ประสิทธิภาพของวิธีการทำความสะอาดที่แตกต่างกัน ในการกำจัดเชื้อแคนดิดา อัลบิแคนส์ ออกจากฐานฟันเทียมชนิดอะคริลิก

THE EFFICACY OF DIFFERENT CLEANING METHODS IN REMOVING CANDIDA ALBICANS FROM ACRYLIC DENTURE BASE

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บทคัดย่อ

สุขอนามัยของฟันเทียมมีส่วนสำคัญในการลดความเสี่ยงการเกิดปากอักเสบเหตุฟันเทียม วิธีการทำความสะอาดฟันเทียมมีหลายวิธี แต่ไม่มีการระบุแน่ชัดว่าวิธีใดเหมาะสมที่จะใช้เป็นข้อกำหนดการทำความสะอาดฟันเทียม วัตถุประสงค์ของการศึกษาคือเปรียบเทียบประสิทธิภาพของวิธีการทำความสะอาดฟันเทียมด้วยสารเคมี โดยเฉพาะการกำจัดเชื้อแคนดิดา อัลบิแคนส์ ออกจากฐานฟันเทียมชนิดอะคริลิก โดยเปรียบเทียบเซลล์มีชีวิตที่เหลืออยู่หลังการทำความสะอาด วิธีทดสอบ เตรียมชิ้นงานอะคริลิกเรซิน 48 ชิ้น นำไปเพาะเชื้อแคนดิดา อัลบิแคนส์ ที่อยู่ในรูปของสารละลาย 1 มิลลิลิตร ในถาดหลุมเพาะเลี้ยง 24 หลุม ที่อุณหภูมิ 37 องศาเซลเซียส 24 ชั่วโมง เพื่อให้เกิดไบโอฟิล์ม จากนั้นนำชิ้นงานแช่ในกลุ่มการทดลองทั้งหมด 8 กลุ่ม ได้แก่ น้ำเปล่า 1 และ 12 ชั่วโมง, โพลิเดนท์ 15 นาที, 1 และ 12 ชั่วโมง และ 0.2% คลอร์เฮ็กซิดีน 15 นาที, 1 และ 12 ชั่วโมง เชื้อมีชีวิตที่เหลืออยู่หลังการทำความสะอาดถูกวัดด้วยค่าความขุ่นและคำนวณเป็นร้อยละเพื่อวิเคราะห์ทางสถิติ ผลการทดลองพบว่า 0.2% คลอร์เฮ็กซิดีน ที่แช่เป็นเวลา 12 ชั่วโมง มีประสิทธิภาพดีที่สุดอย่างมีนัยสำคัญทางสถิติ เมื่อเปรียบเทียบกับทุกกลุ่มการทดลอง ร้อยละของเซลล์ที่เหลืออยู่หลังการทำความสะอาด คือ 13.10±3.15% ($p < 0.05$) จากผลการทดลองสรุปว่า 0.2% คลอร์เฮ็กซิดีน สามารถใช้ทำความสะอาดฟันเทียมเพื่อลดจำนวนเชื้อแคนดิดา อัลบิแคนส์ และยังมีผลรักษาและป้องกันการเกิดปากอักเสบเหตุฟันปลอมร่วมด้วย

คำสำคัญ: ฐานฟันเทียมชนิดอะคริลิก, แคนดิดา อัลบิแคนส์, ปากอักเสบเหตุฟันปลอม, วิธีการทำความสะอาดฟันเทียม

