

Comparison of *Candida albicans* Adhesion and Colonization on All-metal and Metal-ceramic Crowns

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ABSTRACT

Introduction: Candidiasis is responsible for 85% of fungal infections of the oral cavity in humans. There is little data available on the adhesion of *C. albicans* to a variety of crowns. This study aimed to investigate the adhesion and colonization of *C. albicans* to two conventional crowns.

Materials and Methods: In this in-vitro study, first 36 circular disks of two crowns were created: all-metal and metal-ceramic (18 for each crown). The disks were transferred to plates containing the suspensions 1×10^5 cfu/ml of *C. albicans* colonies. After 40, 80, and 120 minutes, the disks were rinsed in sterile normal saline and shaken at low speed to separate yeast cells. This suspension was cultured on Sabouraud dextrose agar culture medium with pour plate method and was then counted. Differences were considered significant when $p < 0.05$, using computer software SPSS version 18 for windows.

Results: In all-metal and metal-ceramic samples, adhesion and colonization of *C. albicans* significantly increased during time ($p < 0.001$). The lowest adhesion and colonization of *C. albicans* was observed in metal-ceramic crowns after 40 minutes, and the highest adhesion and colonization of *C. albicans* in metal-ceramic crowns after 120 minutes.

Conclusion: The results showed that adhesion of *C. albicans* to all-metal crowns was significantly less than metal-ceramic crowns after 80 minutes and 120 minutes.

Key words: *Candida albicans*, Crown, All-metal, Metal-ceramic, In-vitro

HOW TO CITE THIS ARTICLE: Farimah Sardari, Fazeh Khosravi, Comparison of *Candida albicans* adhesion and colonization on all-metal and metal-ceramic crowns, J Res Med Dent Sci, 2019, 7(2): 93-97

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Received: 26/11/2018

Accepted: 20/03/2019

INTRODUCTION

Several microorganisms may exist in the oral cavity, some of which may cause severe diseases. Oral candidiasis, the most common human fungal infection, is one of the important infections of the oral cavity, caused by *Candida* [1,2]. Among different species of *Candida*, the most common (more than 80%) of oral infections are caused by three species: *C. albicans*, *C. glabrata*, and *C. tropicalis* [3]. The prevalence of *C. albicans* in mouth is reported to range from 3% to 48% with a higher prevalence in healthy children and young adults [4].

C. albicans invades the epithelium and specific glycoproteins, and co-agglutination reactions can have a higher affinity for epithelial cell adhesion [5]. The infection is controlled in healthy people by antimicrobial proteins in the saliva and oral microflora [6]. The use of dentures, drug use, hormonal factors, radiation therapy, diabetes mellitus, acquired immunodeficiency syndrome, and salivary disorder like Sjogren's syndromes can predispose the patients to *C. albicans* [1,4,6].

C. albicans can be colonized in different surfaces of the oral cavity, including the tongue, palate, dental plaques, and restorations [7]. Different restorations have been suggested to cover the teeth's lost crown; including all-metal, all-ceramic, and porcelain-fused-to-metal crown(s) (PFMs) [8]. As to the evidence, feldspathic/silica-based crowns had significantly lower survival, especially in posterior region [9], and comparison of different crowns showed the highest survival rates in PFMs (94.4%), densely sintered zirconia PFMs (90.4%), reinforced glass ceramic PFMs (89.1%), and glass infiltrated alumina PFMs (86.2%), respectively [10]. The main aspects of restorations, addressed by researchers, include the aesthetic aspects, survival, and their stability. Although these aspects are also very important, the chance of candidiasis infection is another important factor in superiority of different crowns. As an important factor in colonization of *C. albicans*, studies have investigated the adherence of this fungus to different restorative materials, including resin-composite [11], acrylic resin [12,13], and glass ionomer [14].

The fungal and bacterial community of dental plaque is subjected to physiological and compositional shifts as a result of environmental stresses generated by the placement of dental restoration and this is could lead to

serious complications that result in the failure of the restoration [15,16]. The results of this study could provide the initial *in-vitro* results on colonization of *C. albicans* to different restorative materials. Because of none have compared the adherence between different crowns, especially two conventional restorations, including all-metal and PFMs. Health of support structures is as important as failure of a restoration, even more important. Therefore, this study aimed to investigate the adhesion and colonization of *C. albicans* to two conventional crowns.

