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ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

Comparison of the *Candida albicans* and biofilm formation amount on natural tooth, porcelain and acrylic resin

Doğal diş, porselen ve akrilik reçine üzerindeki Candida albicans tutulumu ve biyofilm oluşumu miktarının karşılaştırılması

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ABSTRACT

Objective: This study compared the retention of the *Candida albicans* and biofilm formation on natural teeth, porcelain and acrylic resin.

Materials and methods: Samples are taken with the sterile ecuvion sticks from the buccal embrasures of the natural tooth, porcelain and acrylic. The biofilm production of candida reproducing strains was determined with microplate method. Samples are settled in 0.5 ml sterile phosphate buffered saline. Reproduction detected colonies defined to species in accordance with their macroscopic and microscopic features and germ tube test in microbiology laboratory.

Results: There was significant differences for retention of *Candida albicans* and biofilm formation on the surface of tooth, porcelain and acrylic ($p<0.05$).

Conclusion: Adherence of *Candida albicans* and biofilm formation on the porcelain significantly less than natural tooth and acrylic, and retention and biofilm formation on the tooth less than acrylic.

Key words: Biofilm, *Candida albicans*, dental materials, tooth.

ÖZET

Amaç: Bu çalışmada doğal diş, porselen ve akrilik rezin üzerinde kandida albikans tutulumu ve biofilim oluşumu kıyaslanmıştır.

Gereç ve yöntem: Örnekler steril eküvion çubuklarla doğal, porselen ve akrilik dişlerin bukkal embrajurlerinden alınmıştır. Kandidaların biofilim oluşumları Mikropleyt yöntemiyle belirlenmiştir. Örnekler 0,5 ml steril fosfat tampon salin solusyonunda bekletilmiştir. Kolonilerin üremesi, türlerin mikrobiyoloji laboratuarında makroskobik ve mikroskobik özelliklerine ve bakteri türlerine göre kıyaslanmasıyla tanımlanmıştır.

Bulgular: Diş, porselen ve akrilik yüzeyinde kandida albikans tutulumu ve biofilim oluşumu bakımından anlamlı bir fark bulunmuştur.

Sonuç: Porselen üzerindeki kandida albikans tutulumu ve biofilim oluşması doğal diş ve akrilik üzerinden ve ayrıca doğal diş üzerinde akrilik üzerinden daha az olmuştur.

Anahtar kelimeler: Biofilm, *Candida albicans*, dental materyaller, diş.

INTRODUCTION

There are many variables between fixed and removable prosthesis like functional, structural and biocompatible differences. Although these variances, prosthesis must be in perfect conditions that do not corrupt the habitat of the oral cavity.[1] In addition prosthesis should not harm the neighborhood soft and hard tissues. There have been done many investigations about the relationships between the prosthesis and their environmental tissues.[2,3] According to studies, the most appropriate, healthy and perfect prosthesis could lead to change the flora of the oral cavity.[4,5]

There are many kinds of microorganism in the oral flora. Candida and species are also get involved in this flora. *Candida albicans* is the most pathogen

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species of the Candida family. They are opportunist in weakening of the immune system. Investigations indicate that the *Candida albicans* are more adherent species to plastic surfaces and the mucosa of the oral cavity than the other candida species.[6] With the affected of the oral cavity's mucosa and the environmental tissues by the chemical and pyhsical properties of the prosthesis lead a well environment for reproducing of the Candida's.[7,8] Increasing number of the Candida causes denture stomatitis.[9] Angular cheilitis, itching, inflamation of the oral mucosa, decreasing of the saliva, erythema of the tongue are the symptoms of the oral candidiasis.[10] All these disorders make the person indisposed. The patient could not use the prosthesis comfortable.

Candida species commonly seen in digestive tract, oral mucosa and skin at healthy people. When immune system is depressed by various reasons, candidas start harming the tissues and become a potential risk for human health. *Candida albicans* are the most isolated form of the bacteria from almost of the candidiasis cases.[11]

It is known that *Candida albicans* adhere not only to intraoral tissues but also to dental materials. But it is not known amount of *Candida albicans* colonization and biofilm formation among on porcelain, natural tooth and acrylic resin. The aim of this study find out to amount of colonization of *Candida albicans* and biofilm formation on this materials.

