Clinical and Experimental Health Sciences

Antimicrobial Effect of Polyhexanide on Denture Base and Soft Lining Materials

Dogukan Yilmaz[1], Hakan Akin[2]

[1] Department of Periodontology, Faculty of Dentistry, Sakarya University, Sakarya, Türkiye.
[2] Department of Prosthodontics, Faculty of Dentistry, Sakarya University, Sakarya, Türkiye.

Correspondence Author: Dogukan Yilmaz
E-mail: dogukanyilmaz@sakarya.edu.tr

Received: 30.11.2020 **Accepted:** 06.12.2021

ABSTRACT

Objective: Polyhexanide (PHMB; polyhexamethylene biguanide) is a well-known antiseptic agent; however, no data exist for its application on denture base and lining materials. In the present in vitro study, the aim was to compare viable bacterial or fungal cell numbers and their adhesion on different types of denture base and lining materials in diverse concentrations of PHMB.

Methods: Light-activated urethane dimethacrylate (UDMA), heat-polymerized polymethyl methacrylate (PMMA), heat-polymerized polydimethylsiloxane, and autopolymerizing polyethylmethacrylate discs were prepared for each group (n = 10). 1×10^8 CFU/mL of all the tested species were appended separately to discs, and they were immersed into different PHMB suspensions (0.5%, 1%, 2%, and 5%) for 10 minutes. The antimicrobial activity and number of adherent species on the surface were evaluated.

Results: In the PMMA group, all studied species except *C. albicans, L. acidophilus*, and *S. aureus* were decreased in various concentrations ($p < 0.05$), and all studied species presented a significant decrease in every concentration of PHMB in the UDMA group ($p < 0.01$) in comparison to the control. *N. sicca, K. pneumoniae, S. pyogenes, S. sanguis, C. pseudotuberculosis*, and *S. aureus* ($p < 0.05$) were reduced in the heat-polymerized polydimethylsiloxane group, while all tested species except *B. subtilis* were decreased in the autopolymerizing polyethylmethacrylate group in comparison to the control ($p < 0.01$). Among all tested materials and species, no significant difference was detected in adherent cell number ($p > 0.05$).

Conclusion: PHMB suspension, in various concentrations, can reduce some species of bacterial and yeast cells.

Keywords: Disinfectant, bacterial growth, denture adhesive, denture base.

1. INTRODUCTION

Removable prostheses are still a treatment of choice in cases of edentulism, especially in the older population. Sometimes the fundamental rules may not be sufficient for ideal denture retention and stability, and soft denture lining materials are identified as a viable option to overcome this problem (1). Inadequate oral hygiene related to biofilm formation and plaque accumulation is one of the major problems for denture base and soft lining materials (2). These materials are easily colonized by microorganisms, including pathogenic and opportunistic bacteria and fungi (3,4). The biofilm formation and adhesion of microorganisms on denture base and soft lining materials depend on several factors, including surface roughness, free energy, and hydrophobicity (5,6). This formation and accumulation may lead to tissue inflammation in the oral mucosa and is identified as denture-related stomatitis, which can be associated with a burning sensation, bleeding, unpleasant taste, and halitosis (7).

To overcome this problem, prostheses can be cleaned mechanically, chemically, or through a combination of both (8). Mechanical cleaning may not be as effective as demanded due to the irregular surface texture of the dentures and the limited hand manipulation of the senior population (8,9). According to the literature, different chemical agents have been studied to disinfect denture base and soft materials; however, none of them has demonstrated superiority over any other (10-14). Furthermore, some adverse effects, including allergic reactions and oral mucosal tissue irritations, due to toxic ingredients of related disinfectants have been reported. These toxic ingredients could become incorporated into the denture base and lining materials (15,16).

