values. The highest median XTT values were found for the bone and tissue level implants. These important findings confirm the hypothesis that different types of material and their specific surfaces

interfere considerably with *C. albicans* adherence.^[22-24] In our study, the gingival former and the abutment parts of the implant systems, which had a relationship with the oral mucosa and gingiva, showed significantly lower *C. albicans* adherence and vitality.

To prevent *C. albicans* biofilms from accumulating and to reduce adhesion, several promising inventions have been introduced.^[14,27,28] As the etiology of *Candida*-associated stomatitis is multifactorial with numerous influencing parameters, a better understanding of the essentials of fungal adhesion, gained through the use of *in vitro* methods to study these adhesion processes, is needed. Overall, the conclusion derived from this *in vitro* investigation is that a significant correlation exists between surface roughness and the amount of adhering *C. albicans*. Applied to the clinical setting, implant treatment would be chosen complacency in patients who are prone to oral candidosis, medically compromised patients under immunosuppression, and tumor patients being treated with chemotherapy or radiation.

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Conflicts of interest

There are no conflicts of interest

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