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Abstract

The proper denture hygiene is important to reduce the risk of denture stomatitis. There are several denture cleaning methods recommended to patients, however it is unclear which is the appropriate denture-cleaning regimen. The purpose of this study was to compare the efficacy of cleaning methods in removing *Candida albicans* from acrylic denture base by compared the remaining viable cells after cleaning. The total of 48 acrylic resin specimens were prepared. *Candida albicans* was incubated and cultured as fungal solutions. All specimens were randomly placed in 24-well tissue culture plate with 1 milliliter of fungal solutions for biofilm formation at 37 degree Celsius for 24 hours. After biofilm formations, all specimens were randomly immersed in eight experimental groups of cleaning methods (distilled water as the negative controls 1 hour and 12 hour, Polident® 5 minutes, 1 hour and 12 hours and 0.2% chlorhexidine 15 minutes, 1 hour and 12 hours). The viable cells of *Candida albicans* after cleaning were determined as optical density and the calculated into the percentage for statistically analysis. The results showed that the cleaning method which had the highest efficacy to remove *Candida albicans* from acrylic denture base was using 0.2% chlorhexidine with 12-hours immersion time compared with other experimental groups, the percentage of viable cells after this cleaning method was 13.10±3.15% ($p < 0.05$). The results of this study can be concluded that 0.2% chlorhexidine can used as a routine denture cleanser to reduce *C. albicans* and it also had the therapeutic effect for treated and prevented denture stomatitis.

Keywords: acrylic denture base, *Candida albicans*, denture stomatitis, denture cleaning methods

Introduction

One of the most common dental problems that is usually found in geriatric patients is tooth loss form many etiologies such as poor oral hygiene, improper dental functions and become partially or completely edentulous. Treatment options for replacing the extracted teeth are either removable or fixed prosthesis including dental implant. Removable prosthesis is one of dental treatments for edentulous patients to replace the missing teeth and improve their chewing, phonetics, esthetics and facial appearance. Under the Thai dental health insurance system, acrylic resin-based denture is commonly selected more than metal-based denture with the reason of lower service charge, acceptable function and appearance by patients.

However, acrylic resin cannot be an ideal material for denture base because of its disadvantages, especially the biological effects. The porosities and surface roughness are the disadvantages of acrylic resins that allow microbial accumulation, especially on tissue surface of denture base. The accumulation and colonization of microorganisms with the involvement of *Candida albicans* is the cause of "denture stomatitis". [1, 2]

The etiology of denture stomatitis is multifactorial. Those have been reported include ill-fitting denture causing mucosal trauma, increasing age of dentures and denture wearers, infection from bacteria and fungi, and poor oral hygiene [3]. There are several denture cleaning methods recommended to patients, however it is unclear which is the appropriate denture-cleaning regimen.

Mechanical and chemical cleaning methods are usually advised to patients to clean plaque and debris from the dentures. Several studies suggested denture cleaning methods to remove *Candida albicans* from acrylic resin denture base, such as cleaning with running water and brush daily, soaking in a denture cleaning solution such as Polident® or soaking in chlorhexidine solution. There are evidences showing that only mechanical cleaning is insufficient to remove denture plaque, so chemical cleaning is needed. [4] However, it cannot be summarized which one is the best cleaning method and there is a lack of evidence about comparative effectiveness of cleaning in acrylic denture base. Accordingly, the aim of this study is to compare the efficacy of denture cleaning methods in removing *Candida albicans* from acrylic resin denture base.

Materials and Methods

Specimen preparation

Cylindrical clear acrylic resin (Rodex, SPD, Italy) with diameter 15 mm and 20 mm in length were fabricated by the loss-wax technique with the cylindrical silicone in the plaster stone mold and metal flask. Heat-activated acrylic resin was mixed according to manufacturing's recommendation and pack into a flask with 1200 Psi of hydraulic press. Acrylic resins were polymerized with conventional heat method. Specimens were deflasked after cooled overnight at room temperature, then finishing and polishing the specimens with standard procedures. Cylindrical acrylic resins were cut into 2 mm of thickness by low speed cutting machine (IsoMet™ Low Speed, Beuhler,USA) using 200 rpm of speed and 50 g load then polished with sand paper number 500, 800 and 1000, respectively. Before culturing with *C. albicans*, all specimens were disinfected in 70% alcohol for 10 minutes, washing with distilled water and then sterilized with ethylene gas.

