Original Article

Comparison of Candidal and Bacterial Adherence to Denture Base Acrylic Resins

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Abstract

Background and Aim: Futura Gen and GC Reline Hard denture chairside reline material shave recently been introduced to the dental market. Although yet to be scientifically proven, these products are claimed to have optimal characteristics. This study aimed to compare the adherence of Candida strains and bacteria to denture base acrylic resins.

Materials and Methods: In this in-vitro experimental study, 24 discs were fabricated of Futura Gen, GC Reline Hard and Meliodent reline acrylic resins. Specimens were inoculatedwith three Candida strains and Streptococcus mutans at three time points and after staining,their optical absorbance (microbial adhesion and biofilm formation) was calculated using spectrophotometry. Two-way ANOVA and Tukey's HSD test were applied for statistical analysis.

Results: Absorbance (optical density)of C. albicans strains on Futura Gen, GC Reline and Meliodent hard reline acrylic resins was 0.022, 0.011 and 0.028 at one hour, 0.057, 0.022 and 0.062 at 24 hours and 0.101, 0.051 and 0.11 at one week, respectively. Absorbance of C. glabrata on the mentioned resins was 0.012, 0.008 and 0.016 at one hour, 0.039, 0.029 and 0.044 at 24 hours and 0.075, 0.068 and 0.081 at one week, respectively. Absorbance of C. dubliniensis on these resins was 0.026, 0.035 and 0.027 at one hour, 0.064, 0.066 and 0.067 at 24 hours and 0.11, 0.12 and 0.13 at one week, respectively. Absorbance of standard strain of S. mutans was 0.027, 0.014 and 0.035 at one hour, 0.064, 0.026 and 0.064 at 24 hours and 0.11, 0.05 and 0.11 at one week, respectively. Candidal and bacterial adhesion to denture base acrylic resins was not significantly different at the understudy time points (P>0.05).

Conclusion: Except for C. dubliniensis, the lowest absorbance belonged to Meliodent. However, absorbance increased over time. Optical absorbance of S. mutans was lower in Meliodent. Among yeast strains, the highest absorbance belonged to C. dubliniensis.

Key Words: Absorbance , adhesion , candida albicans , streptococcus mutans , acrylic resin

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Received: 19 May 2012
Accepted: 18 Oct 2012

Journal of Islamic Dental Association of IRAN (JIDAI) Summer 2013 ;25, (2)

Introduction

Acrylic resins have the widest application in denture fabrication [1]. Adherence of microorganisms especially yeasts to acrylic resins is an important issue compromising the service and efficacy of prostheses. Adhesion of Candida strains to mucosa and denture and formation of biofilm are responsible for development of Candida stomatitis [2, 3]. Adherence is the first step in the process of infection; which is followed by biofilm formation. Biofilm formation occurs in three phases including adherence of microorganisms to the surface,

growth and secretion of extracellular polymers (formation of mature biofilm) and eventually formation of a scaffold on the surface. In comparison of bacterial and yeast biofilm,extracellular polymers in yeast biofilm have lower protein and carbohydrate content and higher amounts of glucose and galactose [4]. Denture stomatitis is among the most important types of Candida infections. Yeasts in this infection are usually present on the palatal surface of denture. Denture base acrylic resin is a suitable environment for growth and colonization of Candida strains. In the clinical setting, C. albicans is more frequently observed than other Candida strains due to the possession of a wide array of virulence factors. C. albicans has been isolated from the mucosa of healthy individuals more than other species and candidiasis is an opportunistic infection that usually occurs in physiologically and immunologically compromised hosts. However, these facts cannot deny the pathogenicity of Candida strains especially C. albicans [5,6]. Phenotypic switching is the most important property of C. albicans responsible for its compatibility with different environments. C. albicanshas the ability to formtrue hyphae or pseudohyphae and chains andclusters of yeast cells called blastoconidia when grown on corn meal agar for 3 days [5, 7]. C. dubliniensis was first identified and distinguished from C. albicans in 1995. This strain is very much similar to C. albicans in many morphological and physiological characteristics and used to be identified as a C. albicans strain for a while. However, a few differences exist between the two that distinguish them from one another. In terms of phenotypic characteristics, both strains grow on specific culture medium at 37-39°C but C. dubliniensis in contrast to C. albicans has limited or no growth at 42°C. An important difference between the two is the inability of C. albicans to express B-glucosidase activity. C. dubliniensis in contrast to C. albicans increases the production of extracellular proteaseenhancing its adherence to buccal mucosa epithelial cells in the oral cavity. Two important characteristics shared by these two strains are the formation of germ tube and chlamydospores [8]. Recent studies on C. dubliniensis have specified it as an opportunistic pathogen isolated from the oral cavity of AIDS patients and cases of denture stomatitis. It quickly gains resistance to fluconazole [9, 10].

