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ORIGINAL ARTICLE

Study of Adhesion Candida Species Isolated from the Mouths of Individuals to Acrylic Discs with a Various Concentration of Silver Nanoparticles

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ABSTRACT

Background: Acrylic material used to make dentures have adherence ability to microorganisms and Long-term use from this leads to their colonization and growth and causes infection at mucosal surfaces of the prosthesis. Since silver nanoparticles can have long lasting antimicrobial effects, a more recent method is recommended which utilizes acrylic disc with silver nanoparticles. So we decided to evaluate and compare adhesion of candida species to acrylic discs with a various concentration of silver nanoparticle in two different time periods. Materials and Methods: In this analytical - laboratory study acrylic discs with 0%, 2%, 5% and 10% concentration of silver nanoparticles were prepared and then were put adjacent to the suspension of candida cells isolated from the mouths of individuals in the two period of 40 and 120 minutes and their adhesion to discs was measured and compared. At the end data was analyzed by using chi-square and Kruskal-Wallis test. Results: In this study, the average adhesion in the concentration of control (acrylic disc without silver nano-particles with concentration 0%) in durations of 40 and 120 minutes, was 49.66 and 63.00 respectively. The average adhesion in concentration 2% in duration of 40 and 120 minutes, was 36.21 and 45.38 respectively. The average adhesion in the 5% concentration in duration of 40 and 120 minutes, was 24.83 and 30.69 respectively. The average adhesion in the 10% concentration in duration of 40 and 120 minutes, was 13.66 and 18.10 respectively. Therefore, the effect of time and concentration on adhesion was significant (P value= 0/000< 0.05). Conclusion: Adhesion of candida species to acrylic discs was dramatically reduced by increasing concentration of silver nanoparticles but increased with the passage of time.

Keywords; *Candida species, Acrylic disc, Silver nanoparticle, Adhesion*

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INTRODUCTION

Stomatitis due to Full denture is usual form of oral candidiasis. it is seen as inflammation developed in places under maxillary denture. It is associated with inflammation of the corners of the lips. At least, in 70% of patients with clinical signs of denture stomatitis, excessive growth of the fungus in the mouth can be seen. Its most likely cause includes settle of the candida species in oral mucosa [1].

Specification of host mucosal epithelial cell receptors is an important structure in adhesion of fungus to surface of epithelial cells of host mucosal and is known as the first step in high translocation of Candida in the mouth [2]. Adhesive ability of Candida species to various prosthetic devices such as adhesion to denture acrylic surface is considered as the exclusive character of this yeast [3]. In addition to the trauma caused by denture on the mucosa, it can decrease the tissue resistance to infection and increase in

epithelium porous to Candida antigens and the fungi toxins and consequently leads to denture-induced stomatitis [4]. Although the main material in replacing lost tissue in dental patients is acrylic resin, has not been much attention to antimicrobial properties of acrylic resins.

Given that current treatments for candidiasis, are Nystatin, fluconazole and amphotericin B in severe cases, some problems such as drug resistance and bad effects of using drugs for these types of topical treatments must be considered [5]. On the other hand, patients with low movement ability as well as those who have poor memory and do not have cyclical visual abilities and do not enforce the orders of a doctor, using fungicide in acrylic could play role in preventing denture-induced stomatitis (6). Silver nanoparticles attached to the cell membrane and penetrate into the cell. Cell membrane proteins containing sulfur proteins and silver nanoparticles not only react with this protein, but also react with compounds containing phosphorus, including DNA. Nanoparticles also attack the respiratory chain, leading to cell death. The nanoparticles release silver ions that will lead to increase their antifungal activity [7, 8, 9]. Nasrollahi and colleagues at the research showed that silver nanoparticles by create a discontinuity in the structure of cell membranes and prevent the germination process, applies its antifungal effect [10]. Kim and colleagues showed that silver nanoparticles may create discontinuity in the structure of cell membranes and exert their antifungal effect. Silver nanoparticles have strong antimicrobial effect on Candida albicans, which is similar to that of the antifungal effect of amphotericin B (as control), respectively. While the hemolytic effect of silver nanoparticles on red blood cells is less than amphotericin B [11]. In a study by Nozari in University of Medical Sciences in Iran, was found that when fluconazole is Along with Silver Nano , its result is very desirable in the treatment of chronic candidiasis [12]. Monterio showed that polymers containing silver could prevent bacteria aggregation in interior and exterior surfaces [13]. As well, silver is effective against a broad spectrum of grown bacteria and biofilms. however, silver concentration as an important structure in its effect was stated. In addition, silver nanoparticles show better effectiveness in smaller sizes due to increasing level without decreasing mechanical specifications [13].