Polyhexanide (polyhexamethylene biguanide; PHMB) is a biocidal cationic polymer (17). It is an antiseptic agent that has been used for many years in different applications in medicine for its broad antibacterial and antifungal activity (17-19). It has been commonly accepted that the antimicrobial activity is due to the ability of PHMB to perforate the bacterial phospholipid membrane, leading ultimately to bacterial death (20). It is one

DOI: 10.33808/clinexphealthsci.833576

of the most promising agents with low cytotoxicity and high tissue compatibility (20,21). Studies have indicated that PHMB inhibits plaque regrowth and reduces oral bacterial count, thus it may be a good option for preventive applications (22,23). To the best of the author's knowledge, despite the advantages of PHMB, no data currently exists in the literature regarding its application as a disinfectant agent on denture base and lining materials. In the present in vitro study, the aim was to compare viable bacterial or fungal cells and their adhesion on different types of denture base and lining materials in diverse concentrations of PHMB. The null hypothesis that PHMB has a reduced tendency for viable cell number and the adhesion of bacterial or fungal microorganisms on denture base and soft lining materials was tested.

2. METHODS

In this study, light-activated urethane dimethacrylate (UDMA) denture base material (Eclipse, Dentsply Trubyte, York, PA, USA), heat-polymerized polymethyl methacrylate (PMMA) denture base material (Meliodent, Bayer Dental, Newbury, UK), heat-polymerized polydimethylsiloxane (Molloplast® B, Detax GmbH & Co., Ettingen, Germany) soft lining material, and autopolymerizing polyethylmethacrylate (Visco-gel, Dentsply Trubyte, York, PA, USA) soft lining material were tested.

2.1. Specimen preparation

Specimens for each material were fabricated by investing wax patterns (12 × 12 × 3 mm) in a cylindric-shaped stone mold. The heat-cure PMMA resin specimens were prepared within a dental flask and cured in a manner similar to that used in conventional denture construction according to the manufacturer's instructions.

UDMA specimens were preheated for 2 minutes in a special oven (Eclipse Conditioning Oven, Dentsply Trubyte, York, PA, USA) to 55 ºC. A separating agent (Al-Cote, Dentsply Trubyte, York, PA, USA) was then applied onto the stone mold, and the warmed resin was adapted into the mold by using finger pressure. After cooling, specimens were removed from the mold, and remnants were removed. To prevent inhibition of polymerization by oxygen, the specimens were warmed in a 55 ºC oven (Eclipse Conditioning Oven, Dentsply Trubyte, York, PA, USA) for 1 hour, and coated with an air barrier coating (Eclipse ABC, Dentsply Trubyte, York, PA, USA). Then, specimens were processed in a light-processing unit (Eclipse Processing Unit, Dentsply Trubyte, York, PA, USA) for 10 minutes. At the end of polymerization, any excess resin was removed using finer grades of silicon carbide paper (320, 600, and 1200 grit), and the polishing was performed with a felt wheel and diamond paste.

Soft lining material samples were prepared according to the manufacturer's instructions. To explain briefly, a separator was applied where bonding was not desired. Temporary soft denture liners were packed into molds and closed under pressure. Polymerization was achieved, in a water bath at 100 ºC for 2 hours for heat-polymerized polydimethylsiloxane, and proximately for 2 or 3 minutes from the start of mixing for the autopolymerization of polyethylmethacrylate. Undesired excess resin was trimmed by sharp instruments. Polyhexanide (Fagron GmbH & Co. KG, Hamburg, Germany) was diluted in water of standardized hardness (WSH; according to DIN EN 1040) to the final test concentrations. All further dilutions were prepared with 40% DMSO/WSH. The suitability as solvent of 40% DMSO/WSH regarding inefficacy was demonstrated using the quantitative suspension test as well as the microdilution test, according to Koburger et al. (24).

2.2. Cell culture and adherence assay

In order to assess bacterial and fungal adhesion, denture base and soft lining disc specimens (n = 10) were sterilized with ultraviolet light and kept in Petri dishes. The bacterial and fungal strains used in this study are presented in Table 1. In the present study, the cell culture and adherence assays were modified and performed according to Pavan et al.'s technique (25). Briefly, bacterial strains were grown onto Mueller Hinton agar (CM337, Oxoid Deutschland GmbH, Wesel, Germany) plates at 37 ºC. Fungal strains were grown onto Sabouraud dextrose agar (CM41, Oxoid Deutschland GmbH) at 30 ºC. 0.5 McFarland suspensions corresponding to $1x10^8$ CFU/mL of all the tested bacteria and fungi were appended separately to all test discs. Discs were placed into the incubators (EN400, NÜVE, Ankara, Turkey) at 90% humidity and 37 ºC. For fungal suspensions, each disc was incubated at 90% humidity and 30 ºC. After 12 h, 24 h, and 36 h of incubation, discs were immersed into PHMB suspensions of 0.5%, 1%, 2%, and 5% concentrations for 10 minutes. The disks were removed from the PHMB suspensions and transferred to microtubes containing sterile saline, and after vortexing, the suspensions were diluted and plated into the blood agar base (Merck KGaA, Darmstadt, Germany) to define the number of bacteria or fungi, and their quantity was determined by counting the colonies.