Candida albicans cultured and biofilm formation on specimen

C. albicans (SC5314) from the frozen stock was cultured on yeast peptone dextrose (YPD) agar and incubated at 30°c for 48 hours in the incubator (MyTemp™, Benckmark Scientific, USA). A single colony of this culture was inoculated into 10 ml of liquid YPD and incubated at 30°c overnight in the orbital shaker (WiseCube®, CS witeg, Germany). After that, the cultures were adjusted to optical density (OD) 0.1. The cultures were incubated for 4-6 hours until log phase at OD 0.4-0.6 at 600 nm as measured by spectrophotometer (GENESYS™ 20, Thermo Fisher Scientific, USA). The cell suspension was adjusted for the following experiments.

Each acrylic resin specimen was placed in 24-well tissue culture plates with 1 ml of *C. albicans* suspension and incubated at 37°c for 24 hours. All procedures were carried out in a biological safety cabinet.

Each contaminated specimen (n=48) will be randomly place in a new 24-well tissue culture plate and randomly assign to one of the following cleaning methods (n=6 per group) by immersing the specimen in denture cleaning solutions as listed. There are total 8 experimental groups including negative control as the following and summarized in the Table 1.

Group I: distilled water for 1 hour (negative control for 1 hour, 5 minutes and 15 minutes experimental groups)

Group II: distilled water for 12 hours (negative control for 12 hours experimental groups)

Group III: distilled water with denture cleansing tablet (Polident®, GlaxoSmithKline, Thailand) for 5 minutes, following the manufacturing's instructions

Group IV: distilled water with denture cleansing tablet (Polident®, GlaxoSmithKline, Thailand) for 1 hour

Group V: distilled water with denture cleansing tablet (Polident®, GlaxoSmithKline, Thailand) for 12 hours

Group VI: 0.2% chlorhexidine (Faculty of Dentistry, Chulalongkorn University, Thailand) for 15 minutes

Group VII: 0.2% chlorhexidine (Faculty of Dentistry, Chulalongkorn University, Thailand) for 1 hour

Group VIII: 0.2% chlorhexidine (Faculty of Dentistry, Chulalongkorn University, Thailand) for 12 hours

Table 1 Experimental groups

Group	Cleaning Methods	Durations
I	distilled water (negative control)	1 hour
II	distilled water (negative control)	12 hours
III	distilled water with denture cleansing tablet (Polident®)	5 minutes
IV	distilled water with denture cleansing tablet (Polident®)	1 hour
V	distilled water with denture cleansing tablet (Polident®)	12 hours
VI	0.2% chlorhexidine	15 minutes
VII	0.2% chlorhexidine	1 hour
VIII	0.2% chlorhexidine	12 hours

MTT colorimetric assay

MTT colorimetric assay is the cleavage of MTT (3-(4, 5-dimthylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) into the formazan crystals which has a purple color by mitochondrial activity of living cells enzyme. The amount of formazan is corresponded with the number of viable cells.

To examine the viable cells of *C. albicans* in acrylic resin after cleaning, MTT solutions were prepared and warmed at 37°c before use. Each specimen was incubated with MTT at 37°c for 3 hours. The formazan crystals were formed in the viable cells and were dissolved by submerged in dimethyl sulfoxides (DMSO). The optical density (OD) of the solutions were determined at 540 nm using microplate spectrophotometer (Epoch 2, BioTek®, USA). Dimethyl sulfoxides without specimen was used for the blank control. The OD of viable cells after cleaning were calculated into the percentage as equation:

% of viable cells = (OD of each specimen/ mean of control group) x 100

Statistical analysis

All statistical computations were performed by SPSS software (IBM SPSS statistics for windows, version 22.0). The percentage of viable cells after cleaning compared with the negative control groups were presented as means and standard deviations. Normality of the data will be determined by Shapiro-Wilk then the data was determined by one-way of ANOVA followed by Tukey post-hoc test. A p-value < 0.05 was considered statistically significant.