Casemiro et al showed that adding 5% and 2.5% silver nanoparticles to acrylic resin impacts on Candida albicans. Mechanical specifications of acrylic resin depend on percentage level of mixed nanoparticles [14]. Young nam *et.al* showed that dentures containing silver nanoparticles have specific outstanding antifungal properties [15].

Sadeghi et.al in their investigation showed that acrylic resin includes 0.5 mg/ L of silver nanoparticles with powerful effect of removing germs against *Escherichia coli* [16]. Therefore, in this study, we tried to produce some prostheses by mix up the silver nanoparticles in dental acrylic with antifungal impact. The new resin, after being in the mouth with the passage of time, releasing silver particles, which are particles that have strong antifungal effects can reduced denture-induced stomatitis (17). Evaluation of adhesion of Candida species with different acrylic discs is with or without various concentrations of silver nanoparticles in two time periods as 40 and 120 minutes.

MATERIALS AND METHODS

The laboratory analytical study was done on 56 species of candidates separated from the mouths of people was conducted through the following steps:

Preparation of fungal samples for testing:

Candida species detected on Sabouraud dextrose agar and were incubated at 30 ° C for 24 hours.

Prepare a suspension of yeast cells:

24-hour yeast cells were taken up by a loop of the medium. Bolts into tubes containing physiology serum transferred and was uniform by mixer in sterile conditions. With the help of a spectrophotometer, at a wavelength of 520 nm and optical density of 0.38, yeast suspension with a cell density 1×10^{7} CFU / ml resulted respectively [18].

Preparing acrylic samples without silver nanoparticles (control samples):

For preparing acrylic samples without silver nanoparticles (control samples) cylindrical parts with a diameter of ten millimeters and a thickness of four millimeters were used (Figure 1). First, gypsum and water were mixed according to the manufacturer's instructions. Then, it was poured up to 10 mm of upper edge of dish. After hardening plaster, silicon (Poti) was placed on it with a thickness of about ten mm. While Poti is hardening, ten cylinders were placed inside it so that the entire thickness of the cylinder was buried in Poti. Then, upper part of dental flask was placed on staddle part and was poured inside plaster dental flask and was placed under hydraulic press machine. Metal cylinders got out after 3 hours and were prepared for making acrylic plaster appropriate for factory. The mixture was placed in the empty spaces when was at dough stage. Finally, dental flask was placed under press machine. Then it was placed inside warm bath with 72°C temperature for 6 hours. After 34 hours in room temperature,

dental flask was opened. Samples were brought out of plaster. By acrylic cutter, additions were cut and was prepared and put into physiology saline [19] (picture 2).

Preparing acrylic samples with various concentration of silver nanoparticles:

To estimate weight of each sample, 50 samples without silver nanoparticle were prepared. Average weight of each sample was calculated as 0.4 gram.

Nano colloid weight of silver particles required for preparing an acrylic sample including 2% silver nanoparticles:

Weight of each acrylic sample 0.4 gram × 0.02 weight of silver nanoparticles gram / weight of each acrylic sample = 0.008 gram

Nano colloid weight of silver particles required for preparing 10 acrylic samples including 2% silver particles.

0.008 gram × 10 = 0.08 gram

Colloid volume required to produce the 10 samples containing 2% acrylic silver nanoparticles:

silver nanoparticles 0.08 gram × Colloids of silver nanoparticles 1.5 ml / Colloids of silver nanoparticles 2 gram = 0.06 ml

According to the above equations, volume of colloids required for the preparation of 10 acrylic samples each weighing 0.2 g for concentrations 2, 5, 10% to weights are as 0.06 ml, 0.15 ml, and 0.3 ml. each one with 53.3 cc acrylic resin powder and 26.6 cc acrylic liquid mixed and glass spatula was added to it until homogenizing it. In the next stage, it was poured inside embedded spaces into dental flask. Other stages continued such as samples without silver nanoparticles [19].

Adjacent samples with a yeast suspension:

Acrylic discs were adjacent to 1 ml of fungal suspension with 1×10^{7} cell concentration of cell into microtubes. In order to minimize slippages of test time, a negative testifier is prepared per each sample series (Physiology Saline and acrylic disc). Then, microtubes were placed into incubator shakers, and placed at 37 ° C as 120 and 40 minutes.