Moreover, 0.5 McFarland suspensions of all the tested bacteria and fungi were placed into the remaining discs, and then kept in the incubator for 6 h. To remove the loosely adherent cells, the specimens' surfaces were gently rinsed with 1 mL of phosphate-buffered saline (PBS; Sigma-Aldrich, St. Louis, MO, USA) at a distance of 10 cm for 1 min. Adherent micro-organisms on the surface of the specimens were counted in blood agar plates (Merck KGaA).

Table 1. *The tested bacterial and fungal strains in present study*

Number	Names	Codes	Gram Stain	Morphology
1	*Neisseria sicca*	ATCC-9913	Gr (-)	Diplococcus
2	*Streptococcus mutans*	ATCC-21752	Gr (+)	Coccus
3	*Klebsiella pneumoniae*	ATCC-10031	Gr (-)	Bacillus
4	*Bacillus subtilis*	ATCC-6633	Gr (+)	Bacillus
5	*Streptococcus pyogenes*	ATCC-19615	Gr (+)	Coccus
6	*Candida albicans*	ATCC-10231	Gr (+)	Yeast
7	*Lactobacillus acidophilus*	ATCC-11975	Gr (+)	Bacillus
8	*Streptococcus sanguis*	ATCC-10557	Gr (+)	Coccus
9	*Proteus vulgaris*	ATCC-7829	Gr (-)	Bacillus
10	*Corynebacterium pseudotuberculosis*	ATCC-19410	Gr (+)	Bacillus
11	*Escherichia coli*	ATCC-11229	Gr (-)	Bacillus
12	*Candida tropicalis*	ATCC-750	Gr (+)	Yeast
13	*Staphylococcus aureus*	ATCC-25923	Gr (+)	Coccus

2.3. Statistical analysis

In the post-hoc power analysis, the alpha error was accepted as 0.05 in order to control Type I error, and the power ranged from 77.1% to 99.9%. This analysis was performed by G* Power 3.0.10 Franz (Faul, Universitat Kiel, Kiel, Germany). Statistical analysis was performed using one-way analysis of variance (ANOVA), and wherever appropriate, subsequent post-hoc analysis was performed using the Tukey test (a = 0.05). Log-transformed values were used due to positively skewed distribution. Statistical data were processed using IBM SPSS 24.0 software (Armonk, NY, USA) for Windows. The statistical significance was set as 0.05.

3. RESULTS

The viable cell number of related microorganisms on the denture base and soft lining materials, after immersion in different concentrations of PHMB suspensions, are presented in Tables 2 through 5. To state the results briefly, in PMMA samples, the number of *Candida albicans*, *Lactobacillus acidophilus*, and *Staphylococcus aureus* species did not demonstrate any significant difference in suspensions of PHMB with different concentrations ($p > 0.05$). In contrast, in UDMA samples, the viable cell number of all studied microorganisms presented a significant decrease in every concentration of PHMB suspension in comparison to the control samples ($p < 0.01$). Among the dental liner specimens, in heat-polymerized polydimethylsiloxane, different concentrations of PHMB suspension reduced the number of *Neisseria sicca* ($p < 0.05$), *Klebsiella pneumoniae* ($p < 0.05$), *Streptococcus pyogenes* ($p < 0.05$), *Streptococcus sanguis* ($p < 0.05$), *Corynebacterium pseudotuberculosis* ($p < 0.05$), and *Staphylococcus aureus* ($p < 0.05$). PHMB suspensions with different concentrations decreased all tested microorganism species except *Bacillus subtilis* in autopolymerizing polyethylmethacrylate samples in comparison to the control suspension ($p < 0.01$). No significant difference was observed between the different concentrations of PHMB suspensions in the reduction of viable bacteria and yeast numbers on tested denture base and soft lining materials ($p > 0.05$).

