M D J

Biosurfactant as a preventive agent on acrylic resin

Dr. Inas Abdul Sattar Salman.*

Abstract

Objective. Current study was aimed to evaluate the activity of biosurfactants and other cleansing solutions of the acrylic resin on growth of Candida albicans and Streptococcus mutans.

Study design. Heat cure acrylic resin discs were prepared and precoated with four types of liquids: water, biosurfactant, chlorhexidine mouth wash and denture cleanser. The water was used as control. The discs kept in the solutions overnight after that the antimicrobial assay was tested against Candida albicans and Streptococcus mutans. Also reduction of growth and biofilm formation of C. albicans on denture acrylic discs was tested by using biosurfactant.

Results. Precoating with biosurfactant caused a greater inhibition in the growth of both types of the microorganisms than chlorhexidine and denture cleanser, with inhibition zone diameter 25mm and 15mm against Streptococcus mutans and Candida albicans respectively , 96 % of Candida albicans growth reduction on denture acrylic discs. Also biosurfactant showed reduction in biofilm formation of Candida albicans on denture acrylic discs.

Conclusions. This study demonstrated that biosurfactant was the best preventive agent for acrylic resin compared with other mouth washer and denture cleanser.

Keywords: acrylic resine, biosurfactant, Candida albicans, Streptococcus mutans.

Introduction

Acrylic resin is the plastic material widely used in dentistry to make the denture base. Placement of the removable prosthesis in oral cavity leads to time-related direct and indirect changes in the oral environment. Direct mucosal reactions often result from mechanical irritation together with an accumulation of microbial plaque on the dentures plus an infrequent toxic or allergic reaction to constituents of the denture material.[1] There are several sequelae caused by wearing removable prosthesis from these sequelae is the plaque accumulation.[2]

The porous surface texture of heat-cured acrylic resin favors the accumulation of dental plaque and creates an environment for candida colonization that irritates the denture bearing area.[1]

Candida species are normally present in apparently healthy persons.[3] Wearing of dentures is one of the most important factors affecting candida carriage. The denture cause alteration in the ecological environment of the prosthesis covered area. The denture act as a reservoir and produce good environment for colonization and adhesion of candida. The concentration of exotoxins and metabolic product of fungal colonies may irritate the oral mucosa.[4,5]

* College of Dentistry, Al-Mustansiriyah University.

C.albicans is not the only cause of the denture stomatitis but it is due to biofilms of other microorganisms that may involve S. mutans[6,7]. Streptococcus mutans is one of acrylic denture surfaces members which compete with C. albicans for sites of biding and it can encourage adhesion of yeast.[6]

Several microorganisms (fungi and bacteria) accumulate on the surface of the acrylic denture and may be difficult to clear even by strong mechanical cleansing or chemical compounds.[8] The close association of bacteria and fungi surrounded by their metabolites and components of saliva may be the definition of denture biofilm.[9] When dentures shabby in the mouth a microbial biofilm will develops , which similar to dental plaque. This biofilm result in a condition known as candidiasis. Many wearers of dentures are affected by candida infections in oral association with prosthesis stomatitis[10].

Chlorhexidine (CHX) is one of the disinfectants, which used against candidiasis[11]. Denture cleansers also used in preventing the denture that related stomatitis by controlling the growth of microorganisms on the dentures when the dentures cleaned by these cleansers when they are out of the mouth.

Biosurfactant is biological molecules secluded from microbes and considered a new drugs. They are produced from different bacteria and fungi. The biosurfactant consist of a hydrophilic and hydrophobic component. The hydrophobic non-polar part composed of fatty, saturated or unsaturated acids. The hydrophilic part is consist of carbohydrates, amino acids, cyclic peptides or phosphates[12,13]. There are several applications for the biosurfactants in pharmaceutical, bioremediation, petrochemical, agriculture, cosmetics, detergent and food industries[14].

Biosurfactant in field of pharmaceutical used for stem fibroblast metabolism stimulation [15] and can be a viewed as potential cancer therapeutics[16]. Biosurfactant is considered as antiadhesive biological coatings for medical materials, and to impede the growth of pathogenic biofilm on medical insertional materials, thus reducing infections in hospital and use of chemicals[16,17].