MATERIALS AND METHODS

Study design

This present *in-vitro* study is done in the immunology of infectious diseases research center, Rafsanjan University of Medical sciences, Rafsanjan, Iran from January to May 2015. Two types of all-metal and PFMs were prepared in circular-shaped (Dibateb Co., Tehran, Iran) discs with 5 mm diameter, 1 mm thickness (Figure 1) [17] and used to experiment the formation of biofilms. *C. albicans* was cultured on Sabouraud-dextrose agar medium (HiMedia, Netherlands) and incubated at 30°C for two days for preparation of new fungal colonies; then one colony of the mentioned fungus was placed in 100 cc of sterile Sabouraud-dextrose broth medium (liquid culture medium, HiMedia, Netherlands) and incubated on a shaker (Behdad, Tehran, Iran). Then a suspension of 1×10^5 cfu/ml (colony forming unit per ml) colony of *C. albicans* was counted. A total of 36 discs of two crown types (18 of each) were prepared as test specimens (Dibateb Co., Tehran, Iran), and three groups of six discs were placed in a plate containing *Candida* suspension for 40, 80, and 120 minutes separately; they were dipped in this suspension at the mentioned times and placed at 37°C.



Figure 1

Figure 1: The circular-shape of two different disks (ceramic and metal crown)

After the necessary time (40, 80 and 120 minutes), the specimens were removed and after a simple three-second washed with sterile physiological serum, they were placed in 1 ml sterile physiological serum (Razi Vaccine and Serum Research Institute, Karaj, Iran) and uniformly

shaken for one minute with low circulation of vortex device (Behdad, Tehran, Iran) to allow the probable yeast cells adhering to the specimens be detached and suspended in the physiologic serum (Razi Vaccine and Serum Research Institute, Karaj, Iran). Subsequently, a certain amount (0.01 ml) of this suspension was cultured on the culture medium of Sabouraud-dextrose agar (HiMedia, Netherlands) with pour plate method [13]. The plates were placed in a 37°C-incubator for 48 hours. After the incubation time, the number of colonies was counted which was representative of the adhesion of *C. albicans* to the test specimens (Figure 2).



Figure 2: Colony count of *C. albicans* base of pure pale method

By multiplying the number of colonies counted in dilution amount, the final number of candida colonies per each disk, within a 1 ml sterile physiologic serum (Razi Vaccine and Serum Research Institute, Karaj, Iran), was calculated at 40, 80 and 120 minutes as cfu/ml. It should be noted that each colony is equivalent to one living cell. In the end, the results were recorded for each group at three times in the relevant checklist and given to the analyst, who was unaware of the groups.

Statistical analyses

The collected data was input into the statistical software IBM SPSS Statistics for Windows version 18.0 (IBM Corp. 2009. Armonk, NY: IBM Corp) used for analysis. Results of numeric variable were presented as mean \pm standard deviation (SD) and that of nominal variables by frequency (percentage) for categorical variables. One-sample Kolmogorov-Smirnov test showed that the data are normally distributed in the two groups ($p > 0.05$). Equality of variances was also confirmed by the Levene's test ($p = 0.180$). Comparison of colony count in each group among the three time intervals was tested by one-way repeated measures ANOVA. For all statistical tests, p -values of 0.05 or less were considered statistically significant.

RESULTS

The mean colony counts of *C. albicans* on all-metal and PFMs crowns at three intervals are shown in Table 1. As the results of two-way ANOVA test showed, the effect of type of crown was statistically significant on the colony counts and regardless of the time, mean colony count of

C. albicans had significant differences in the vicinity of two crown types (df=1, F=685.120, p<0.001). Also, the effect of time was significant and regardless of the crown type, mean colony count of *C. albicans* had significant differences in the measured intervals and increased with time (df=2, F=3808.908, p<0.001) (Figure 3).

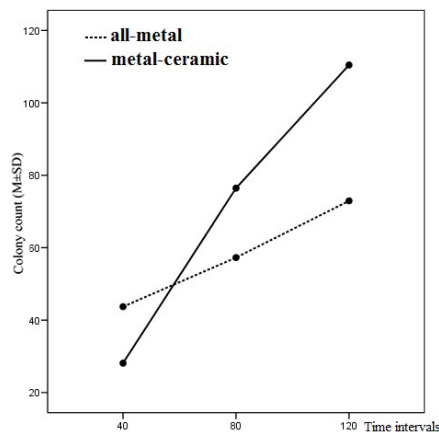


Figure 3: The trend of changes in mean colony count of *C. albicans* in the two groups and three time intervals

Time and crown type had a mutual effect and the slope of changes in mean colony count of *C. albicans* were significantly different in the measured intervals in two crown types (df=2, F=887.392, p<0.001). Although colony count of *C. albicans* after 40 minutes was higher in all-metal crowns compared to PFMs, it significantly increased in PFMs in the next two intervals (80 and 120 minutes) (Table 1).