Check the adhesion of candida cells to Acrylic discs:

After passing the required time (40 and 120 minutes), the samples got out. After a simple washing during 3 seconds with 2 ml Physiology Saline of Sterile, discs were replaced to a pipe including 3 ml Sterile Physiology saline. Vertex devices was shacked for one minutes slowly until separating possible yeast cells attached to samples and Suspending it in Physiology saline. In the next stage, 20 Landa were harvested on Sabouraud Dextrose Agar, and incubated it for 48 hours in 37 ° C. After heating time, number of the formed colonies were counted. This number of colonies represented candida adhesion to the samples of test [20]. Chi-Square , Kruskal-Wallis One way Anova, Two way Anova tests were used to analyze data.

RESULTS

In 56 candida species, separated from the mouth of people in this study, frequency distribution of candida species included respectively as 27 items of Albicans species(48.2%), 20 items of Glabrata species (35.7%), 5 items of Tropicalis (8.9%) , and 4 items of Krusei species (7.1 %) (table 1). According to table1 and results of Chi Square test, P-Value was 0.000 <0.05 represents significant difference between candida species in people. According to table 2, and using one-way Anova, it was concluded that tropicalis species with 42.96 average has the most amount of adherence, and krusei species with average of 29.40 has the less amount of adhesion. Glabrata and Albicans species adherence were respectively as 42.18 and 30.78. P Value was 0.000 <0.05 indicates significant difference in adherence level in candidates groups. According to table 3, adherence amount in concentration of control (acrylic resin without silver nanoparticles) was 56.33 and in concentrations 2%, 5%, 10% of nanoparticles was respectively as 40.79, 27.76, 15.88.

According to table 3, impact of concentration on adherence was significant (P Value = 0.000 < 0.05). The adhesion average was not the same in four groups. In this investigation, according to table 4, average concentration of adhesion control during 40 minutes was 49.66, and during 120 minutes was 63.00. Adhesion average in 2% concentration during 40 minutes and 120 minutes were respectively as 36.21 and 45.38. Adhesion average in 5% concentration during 40 minutes and 120 minutes were respectively as 24.83 and 30.69. Adhesion average in 100% concentration and during 40 minutes and 120 minutes were respectively as 13.66 and 18.10. In addition, effect of time on adhesion was significant. Adhesion average was not the same in two times (P Value = 0.000 < 0.05).

DISCUSSION

Oral candidiasis is the most common mucous lung infection in contact with the prosthesis [21], as it includes 72% of denture consumers [22]. In complete denture holders, especially in the upper jaw

denture-induced stomatitis causes [23] for some reasons such as loss of harmony between prosthesis and jaw, the emergence of trauma to the jaw when eating, oral hygiene and the presence of microorganisms such as Candida albicans in the mouth [23]. Organization of biofilms in the creation of denture-induced stomatitisis seen in 65% of edentulous patients and depends on the number of structures, especially unevenness and roughness of the surface being penetrated [5, 14, 21 and 24]. To evaluate the toxicity of silver, an experiment was conducted on rat spermatogonial stem cells. It was founded if concentration of silver nanoparticles is between 5 µg/ml to 10 µg/ml, it results in Necrosis and apoptosis of rat spermatogonial stem cells [25]. Anyway, silver is not considered as dangerous heavy metals which is dangerous for public health [26].

According to table 2, representing significant difference between adhesion level in candidates groups, Tropicalis and Glabrata species had the most adhesion level and Albicans species had the less adhesion level to the acrylic resin. In this study, increasing the adhesion of Tropicalis and Glabrata species to acrylic resin represents significance of non-Albicans species in creating denture stomatitis. Luo et.al in their study showed that Glabrata species adhesion than Albicans adhesion increased significantly (27). Kumar et.al in their investigation showed that adhesion of Candida species depended on catheters which was in consistent with our study [28]. According to table 3, the most and the least adhesion amount are respectively with 0% concentration and 10% concentration. So adhesion of the Candida species depends on type of acrylic. Increasing concentration of silver nanoparticles results in decreasing adhesion of candida species to acrylic resin significantly. In addition, according to table 3, adhesion means in time intervals of 120 minutes than 40 minutes increases. By passing time, adhesion of candida species isolated of mouth to acrylic resin increases significantly. According to table 4, the most amount of adhesion was in 120 minutes time duration and in concentration 0% of nanosilver. The less adhesion was in 40 minutes in concentration 10% of nanosilver. Therefore, there is a significant direct relationship between acrylic discs adhesion and increasing time. Also, it has a negative relationship with increasing nanosilver concentration. According to results of investigation by Acosta-Torres *et.al*, it was determined that Nano Silver drives dramatically reduces the adhesion of Candida Albicans that was consistent with our study [29]. Ghahremanloo showed that in acrylic resins by increasing the concentration of silver nanoparticles, antifungal effect increases which is in consistent with our study [19].