C. glabrata is a common strain isolated from denture stomatitis and vaginitis patients [10]. In the recent years, its prevalence in human diseases has greatly increased which may be attributed to its resistance to fluconazole. A specific characteristic of this strain is the absence of hyphae or pseudohyphae formation in cornmeal agar medium [5-7].

Aside from the microorganism strain, other variables such as time, quality and composition of acrylic resin and its surface roughness may play a role in adhesion of microorganisms and consequently lead to variable degrees of stomatitis. As reported in electron microscopy analyses, Candida biofilms mainly form along the cracks and imperfections of the denture acrylicsurface [11].

Since no published study is available on Candidal adhesion to Futura Gen and GC Reline hard lining material, the present study was designed aiming at determining the Candidal and bacterial adhesion to Futura Gen and GC Reline hard acrylic resins in comparison to conventional heat-cured acrylic resin (control group).

Materials and Methods

In this in-vitro experimental study, absorbance of understudy microorganisms adhered to GC Reline hard (GC American Inc., America), Meliodent (Heraeus Kulzer, Germany) and Futura Gen (Schutz, Germany) acrylic resin specimens was measured. Acrylic discs were fabricated and inoculated with standard strains of C. albicans (ATCC 14053), C. glabrata (ATCC 90030), C. dubliniensis (ATCC MYA-2975) and S. mutans (ATCC 35668) at different time points. Absorbance (optical density) was then measured using spectrophotometry.

In order to prepare heat-cured acrylic discs (Meliodent), dies measuring 1cm in diameter and 1 mm in thickness were flasked to form molds. Heat-cured acrylic resin was packed inside the molds and flasking steps were carried out. Heat-cured acrylic resin was heated for 9 hours in water with constant temperature of 165°F (equal to 73.5°C).

In order to fabricate GC Reline specimens, acrylic powder (polymer) and liquid (monomer) were mixed according to the manufacturer's instructions, packed into the prepared molds, pressed and allowed to complete polymerization at room temperature.

Futura Gen (F) specimens were fabricated by using the UniPressinjection system (Schutz-Dental GmbH, Rosbach, Germany) according to the manufacturer's instructions. Specimens with voids, irregularities or incomplete polymerization were excluded from the study. Fabricated discs of the three acrylic resins were polished and finished as follows:

Specimens were first polished by abrasive paper and light manual pressure and then with a slurry of medium grit pumicemixed in a 1:1 ratio with water and a cloth wheel for one minute. Polishing was continued withfine grit pumice. Eventually, high shine buff was used with polishing brown Tripoli compound for 60 seconds. The quality of polishing of acrylic resins was checked by profilometer and specimen sizes were measured by digital caliper.

Candida strains were cultured in Sabouraud dextrose agar medium while S. mutans was cultured in brain heart infusion (BHI) agar. In the next phase, culture media such as Sabouraud dextrose agar, BHI agar, Tryptone Soya broth, and RPMI 1640 medium were used. Afterwards, a suspension of respective microorganisms was prepared in RPMI-1640 medium buffered with MOPS [3-(N-morpholino) propanesulfonic acid]. Candida stocks were used to prepare microbial suspension with 0.15 optical density at 530 nm. This turbidity is equal to $1x10^5$ yeasts/ml. S. mutans suspension with optical density of 0.1 at 530 nm was also prepared. This turbidity is equal to $1.5x10^8$CFU/ml. Acrylic discs were then placed as pairs in sterile 24-well cell culture plates.

One ml of microbial suspension was added to each well and plates were stored in an incubator at 37°C. The extent of biofilm formation was measured at one hour, 24 h and one week. The well containing acrylic discs and culture medium with no microorganism was considered as the control (blank).