Table 1: Comparison of mean colony count of *C. albicans* in three time intervals between two groups of all-metal and metal-ceramic crown

Group		40 minutes	80 minutes	120 minutes	p-value*
All-metal	Min-max	41-47	54-61	70-76	<0.001
	Mean ± SD	43.72 ± 1.99	57.28 ± 2.22	72.94 ± 1.89	
Metal-ceramic	Min-max	24-32	72-81	104-117	<0.001
	Mean ± SD	28.11 ± 2.37	76.44 ± 3.07	110.44 ± 4.09	
p-value**		<0.001	<0.001	<0.001	-

*Comparison between the three time intervals by one-way ANOVA; **Comparison between the two groups by independent two-sample t test

DISCUSSION

The results of the present *in-vitro* study on PFM crown and all-metal crown showed statistically significant differences in mean colony count of *C. albicans* in different time intervals. The maximum time was considered 2 hours in this study (120 minutes) and the results of mean colony count of *C. albicans* were compared between the groups of crown types and among the three time intervals (40, 80, and 120 minutes). The lower growth of *C. albicans* over time in all-metal crowns than of PFMs could be due to the better ability of polishing in metal, compared to ceramic; apparently, the metal bubbles are easily observed and can be treated by

the clinician [18]. The higher colonization of *C. albicans* in the first 40 minutes in all-metal crowns could also be due to the ion release in the first phase from metal [19]. Nevertheless, as far as the authors are concerned, no study has compared the fungal growth between these two crowns, for their results to be comparable to ours.

As the results indicated, the crown type and time had both significant increasing effects on mean colony count of *C. albicans* and also mutual effects with each other. The mean colony count of *C. albicans* was higher in all-metal crowns after 40 minutes while the mean colony count of *C. albicans* significantly increased in PFMs after 80 and 120 minutes. These results indicated that PFMs had a higher fungal growth compared to all-metal crowns after two hours.

Several studies have reported the increased attachment of *C. albicans* to artificial materials, such as dental prostheses, catheters, and restorative materials, suggested to be due to the biofilm formation of candida and better surface adherence [20-22]. Burgers et al. [23] studied the adherence of *C. albicans* in composite resin materials (compomer, ormocer, novel silorane, and conventional hybrid composites) and showed less adhesion of fungi in compomers and ormocers. Lawaf et al. [14] showed high adherence of *C. albicans* strains to glass ionomer fragments, especially in the absence of human whole saliva. Although, the results of these studies confirm the general results of the present study on higher adherence of *C. albicans* to restorative materials [11], the results are not comparable with regard to types of restorative materials.

According to the general opinion, the adhesion of *C. albicans* to Herkulin (resin restorative materials) is reduced by whole saliva and secretory immunoglobulin A [11,14,24,25]. Also, other materials in saliva have been suggested to detach *C. albicans* from the cell wall in plastic plates [26]. These results emphasize on the management of *C. albicans* in patients with conditions reducing the saliva flow or pH [27], such as acquired immunodeficiency syndrome, and salivary disorder like Sjogren's syndromes [6]. As to the evidence, diabetic patients may have altered saliva flow, therefore, paying attention to *C. albicans* in these patients and including the clinical assessment and educating patients on appropriate care are valuable [28]. The role of saliva in reducing colonization of *C. albicans* can have treatment targets, as *Candida* strains have high resistance to different antibiotics [29,30].

Generally, there is little information about the colonization and adherence of *C. albicans* to different restorative materials. In the present study, the colonization of *C. albicans* to two conventionally used crowns were compared to provide valuable information on the choice of the most appropriate crown material; nevertheless, this study may have also some limitations. The most important limitation of this study could be the fact that the presence of saliva has not been evaluated. As to the evidence, the presence of saliva significantly reduced the colonization and adhesion of *C. albicans* to

restorative materials [11,14,24]. Meanwhile, the crown materials in the present study has not been used and investigated in other studies [11,14,24] and further research is required to illuminate different aspects of *C. albicans* colonization in all-metal and PFMs.

CONCLUSION

Base of our survey, the adhesion of *C. albicans* to all-metal crowns was significantly less than metal-ceramic crowns after 80 minutes and 120 minutes. Further studies are required to evaluate the colonization of *C. albicans* to all-metal and PFMs in the presence of whole human saliva.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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