CONCLUSION

1. Adhesion of candida genus depends on acrylic species. Increasing concentration of silver nanoparticles, reduces adhesion of candida genus to acrylic resin significantly.
2. Adding silver nanoparticles to acryl results in changing color of acryl from pink to brown.

In this investigation, adhesion level of candida genus to acrylic discs and acrylic discs with silver nanoparticles depend on factors such as candida genus and concentrations 2%,5%,10% of silver nanoparticles. In this study as silver nanoparticles were trapped into acryl samples, in short-time were not able in applying an antimicrobial effect. Only superficial nanoparticles applied antimicrobial effect. Naturally, if the nanoparticles are mixed with a suspension and then be exposed to the fungus, may impose a greater effect than placing inside solid acrylic samples. On the other hand, recent researches showed that if the silver is saturated into polymer, its antimicrobial effect is more than covering as a superficial layer on acryl, maybe because Superficial silvers usually are passive by anion proteins probably(30 and 31). Another unfavorable result was due to the integration of silver nanoparticles with acrylic discs, increasing the turbidity of poly methyl methacrylate. Adding more percentage of silver nanoparticles to resin, results in more opaque. Results of other studies were similar to this study (14). Hence, more investigations are required to prevent reducing base transparency of acrylic resin including silver nanoparticles in a way avoiding beauty of prostheses.

Table 1- Frequency distribution of Candida specises

Candidate specises	Frequency	Percentage	One sample Chi Square
Tropicalis	5	8.9	X2=27.57 P value = 0.00
Krusei	4	7.1	
Albicans	27	48.2	
Glabrata	20	35.7	
Total	56	100	

One sample Chi Square test showed significant difference in the number of species observed in individuals.

Table 2- comparison of adhesion the average in the Candida species groups

Candidate species	Number of adhision assessment	adhesion mean	SD	One way Anova
Tropicalis	50	42.96	18.23	F=11.64 P value = 0.00
Krusei	100	42.18	18.42	
Albicans	100	30.78	15.55	
Glabrata	40	29.40	18.19	

One way Anova showed a significant difference in adhision level in Candidate groups.

Table 3- Mass Effect and time on adhesion

Variable		Adhesion assessment number	Adhision	SD	Two way Anova
Time	40	116	29.39	120.01	F=34 P value = 0.00
	120	116	31.09	16.9	
Concentration	Measures	58	56.33	14.71	F=152.53 P value = 0.00
	2%	58	40.79	11.95	
	5%	58	27.76	10.26	
	10%	58	15.88	8.18	

Two way Anova test represented significant difference in concentration time on adhesion.

Table 4. Mean and standard deviation of adhesion based on concentration and time

Concentration	Time	N	Mean	Std. Deviation
Control	40	29	49.66	12.644
	120	29	63.00	13.743
2%	40	29	36.21	10.890
	120	29	45.38	11.350
5%	40	29	24.83	10.082
	120	29	30.69	9.754
10%	40	29	13.66	7.475
	120	29	18.10	8.377

Adhesion average was not the same in two times (P Value = 0.000 < 0.05)

Figure 1: metallic drums (stainless steel)

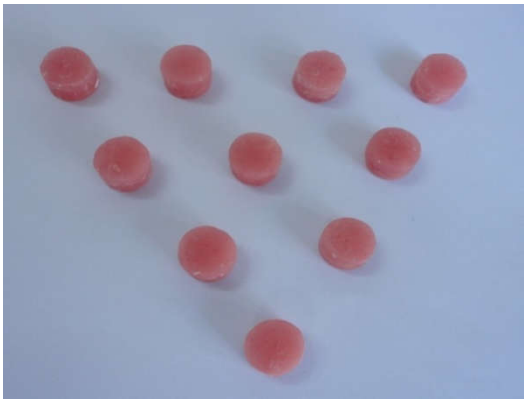

Figure 2: final acrylic samples without silver nanoparticles.

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