The aim of current study was to determine the antibacterial and antibiofilm activity of biosurfactant against Candida albicans and Streptococcus mutans isolated from patient's mouth and coating hot cure acrylic resin by biosurfactant as a preventive agent.

Materials and Methods

Preparing denture resin discs

Acrylic denture resin discs with 1cm diameter and 3mm thickness were prepared by the use of wax pattern. Wax pattern produced by cutting the cylinder of syringe at a length of 3mm. The proportioning and mixing of the acrylic powder and monomer followed the manufacturer's instructions. Polymerization was carried out by processing the resin at 74^{o}C for 1.5hour and then at 100^{o}C for 30 minutes according to manufacturer's instructions. After complete curing the flask was removed from water bath and allowed to cool slowly for 30 minutes at room temperature then the flask opened and acrylic specimens were removed (each flask contain 4 acrylic discs) (figure 1).

Immersion solutions

Four types of immersion solutions were used: water as control, biosurfactant (produced locally from Leuconostoc mesenteroides

ssp.cremoris, obtained from Biology department, Al-Mustansiriyah University - Iraq), chlorhexidine mouth wash (Listerine/US) and denture cleanser tablet (Lacalut DENT Cleaning tabs/Germany) dissolved in water. The acrylic discs kept overnight in these solutions to simulate the time of keeping dentures out of the patient mouth. At morning, these discs were placed for farther study.

Microorganisms:

Candida albicans and Streptococcus mutans were used in present study obtained from of Biology department - Mustansiriyah University - Iraq)

Antimicrobial assay

Kirby – Bauer method was used for antimicrobial assay of precoated acrylic discs that immersed in several immersion solution included water, biosurfactant, and chlorhexidine mouthwash and denture cleanser. Microbial suspension was prepared then turbidity was adjusted to obtain approximately 0.5 MacFarland standard. Microbial suspension was transferred carefully and evenly spread on De-Man Rogosa Sharpe (MRS) agar for S.mutans and brain heart infusion agar for C. albicans, left to dry completely at room temperature. The precoated PMMA denture acrylic discs were placed on the agar. Later the plates were incubated for 24 h/37^{o}C. Inhibition zones that developed around the discs were measured by millimeter (mm) using a metric ruler.

Reduction of C. albicans growth in denture acrylic discs by biosurfactant:

Reduction of C. albicans growth in denture acrylic discs by biosurfactant solution was determined using Co-culture assay[18].

The candida culture was grown in acrylic discs with biosurfactant solution, the control included candida culture in acrylic discs with brain heart infusion broth. Co-cultures of acrylic discs with biosurfactant solution and control treatment were incubated for 24 h at 37^{o}C. After incubation 1ml of each cultures were serially diluted. Then 0.1ml from each dilution sample was spreader on brain heart infusion agar plates. The plates were incubated at37^{o}C for 24 hrs. Colonies of candida were counted and the inhibitory effect of biosurfactant was evaluated and calculated the percent of reduction of C. albicans using the following equation described as Gosh et al.[19]:

$$R(\%)=[A-B]/A \times 100$$

R: reduction rate, A : number of 85hermop colonies from acrylic disc in control , B: number of 85hermop colonies from acrylic disc treated with biosurfactant

Antibiofilm activity of biosurfactant in denture acrylic discs:

The effect of biosurfactant on formation of C. albicans biofilm in denture acrylic discs was examined. The acrylic discs kept overnight in biosurfactant solution, a control acrylic disc kept in water. At morning these discs were put on filter paper to removed solution and drying at room temperature. The dried disks were put in 10 ml of brain heart infusion broth that inoculated with C. albicans, and then they incubated for 24 h at 37^{o}C. After incubation, the broth was decanted then all discs were stained with crystal violet (for 30 min) at room temperature. After staining acrylic discs were washed to remove excess stain and washed three times with 95% ethanol, then ethanol was collected for measuring the absorbance of each piece at 630nm using spectrophotometer and inhibition of

biofilm formation percentage was determined by using the equation described by Namasivayam et al.[20]