MATERIALS AND METHODS

One hundred and fifty six patients that applied to Mustafa Kemal University Faculty of Dentistry were chosen for this study.

All the patients are informed and their confirmations are receipt before the study. Samples are taken with the sterile ecuvion sticks from the buccal embrasures; from both of acrylic and natural teeth on the patients with removable partial dentures and both of ceramic and natural teeth on the patients with fixed prosthesis.

Collected samples are settled in 0.5 ml sterile Phosphate Buffered Saline and delivered immediately to laboratory. Tubes contain swab, vortexed for 30 second quietly. Later on, the swabs removed and samples were centrifuged. Obtained pellets again resuspensioned in 50 µl buffer. This suspension quantitatively inoculated to Sabouraud dekstroz agar medium that includes chloramphenicol. Also, the inoculation of ecuvion sticks are done. In oven; at 35-37°C for 24-48 hours incubated. End of time, reproduction detected colonies defined to species in accordance with their macroscopic and microscopic features and germ tube test. Pursuant to germ tube test the positive resultants defined as *Candida albicans* and negatives defined as non-albicans Candida species. Results evaluated quantitavely, calculating the number of bacteria in mililiter, in the order to Colony Forming Units/ml. The biofilm production of Candida reproducing strains is determined with microplate method.

Determination of biofilm production with microplate method

The method for coagulase negative staphylococcus defined by Christensen and his colleagues,[12] modified and used. *Candida albicans* strains produced at Sabouraud Dextroz Agar (SDA) medium at 37°C for 48 hours. Subsequently, diluted in proportion as 1/100 at Sabouraud Dextroz Broth (SDB) includes 2% glucose. This dilution was put 200 µl on the each wells of sterile flat based microplate and incubated at 37°C for 48 hours. End of period, content of each well evacuated and washed 4 times with PBS. Following this, in each well placed with 200 µl methylene blue and staining ensured in room temperature within a hour. Then wells washed 3 times with distilled water and microplates are left reverse to dry on drying papers.

In this study, sterile SDB is used as negative control. Microplates optic densities read at 492nm wave length micro ELISA reader. (Tek TIME, Organon Teknika, France). This processes made 3 times for each strain, and the arithmetic mean of read values of optic densities are receipted.

The value of optic density of strains bigger than 0.240 are assessed as potent adherent, 0.120-0.240 strains are adherent and strains below 0.120 are assessed as adherent negative.

This study is validated by ethics committee of our hospital.

Statistical analysis

One way Anova (SigmaStat version 3.0, SPSS version 11.0, Inc., Chicago, USA) and Turkey post-hoc

tests were used to analyze the differences between the materials of Candida colonization and biofilm formation.The significant level was set at 0.05.

RESULTS

Concerning determined colonization number's arithmetic mean and standard deviations are given at Table 1 according to used material's type.

One Way Anova results are given for determining the colonization differences among groups at Table 2.

There is a statistically significant differences among the materials according to One Way ANOVA test results (p<.05) (Table 2).

Turkey test results were given to determine the source of differences at Table 3.

According to Turkey result, amount of colonization on materials respectively acrylic>natural tooth>porcelain.

Table 1. Arithmetic means and standard deviations of colonized Candida's acording to material

Materials	n	Mean	S.D.
Porcelain	36	8400.00	14793.90
Tooth	78	24138.46	41903.81
Acrylic	42	31742.86	43869.14
Total	156	22553.85	38748.60

Table 2. Colonization differences amoung groups

	df	F	p
Between Groups	2	3.779	0.025
Within Groups	153		
Total	155		

Table 3. Turkey test results were given to determine the source of differences

(I)	(J)	Mean Difference (I-J)	Sig.
Tooth	Porcelain	15738.46	0.104
Acrylic	Porcelain	23342.85*	0.021
	Tooth	7604.39	0.551

* The mean difference is significant at the .05 level.