Table 2. *Viable cell number of related microorganisms on PMMA samples that immersed into PHMB suspensions of 0.5%, 1%, 2%, 5% concentration for 10 minutes.*

	PMMA n:10														
	12h					24h					36h				
	0.5 %	**1%**	**2%**	**5%**	**C**	**0.5%**	**1%**	**2%**	**5%**	**C**	**0.5%**	**1%**	**2%**	**5%**	**C**
N. sicca	10^6	$\mathbf{10^3}$	$\mathbf{10^2}$	**0**	$\mathbf{10^5}$	10^4	$\mathbf{10^2}$	$\mathbf{10^2}$	**0**	$\mathbf{10^5}$	10^4	$\mathbf{10^1}$	$\mathbf{10^1}$	**0**	$\mathbf{10^6}$
S. mutans	10^6	$\mathbf{10^2}$	$\mathbf{10^2}$	**0**	$\mathbf{10^5}$	10^5	$\mathbf{10^2}$	$\mathbf{10^2}$	**0**	$\mathbf{10^5}$	10^4	$\mathbf{10^1}$	$\mathbf{10^1}$	**0**	$\mathbf{10^6}$
K. pneumonia	10^7	10^6	10^5	$\mathbf{10^2}$	$\mathbf{10^5}$	10^6	10^5	10^4	$\mathbf{10^1}$	$\mathbf{10^6}$	10^5	10^4	10^3	$\mathbf{10^1}$	$\mathbf{10^7}$
B. subtilis	$\mathbf{10^7}$	$\mathbf{10^6}$	$\mathbf{10^1}$	$\mathbf{10^1}$	$\mathbf{10^4}$	$\mathbf{10^2}$	$\mathbf{10^2}$	$\mathbf{10^1}$	$\mathbf{10^1}$	$\mathbf{10^4}$	$\mathbf{10^2}$	$\mathbf{10^1}$	**0**	**0**	$\mathbf{10^7}$
S. pyogenes	10^6	$\mathbf{10^2}$	$\mathbf{10^2}$	**0**	$\mathbf{10^4}$	10^5	$\mathbf{10^2}$	$\mathbf{10^2}$	**0**	$\mathbf{10^5}$	10^4	$\mathbf{10^1}$	$\mathbf{10^1}$	**0**	$\mathbf{10^6}$
C. albicans	10^5	10^2	10^2	0	10^3	10^1	0	0	0	10^3	0	0	0	0	10^7
L. acidophilus	10^6	10^3	10^2	0	10^3	10^2	10^2	10^2	0	10^3	10^2	10^2	10^1	0	10^6
S. sanguis	10^6	10^6	**0**	**0**	$\mathbf{10^4}$	10^4	10^4	**0**	**0**	$\mathbf{10^4}$	10^2	10^2	**0**	**0**	$\mathbf{10^7}$
P. vulga	10^7	10^7	$\mathbf{10^5}$	**0**	$\mathbf{10^6}$	10^6	10^5	**0**	**0**	$\mathbf{10^7}$	10^5	10^4	**0**	**0**	$\mathbf{10^7}$
C. pseudotuberculosis	10^7	10^5	10^5	$\mathbf{10^2}$	$\mathbf{10^5}$	10^6	10^4	10^4	$\mathbf{10^1}$	$\mathbf{10^6}$	10^5	10^4	10^3	$\mathbf{10^1}$	$\mathbf{10^7}$
E. coli	$\mathbf{10^5}$	$\mathbf{10^3}$	$\mathbf{10^2}$	$\mathbf{10^2}$	$\mathbf{10^6}$	$\mathbf{10^5}$	$\mathbf{10^2}$	$\mathbf{10^2}$	$\mathbf{10^2}$	$\mathbf{10^7}$	$\mathbf{10^4}$	$\mathbf{10^2}$	$\mathbf{10^2}$	$\mathbf{10^2}$	$\mathbf{10^7}$
C. tropicalis	10^5	$\mathbf{10^3}$	$\mathbf{10^2}$	$\mathbf{10^2}$	$\mathbf{10^4}$	10^5	$\mathbf{10^3}$	$\mathbf{10^2}$	$\mathbf{10^1}$	$\mathbf{10^5}$	10^4	$\mathbf{10^2}$	$\mathbf{10^2}$	$\mathbf{10^1}$	$\mathbf{10^6}$
S. aureus	10^5	10^4	10^4	10^2	10^4	10^5	10^4	10^4	10^2	10^5	10^3	10^3	10^3	10^2	10^5

PMMA: Heat-polymerized polymethyl methacrylate; PHMB: Polyhexanide; C: Control; h: Hours. Numbers with bold indicate statistically significant difference in comparison to control (p<0.05).