%Inhibition of biofilm formation = $\frac{O.D\ control - O.D\ treatment}{O.D\ control} \times 100$

Control: disc kept in water , treatment: disc kept in biosurfactant

Results

Antimicrobial effect of precoated denture acrylic discs that immersed in several immersion solution included water , biosurfactant, chlorhexidine mouth wash and denture cleanser were determined against S.mutans and C. albicans , the results showed that biosurfactant had the best antimicrobial effect with inhibition zone diameter 25mm and 15mm against S.mutans and C. albicans respectively, followed by chlorhexidine mouth wash with inhibition zone 10mm against S.mutans , while no inhibition recorded against C. albicans (Table 1 , Figure 2).

The candida growth in denture acrylic disc was inhibited by biosurfactant which showed high inhibition effect with 96% reduction of C.albicans growth.

Anti biofilm effect of biosurfactant on denture acrylic disc was observed against C.albicans, with biofilm inhibition 5% .

Discussion

Increased resistance of pathogenic microorganisms against drugs necessity the demand for new antimicrobial and antifungal agents[21.] One of the effective and safe alternative to synthetic medicines is the biosurfactant[22,23].

Lipopeptide biosurfactants are end products of microbes which have antifungal, antibacterial and anti-adhesive properties[23]. Biosurfactant showed inhibitory effect against several pathogenic bacteria[25,26]. Haque et al.[23] showed that 80% of candida albicans growth was inhibited at 60 µg/ml biosurfactant and the complete inhibition of Candida growth was recorded at higher concentration .

Antimicrobial activity mechanisms for many biosurfactants included distortion of the cells and berk of cell membrane integrity[16].

The coating of silicone elastomer disks with a lipopeptide biosurfactant led to the reduction of Candida albicans biofilm formation[28].

The presence of biosurfactant-releasing S.thermophilus B cells on the silicone rubber was effective in reducing adhesion of C. albicans[29]. Biosurfactants from Streptococcus Thermophiles and L. lactis had an inhibitory effect on biofilm formation of pathogenic bacteria on voice prostheses[30]. The pretreatment of polystyrene surfaces and other medical instrument with biosurfactant decreased the adhesion of C. albicans[31]. Ismaeel et al.[32] observed the ability of biosurfactant to biofilm inhibition in contact lenses. Biosurfactant showed inhibition activity against biofilm formation and adherence of gram positive and gram negative bacteria[25].

Also biosurfactant antibiofilm effect on catheters was observed against pathogenic bacteria[26]. The chemical and physical conditions of the biofilm environment changed by the use of biosurfactant in addition to its direct effect against the pathogens[33]. The amphiphilic effect of the biosurfactant cause the accumulation of it at the surface of the acrylic. The first stage of biofilm formation is the adhesion so prevention of the adhesion may lead to reduction of the biofilm formation. The ability of the biosurfactant to adsorb on the acrylic surface may interfere with the microbial adhesion and desorption

processes. Also the molecules of the biosurfactant consist of lipopeptides. So their anti Candidal and antimicrobial activity may be due to the capacity of these molecules to act against the microbs These anti-adhesive and antimicrobial properties make biosurfactant suitable therapeutic choice.

This study demonstrated that biosurfactant was the best preventive agent for acrylic resin compared with other mouth washes and denture cleanser.