After completion of different incubation periods, culture mediawere suctionedand plates were washed with PBS for three times in order for the unattached microorganisms to be extracted from the plates. In the next phase, acrylic discs were gently transferred to new plates. Specimens were dried for 15 minutes and one ml of 0.4% Gram's crystal violet solution was added to the wells and remained for 15 minutes. The residual color stains were extracted and acrylic discs were washed with PBS for three times to remove excess dye. Specimens were dried for 15 minutes and one ml of absolute ethanol was added to the wells containing acrylic discs to dissolve the dyes attached to acrylic discs. In the next phase, absorbance of the obtained dye solution was measured at 630 nm.

Two-way ANOVA and Tukey's HSD test along with SPSS version 15 software were used for statistical analysis of data.

Results

The results of two-way ANOVA for evaluation of the effect of time and type of acrylic resin on degree of Candida adhesion are demonstrated in Table 1. Based on the obtained results, time (P=0.09) and type of acrylic resin (P=0.11) had no significant effect on degree of Candida adhesion to specimens. Increased time and type of acrylic resin could not significantly change the absorbance indicative of Candida adhesion.

As observed in Table 2, the lowest absorbance of C. albicans, C. glabrata and S. mutans was noted in Meliodent resin. Futura Gen and GC Reline hard ranked next, respectively. For C. Dubliniensis, the lowest absorbance was seen in Futura Gen resin. Furthermore, absorbance of understudy microorganisms in all three types of acrylic resins increased by increasing the time from one hour to one week. For S. mutans, the absorbance in the first hour was higher in GC Reline hard than in the other two resins. However, after 24 hours and one week, absorbance of microorganisms in GC Reline and Futura Gen became equal. Among Candida strains, C. dubliniensis had the highest absorbance.

Discussion

Surface characteristics of the acrylic resin, microorganism strain and the mutual interaction of the two are among the most important factors affecting the colonization of microorganisms on tissue and denture surfaces.

In this study, the lowest absorbance of C. albicans, C. glabrata and S. mutans was observed in Meliodent resin. Futura Gen and GC Reline hard ranked next, respectively. For C. dubliniensis, the lowest absorbance was observed in Futura Gen resin.

Considering the fact that a direct association has

Table 1: Results of two-way ANOVA for evaluation of the effect of time and type of acrylic resin on Candida adhesion

-	Sum of squares	Degrees of freedom	Mean squares	F ratio	P .V
Type of acrylic resin	0/526	2	0/224	0/36	0/11
Time	0/725	2	0/356	0/44	0/09
Type of acrylic resin*time	0/14	4	0/05	0/18	0/25
Error	0/526	57	0/001		
Total	1/917	66			

Table 2: Absorbance of microorganisms on different acrylic discs at different time points

-	Time point								
	One hour			24 hours			One week		
Candida strains	**Hard Gc reline**	**Meliodent**	**Futura Gen**	**Hard Gc reline**	**Meliodent**	**Futura Gen**	**Hard Gc reline**	**Meliodent**	**Futura Gen**
C. albicans	0/028	0/011	0/022	0/062	0/022	0/057	0/11	0/051	0/101
C. glabrata	0/016	0/008	0/012	0/044	0/029	0/039	0/081	0/068	0/075
C. dubliniensis	0/027	0/035	0/026	0/067	0/066	0/064	0/13	0/12	0/11
S. mutans	0/035	0/014	0/027	0/064	0/026	0/064	0/11	0/05	0/11

been reported in previous studies between the surface roughness and porosities of the acrylic resin and degree of microbial biofilm formation, (2-3, 13-17), the porosities of the Meliodent acrylic resin may be less than the other two resins. However, complementary electron microscopic investigations are required to confirm it. Radford et al, in their study concluded that Candida strainshave lower adherence to smooth denture surfaces compared to rough surfaces [13].

The present study results revealed that absorbance of microorganismson the three understudy resins significantly increased over time (from one hour to one week). This finding is in agreement with previous study results indicating a direct correlation between time and absorbance [18, 19].