Table 3. *Viable cell number of related microorganisms on UDMA samples that immersed into PHMB suspensions of 0.5%, 1%, 2%, 5% concentration for 10 minutes.*

	UDMA n:10														
	12h					24h					36h				
	0.5 %	1%	2%	5%	C	0.5%	1%	2%	5%	C	0.5%	1%	2%	5%	C
N. sicca	10^3	**10^1**	**0**	**0**	**10^3**	10^1	**0**	**0**	**0**	**10^3**	10^1	**0**	**0**	**0**	**10^4**
S. mutans	**10^1**	**0**	**0**	**0**	**10^3**	**0**	**0**	**0**	**0**	**10^3**	**0**	**0**	**0**	**0**	**10^4**
K. pneumonia	**10^2**	**10^1**	**0**	**0**	**10^3**	**10^1**	**0**	**0**	**0**	**10^3**	**0**	**0**	**0**	**0**	**10^4**
B. subtilis	10^2	**10^1**	**10^1**	**0**	**10^3**	**10^1**	**10^1**	**10^1**	**0**	**10^3**	**10^1**	**10^1**	**0**	**0**	**10^4**
S. pyogenes	**0**	**0**	**0**	**0**	**10^3**	**0**	**0**	**0**	**0**	**10^3**	**0**	**0**	**0**	**0**	**10^4**
C. albicans	**10^2**	**10^1**	**0**	**0**	**10^4**	**10^2**	**0**	**0**	**0**	**10^5**	**10^1**	**0**	**0**	**0**	**10^6**
L. acidophilus	**10^2**	**10^1**	**0**	**0**	**10^3**	**10^1**	**10^1**	**0**	**0**	**10^3**	**10^1**	**10^1**	**0**	**0**	**10^4**
S. sanguis	**10^1**	**0**	**0**	**0**	**10^3**	**0**	**0**	**0**	**0**	**10^3**	**0**	**0**	**0**	**0**	**10^4**
P. vulga	**10^2**	**10^1**	**0**	**0**	**10^3**	**10^1**	**0**	**0**	**0**	**10^3**	**0**	**0**	**0**	**0**	**10^4**
C . pseudotuberculosis	10^3	**10^2**	**0**	**0**	**10^3**	10^2	**10^1**	**0**	**0**	**10^3**	10^1	**0**	**0**	**0**	**10^4**
E. coli	10^5	10^4	**0**	**0**	**10^4**	10^4	10^3	**0**	**0**	**10^5**	10^3	10^2	**0**	**0**	**10^6**
C. tropicalis	**10^2**	**10^1**	**0**	**0**	**10^4**	**10^1**	**10^1**	**0**	**0**	**10^4**	**10^1**	**0**	**0**	**0**	**10^5**
S. aureus	**10^2**	**10^2**	**0**	**0**	**10^3**	**10^2**	**10^1**	**0**	**0**	**10^4**	**10^1**	**10^1**	**0**	**0**	**10^5**

UDMA: light-activated urethane dimethacrylate; PHMB: Polyhexanide; C: Control; h: Hours.
Numbers with bold indicate statistically significant difference in comparison to control (p<0.01).