References

1- Ferrcane J.L. Materials in Dentistry. Principles and Application. 2nd edition. U.S.A.: Lippincott, Williams and Wilkin. 2001,p:262-5.
2- Zarb G, Hobkirk JA, Eckert SE, Jacob RF. Prosthodontic Treatment for Edentulous Patients: Complete Dentures and Implant-Supported Prostheses. 13th ed. India:Elsevier. 2013,p:44-5.
3- Odds F.C. Ecology of Candida and Epidemiology of Candidosis. In: Candida and Candidosis; a review and bibiliography. London:Balliere Tindall; 1988.p:68-92.
4- Budtz-Jorgensen E. The significance of Candida albicans in denture stomatitis.J Den Res.1974;82:151-52.
5- Davenport JC. The denture surface. Br Dent J. 1987;14:217-18.
6- Baena-Monroy T, Moreno-Maldonado V, Franco-Martinez F, Aldape-Barrios B, Quindos G, Sanchez-Vargas LO. Candida albicans, Staphylococcus aureus and Streptococcus mutans colonization in patient wearing dental prosthesis. Med Oral Cir Bucal. 2005;1:E27-39.
7- Linossier A, Vargas A, Villegas R, Chimenos E. Quantitative relationship between salivary level of Streptococcus mutans and Candida albicans in children with Down's syndrome. Med Oral. 2002;7:84-92.
8- Budtz-Jorgensen E. Etiology, pathogeneses, therapy and prophylaxis of oral yeast infections. Acta Odontal Scand. 1990;48:61-9.
9- Baehni PC, Takeuchi Y. Anti-plaque agents in prevention of biofilm-associated oral diseases. Oral Dis. 2003;9:23-29.
10- Ruby J, Barbeau J. The buccale puzzle:the symbiotic nature of endogenous infections of the oral cavity. Can J Infect Dis. 2002;13:34-41.
11- Andrea cochis, Letizia Fracchia, Maria Giovanna Martinotti, Lia Rimondini. Biosurfactant prevent in vitro Candida albicans biofilm formation on resins and silicon materials for prosthetic devices. Oral medicine J 2012;113:755-761
12- Desai JD, Banat IM. Microbial production of surfactants and their commercial potential. Microbial Mol Biol Rev. 1997;61:47-64.
13- Nitschke M, SGV AO C. Biosurfactants in food industry. Trends Food Sci Technol. 2007;18:252-9.
14- Alahmad K. The Definition, Preparation and Application of Rhamnolipids as Biosurfactants. International Journal of Nutrition and Food Sciences. 2015; 4(6): 613-623.
15- Shoeb E, Akhlaq F, Badar U, Akhter J, Imtiaz S. ClassifIcation and Industrial Applications of Biosurfactant. Natural Appl. Sci. 2013; 4(3): 2223-9944.
16- Gudina EJ, Rangarajan V, Sen R, Rodrigues LR. Potential therapeutic applications of biosurfactants. Trends in pharmacological sciences. 2013; 34: 667–675.
17- Rodrigues LR, Teixeira JA, Van der Mei HC, Oliveira R. Physicochemical and functional characterization of a biosurfactant produced by Lactococcus lactis 53 . Colloids Surf B Biointerfaces. 2006;49(1):79-86.
18- Salman JAS. Antibacterial activity of silver nanoparticles synthetized by Lactobacillus spp. Against Methicillin Resistant-Staphylococcus aureus. International Journal of Advanced Research. 2013;1(6):178-184.
19- Ghosh S, Upadhay A, Singh A, Kumar A. Investigation of antimicrobial activity of silver nanoparticle loaded cotton fabrics which may promote wound healing. International Journal of Pharma and Bio Science. 2010;1(3):1-10.
20- Namasivayam SKR, Preethi M, Bharani ARS, Robin G, Latha B. Biofilm inhibitory effect of silver nanoparticles coated catheter against Staphylococcus aureus and evaluation of its synergistic effects with antibiotics. Int J Biol Pharm Res . 2012;3(2): 259-265.
21- Rivardo F, Turner RJ, Allegrone G, Ceri H, Maritinotti MG. Anti-adhesive activity of two biosurfactants produced by bacillus spp. Prevents biofilm formation of human