Results

The means and standard deviations of the percentage of viable cells after cleaning were presented in Table 2. The results of Group IV and V (Polident® 1 hour and 12 hours, respectively) had significantly different compared with both 1–hour and 12–hours negative controls ($p < 0.05$), however Group III (Polident® 5 minutes) had no significantly different compared with all negative controls ($p > 0.05$). There were no significant difference between all of Polident® experimental groups ($p > 0.05$). When compared Polident® and 0.2% chlorhexidine experimental groups, the results showed that Group III (Polident® 5 minutes) had significantly different when compared with all Group of 0.2% chlorhexidine ($p < 0.05$), Group IV (Polident® 1 hour) had no significantly different with Group VI (0.2% chlorhexidine 15 minutes) and VII (0.2% chlorhexidine 1 hour) ($p > 0.05$) ,but it had significantly different with Group VIII (0.2% chlorhexidine 12 hours) ($p < 0.05$) and the results of Group V (Polident® 12 hours) had significantly different with Group VI (0.2% chlorhexidine 15 minutes) ($p > 0.05$), but it had significantly different with Group VII (0.2% chlorhexidine 1 hour) and VIII (0.2% chlorhexidine 12 hours) ($p < 0.05$). For all of 0.2% chlorhexidine experimental groups their results had significantly different when compared with all negative controls ($p < 0.05$). The results between Group VI (0.2% chlorhexidine 15 minutes) and VII (0.2% chlorhexidine 1 hour had no significantly different ($p > 0.05$). For Group VII (0.2% chlorhexidine 12 hours), it had significantly different when compared with negative controls and all others experimental groups, the results showed that it had the lowest percentage of viable cells after cleaning, therefore it had the highest efficacy to removed *C. albicans* from acrylic denture base.

Table 2 The percentage of viable cells after cleaning

Group	Cleaning Methods	Mean±SD* (%)
I	distilled water 1 hour (negative control)	100.00±5.88[a]
II	distilled water 12 hours (negative control)	100.00±5.88[a, b]
III	distilled water with denture cleansing tablet (Polident®) 5 minutes	89.43±3.83[a, b, c]
IV	distilled water with denture cleansing tablet (Polident®) 1 hour	79.50±7.40[c, d]
V	distilled water with denture cleansing tablet (Polident®) 12 hours	84.60±10.37[c, d, e]
VI	0.2% chlorhexidine 15 mins	76.90±9.43[d, e, f]
VII	0.2% chlorhexidine 1 hours	67.96±3.30[d, f]
VIII	0.2% chlorhexidine 12 hours	13.10±3.15

*No significant difference ($p > 0.05$) between groups which noted by the same superscript