Descriptive statistics results of arithmetic mean and standard deviations according to material's type for biofilm formations are given at Table 4.

Table 4. Aritmatic mean and standart deviations according to material's type for biofilm formation's

Materials	N	Mean	Std. Deviation
Porcelain	36	0.04	0.01
Tooth	78	0.06	0.05
Acrylic	42	0.12	0.14
Total	156	0.07	0.09

One Way Anova results for determining the biofilm formation differences among groups are given at Table 5.

One Way ANOVA test result showed that there is a statistically significant differences about formation of the biofilm layer amoung the materials (p<.05).

According to Turkey result, amount of biofilm formation on materials respectively acrylic>natural tooth>porcelain.

Table 5. Biofilm formation differences amoung groups

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.19	2	0.09		
Within Groups	0.94	153	0.01	15.04	0.00
Total	1.14	155			

Table 6. Multiple Comparisons (Turkey HSD)

(I)	(J)	Mean Difference (I-J)	Std. Error	Sig.
Tooth	Porcelain	0.02	0.02	0.42
Acrylic	Porcelain	0.09(*)	0.02	0.00
	Tooth	0.07(*)	0.02	0.00

* The mean difference is significant at the .05 level.

DISCUSSION

Oral health is in a close relation with quality of life. Prosthetic treatments, reestablishes function and can offer satisfactory esthetics but they can unfavorably influence the oral health. Prosthetics must be in ideal conditions and well-planned. It must be noted that inappropriate prosthetics would be more harmful than their benefits.

Materials surfaces are very important factor for the colonization of the microorganisms. There have been many studies on the adhesion of the *Candida albicans* to denture acrylic resin and leads to denture stomatitis.[13,14] On the other hand there is no studies about comparing to adhesion of the *Candida albicans* to tooth, porcelain and acrylic resins.

Biofilm adhesion plays a significant role at candida's pathogenicity.[15,16] The biofilms are cells that in relationship with surface and yeasts and filaments which are surrounded by an extracellular matrix. They are divorced from free cells by their phenotypes.[17,18]

Due to biofilms are the reservoir of the infections, they cause to spread of the infections. Also they are more stronger than free cells against to anti-fungals.[15,6] Biofilms affect the phagocytosis and opsonization of the polymorphonuclear leukocyte by inducing their chemotaxis.[19] Then they become powerful against host immunity.[20]

The effects of prosthesis to oral flora have been investigated previously. In detail, the mechanism of disruption on the oral flora has been stated in these studies .The presumption of generating infection on the periphery oral tissues are compared between the acrylic and ceramic prosthesis, and shown that the acrylic prosthesis are tend to make infection more than the ceramic prosthesis.[21] The components of partial removable prosthesis; clasps, rests, retainers and the wide acrylic base are retention areas for dental plaque and Candidas.[22]

Nevertheless not all mouths with the prosthetic restorations show tissue inflammation related with prosthesis. Yavuz and his friends reported, in a study that imply 50 ceramic crown inner surface culture samples, there is bacterial reproduction in 28 crown, and no any bacterial reproduction in 22 crown.[23]

Candida albicans is adhese the rough surfaces much more than flat and polishing surfaces.[24-26] *Candida albicans* is colonize the prosthetic materials as well as the oral tissues.[27,28] There is a positive correlation among the adhesion amount and colonization, ability of the turning out a disease.[29]

Radford and et al examined prosthetic materials that shows different polymerization feature for adhesion and firstly emphasied significance of the rough surfaces in an early stage of the *Candida albicans* adhesion.[30]

Among many studies concerning the adhesion mechanisms of *C.albicans* to different materials and factors affecting their mechanisms, surface roughness and type of materials are known to be two major factors for the adherence mechanism directly.[7,8]

In conclusion, *Candida albicans* colonized and biofilm formation occurred on acrylic more than natural tooth and porcelain. It may arise from surface roughness or oral hygiene. Because porcelain and natural tooth surface is much more flat than acrylic. Surface roughness of the materials has to decrease and oral hygiene has to be done as possible.

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