bacterial pathogens. Appl Microb Biotechnol. 2009;83:541-53.
22- Harrison JJ, Ceri H, Yerly J, Stremick CA, Hu Y, Martinuzzi R, et al. The use of microscopy and three- dimensional visualization to evaluate the structure of microbial films cultivated in the Calgary biofilm device. Boil Proced Online. 2006;8:194-215.
23- Behal V. Mode of action of microbial bioactive metabolites. Folia Microbial. 2006;51:359-69.
24- Ceresa C, Rinaldi M, Chiono V, Irene C, Gianna A, Letizia F. Lipopeptides from Bacillus subtilis AC7 inhibit adhesion and biofilm formation of Candida albicans on silicone. Anton Van Leeuw. 2016;109:1375–1388.
25- Salman JAS, Alimer DA. Antibacterial and antiadhesive properties of a biosurfactant isolated from lactobacillus Rhamnosus against some bacteria causing Uti in Iraqi women. Int. J. Curr. Res. 2014; 6(3): 5368-5374.
26- Salman JAS, Khudair A. Antibacterial And Antibiofilm Effect of Biosurfactant Produced from Leuconostoc mesenteroides ssp. cremoris against Bacteria from Catheters. World J. of Pharmaceutical Research. 2015; 4(10): 320-333.
27- Haque F, Alfatah Md, Ganesan K, Bhattacharyya MS. Inhibitory Effect of Sophorolipid on Candida albicans Biofilm Formation and Hyphal Growth. Sci. Rep. 2016;6, 23575:1-11.
28- Ceresa C, Rinaldi M, Fracchia L. Synergistic activity of antifungal drugs and lipopeptide AC7 against Candida albicans biofilm on silicone. AIMS Bioengineering. 2017; 4(2): 318-334.
29- Busscher HJ, Van Hoogmoed CG, Geertsema-Doornbusch GI, Van Der Kuijl-Booij M, Van Der Mei HC. Streptococcus thermophilus and its biosurfactants inhibit adhesion byCandida spp. on silicone rubber," Applied and Environmental Microbiology. 1997; 63(10): 3810–7.
30- Rodrigues LR, Van der Mei HC, Teixeira JA, Oliveira R. Influence of biosurfactants from probiotic bacteria on formation of biofilms on voice prosthesis. Appl. Environ. Microbiol. 2004; 70(7): 4408–4410.
31- Janek T, Łukaszewicz M, Krasowska A. Antiadhesive activity of the biosurfactant pseudofactin II secreted by the Arctic bacterium Pseudomonas fluorescens BD5. BMC Microbiology. 2012; 12(1): 24.
32- Ismaeel MCh, Ibrahim KM, Al-Malikey MKh. The effect of surlactin produced by Lactobacillus acidophilus on eye infectious bacteria in rabbits. J. Baghdad for Sci. 2013;10(1):133-143.
33- Kiran GS, Sabarathnam B, Selvin J. Biofilm disruption potential of glycolipid biosurfactant from marine Brevibacterium casei. FEMS Immunol Med Microbial 2010;59:432-8.

Table 1: Antimicrobial effect of precoated PMMA denture acrylic discs

Immersion solution	**Inhibition zone (mm)**	
	Streptococcus mutans	**Candida albicans**
Water	0	0
Biosurfactant	25	15
Chlorhexidine mouth wash	10	0
Denture cleanser	0	0

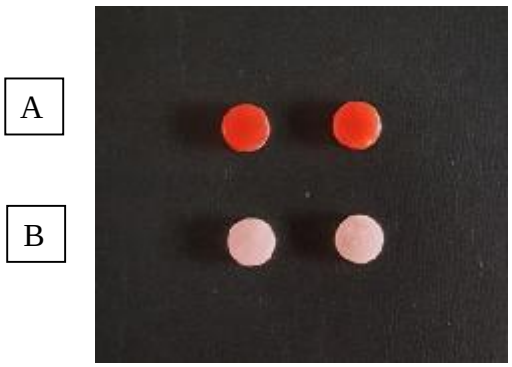

Figure 1 : A: the wax patterns for acrylic discs preparation.
B: acrylic discs after preparation.

-A- -B-

Figure 2: Antimicrobial effect of precoated PMMA denture acrylic discs
1 acrylic disc immersed in biosurfactant
2 acrylic disc immersed in chlorhexidine mouth wash
3 acrylic disc immersed in denture cleanser
4 acrylic disc immersed in water
A: against Streptococcus mutans B: against Candida albicans