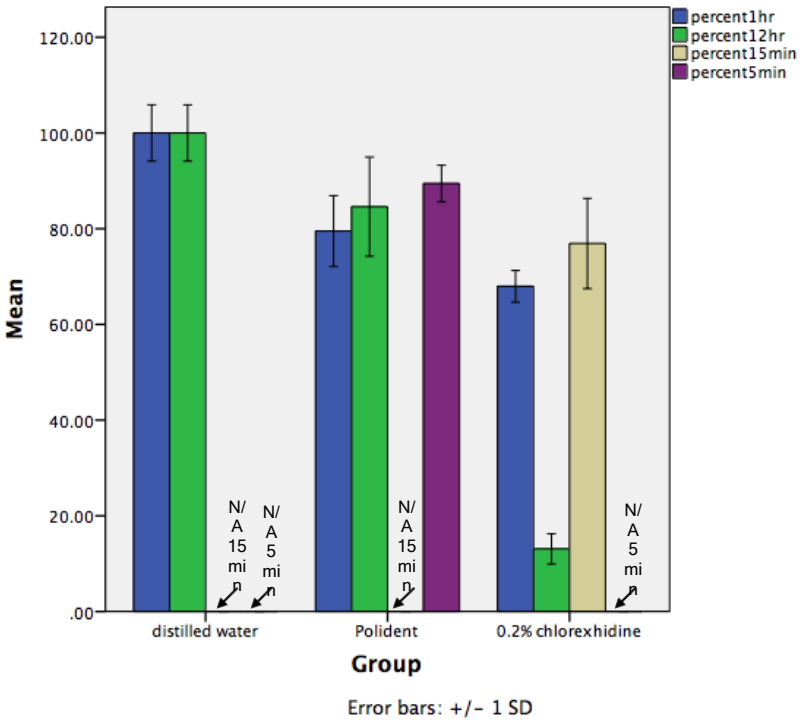

Figure 1 showed the percentage of viable cells after cleaning

Discussion and Conclusion

Denture stomatitis is one of the three most common denture-related problems of Thai geriatric patients. [5] Poor denture care and hygiene is one of multifactorial etiologies of denture stomatitis and it also the critical risk due to the promoting of anaerobic and low pH condition that lead to opportunistic growth of pathogens such as *C. albicans*. [3] As mentioned above, the proper maintenance of denture hygiene is significant for reducing the risk of microbial infection in denture wearers.

This study used denture cleansing tablet (Polident®) which is commonly available in market and 0.2% chlorhexidine as the denture cleanser. Polident® was classed in alkaline peroxides denture cleanser, the oxygen-liberating mechanism of alkaline peroxides was loosened the debris and biofilm and it also removed the light stain. The effervescent effect of alkaline peroxides produced hydrogen peroxides which contained of active oxygen when contacted with water, this effect had important role to removing debris and antimicrobial from oxygen. [6,7,8] The effect of hydrogen peroxides to *C. albicans* is to induce the hyphal differentiation and the increased amount of hydrogen peroxides is the contrast of the biofilm growth situations which occurred in anaerobic conditions. [7,9] This study supported the several previous studies that Polident® can reduced *C. albicans* compared with distilled water, the distilled water referred as patients had no any cleaning their dentures and it would lead to the poor denture hygiene. [7,10] The manufacturer's instructions of Polident® used in this study was 15 minutes. There have been several reports about the immersion time of alkaline peroxides denture cleansers. The study of Shay [11] showed that 15, 30 and 60 minutes of alkaline peroxides immersion time were insufficient, and the study stated that the overnight used of denture cleansers would had more effective. The used of alkaline peroxides denture cleanser did not alter the properties of acrylic resins. [12] There was the report supported

Shay's study, it found that the used of alkaline peroxides denture cleanser for 60 minutes immersion time can be reduced the amount *C. albicans*, but it was not completely removed. [13] On the other hand, the results of overnight used of alkaline peroxides denture cleanser (Polident®) from the present study did not supported the previous studies, there had no different efficacy between 60-minutes and overnight immersion time due to the limited time of effervescent effect.

Another denture cleanser used in this study is 0.2% chlorhexidine which classed in disinfectant cleansers. Disinfectants used for treatment and prevent fungal infection beneath the removeable prosthesis, it not commercially found as alkaline peroxides denture cleanser. [7] Chlorhexidine is widely used for against the wide range of organisms included *C. albicans*. The antimicrobial effect of chlorhexidine is from its positive charged bind to the negative charged of cell wall, then the leakage of cell substances was initiated. [14,15,16,17] When chlorhexidine was exposed to *C.* albicans, the loosened fragment of the cell wall would occur. [15] There were the reported mentioned that chlorhexidine can be used as immersion solution to reduced microbial growth on dental prosthesis and it is also used as a denture cleansing to reduced biofilm. [14, 18] McCourtie, et al [15] demonstrated that the pretreatment of chlorhexidine to acrylic was reduced the adherence of *C. albicans*. The study of Pusateri, et al [17] showed that the biofilm of *C. albicans* on acrylic denture was sensitive to be killed and inhibited growth by chlorhexidine, the study stated that chlorhexidine was the therapeutic application.

The results of this study showed that 0.2% chlorhexidine had more efficacy than Polident® to removed *C. albicans* from acrylic denture base. Therefore, it described that the antimicrobial effect of chlorhexidine was play the important role to reduce the viability of *C. albicans*, whereas Polident® not had this property. In addition, 0.2% chlorhexidine with 12-hours immersion time had the highest efficacy to remove *C. albicans* in this study, it summarized that the long immersion had more antimicrobial effect.

From the results of this study, 0.2% chlorhexidine can used as a routine denture cleanser to reduce *C. albicans* and it also had the therapeutic effect for treated and prevented denture stomatitis.

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