Various Candida strains have been isolated from cases with Candida stomatitis. The most commonly isolated strains have been C. albicans [10, 20, 21], C. glabrata, C. tropicalis and C. dubliniensis, respectively [10]. In the current study, the highest absorbance belonged to C. dubliniensis followed by C. albicans and C. glabrata. This is especially important considering the fact that C. dubliniensis, used to be identified as C. albicans until recently, has newly been isolated from patients with denture stomatitis and quickly develops resistance to fluconazole. Thus, increased adherence observed in the present study confirms the previous study results and possible role of this microorganism in causing stomatitis [10].

Fungal infections occur when the host provides the necessary environmental and nutritional requirements for adhesion, growth and proliferation of yeasts. Therefore, local and systemic predisposing factors are required for the fungal infection to occur. C. albicans is the most prevalent opportunistic fungal pathogen in the oral cavity; which is usually observed in association with denture stomatitis. C. albicans adheres to denture surfaces and proliferates. Previous in-vitro studies revealed that C. albicans adheres to resins, glasses and metal surfaces. However, its exact mechanism of attachment has yet to be fully understood. Adhesion of C. albicans to polymer surfaces is mediated through the electrostatic and London Van der Waals forces. Involvement of electrostatic and hydrophobic forces in formation of adhesion has reported to be variable based on the type of substrate and environment. However, it seems that these forces are necessary for primary adhesion of fungi providing an opportunity to enhance bonding and plaque accumulation on denture surfaces. Surface characteristics primarily affectthe adhesion, attachment and colonization of microorganisms.

Under in-vitro conditions, adherence of C. albicans to denture surfaces was closely associated with hydrophobicity of microorganisms andevident distribution of physicochemical forces. In hydrophobic surfaces such as in PMMA, monomers are exposed on the surface and attach to the hydrophobic components of proteins through a strong hydro-

phobic bond. Electrostatic forces rank second in terms of importance after hydrophobic forces [11, 12]. Presence of saliva, its pH, presence of serum and other microorganisms along with factors such as surface topography and chemical composition of surface can influence the adherence of C. albicans to acrylic resin surfaces [14].

In the understudy three resin types, adhesion of bacteria (S. mutans) was greater than that of C. albicans and C. glabrata and smaller that that of C. dubliniensis. This finding may be attributed to extracellular polymers. In comparison of bacterial and fungal biofilms, extracellular polymers of yeast biofilmhave lower protein and carbohydrate content and higher levels of glucose and galactose [5].

Another influential factor on adhesion of yeasts to acrylic surfaces is the hydrophilic nature of the microorganism. It has been reported that C. strains are relatively hydrophilic and have greater adhesion to wet (high energy) surfaces [22]. Therefore, saliva coated specimens are more susceptible to Candida adhesion compared to other specimens probably due to the increased surface energy. Candida adherence is responsible for development of denture stomatitis [23-25]. The most critical requirement for successful colonization of microorganisms and subsequent infection is the ability of Candida strains to adhere to surfaces [26]. However, the results of in-vitro studies should be generalized to the clinical setting with caution and consideration of their limitations in simulating clinical conditions. On the other hand, acrylic resins are increasingly used for fabrication, reline, and repair of dentures and no biological analysis is performed prior to their application in clinical setting because there is a general consensus that their application has very limited or negligible health risks for patients. In the present study, degree of biofilm formation on three acrylic resins (Futura Gen, GC Reline hard and Meliodent) was assessed and it was found that Meliodent resin had the highest absorbance. On the other hand, the role of C. dubliniensis, a newly identified strain, was documented and confirmed in biofilm formation and stomatitis [27]. Further complementary studies are required to evaluate biofilm formation in the oral environment and assess other biological and physical properties of acrylic resins such as their cell toxicityparticularly in Futura Gen.

Conclusion

1.The lowest absorbance was observed in Meliodent resin. Futura Gen and GC Reline ranked next, respectively. Absorbance increased over time (from one hour to one week).

2.For S. mutans, the absorbance in the first hour was higher in GC Reline hard than in the other two resins. However, after 24 hours and one week, absorbance of microorganisms in GC Reline and Futura Gen became equal. Among Candida strains, the highest absorbance belonged to C. dubliniensis.

Acknowledgement

The authors would like to express their gratitude to the Research Deputy of Shiraz University of Medical Sciences and the Biomaterial Research Center for funding this manuscript.

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