Table 4. *Viable cell number of related microorganisms on Heat-Polymerized Polydimethylsiloxane samples that immersed into PHMB suspensions of 0.5%, 1%, 2%, 5% concentration for 10 minutes.*

	Heat-Polymerized Polydimethylsiloxane n:10														
	12h					24h					36h				
	0.5 %	1%	2%	5%	C	0.5%	1%	2%	5%	C	0.5%	1%	2%	5%	C
N. sicca	10^5	10^5	10^4	**10^4**	**10^5**	10^4	10^4	10^4	**10^3**	**10^5**	10^4	10^4	10^3	**10^2**	**10^6**
S. mutans	10^4	10^4	10^3	10^3	10^3	10^3	10^3	10^2	10^2	10^4	10^1	10^1	0	0	10^5
K. pneumonia	**10^4**	10^4	10^4	10^3	**10^4**	**10^3**	10^3	10^3	10^2	**10^5**	**10^2**	10^2	10^2	10^1	**10^6**
B. subtilis	10^4	10^4	10^3	10^3	10^3	10^3	10^3	10^2	10^2	10^4	10^2	10^2	10^1	0	10^5
S. pyogenes	10^4	10^4	10^3	**10^2**	**10^3**	10^3	10^3	10^2	**0**	**10^4**	10^2	10^2	0	**0**	**10^5**
C. albicans	10^3	10^3	10^3	10^2	10^3	10^2	10^2	10^2	0	10^4	10^1	10^1	0	0	10^5
L. acidophilus	10^3	10^3	10^2	10^1	10^2	10^2	10^2	10^1	0	10^3	10^1	0	0	0	10^4
S. sanguis	10^2	**10^2**	**10^1**	**0**	**10^2**	10^1	**10^1**	**0**	**0**	**10^3**	10^1	**0**	**0**	**0**	**10^4**
P. vulga	10^3	10^3	10^2	10^2	10^3	10^2	10^2	10^1	10^1	10^4	10^1	0	0	0	10^4
C . pseudotuberculosis	10^3	10^3	**10^2**	**10^1**	**10^3**	10^2	10^2	**10^1**	**0**	**10^4**	10^1	10^1	**0**	**0**	**10^4**
E. coli	10^4	10^4	10^3	10^3	10^3	10^3	10^3	10^2	10^1	10^4	10^2	10^2	10^1	0	10^4
C. tropicalis	10^3	10^3	10^3	10^2	10^3	10^3	10^3	10^2	10^2	10^4	10^2	10^2	10^1	0	10^4
S. aureus	10^4	**10^3**	**10^2**	**10^1**	**10^3**	10^2	**10^2**	**0**	**0**	**10^4**	10^1	**0**	**0**	**0**	**10^4**

PHMB: Polyhexanide; C: Control; h: Hours. Numbers with bold indicate statistically significant difference in comparison to control (p<0.05).

Table 5. *Viable cell number of related microorganisms on Autopolymerising polyethylmethacrylate samples that immersed into PHMB suspensions of 0.5%, 1%, 2%, 5% concentration for 10 minutes.*

	Autopolymerising polyethylmethacrylate n:10														
	12h					24h					36h				
	0.5 %	1%	2%	5%	C	0.5%	1%	2%	5%	C	0.5%	1%	2%	5%	C
N. sicca	10^3	**10^2**	**10^2**	**10^2**	**10^4**	10^3	**10^2**	**10^1**	**10^1**	**10^4**	10^2	**10^1**	**10^1**	**0**	**10^5**
S. mutans	**10^3**	**10^2**	**10^2**	**10^1**	**10^4**	**10^3**	**10^2**	**10^2**	**0**	**10^5**	**10^2**	**10^1**	**10^1**	**0**	**10^5**
K. pneumonia	10^5	10^4	**10^3**	**10^3**	**10^4**	10^5	10^4	**10^2**	**10^2**	**10^5**	10^4	10^3	**10^2**	**10^2**	**10^5**
B. subtilis	10^3	10^3	10^3	10^3	10^3	10^3	10^3	10^2	10^1	10^4	10^3	10^2	10^1	10^1	10^5
S. pyogenes	10^4	**10^3**	**10^3**	**10^1**	**10^4**	10^3	**10^3**	**10^3**	**0**	**10^5**	10^3	**10^2**	**10^2**	**0**	**10^5**
C. albicans	10^3	10^3	**10^2**	**10^1**	**10^3**	10^2	10^2	**10^2**	**0**	**10^4**	10^1	10^1	**10^1**	**0**	**10^5**
L. acidophilus	**10^2**	**10^2**	**10^2**	**10^1**	**10^3**	**10^2**	**10^2**	**10^2**	**0**	**10^4**	**10^2**	**10^1**	**10^1**	**0**	**10^4**
S. sanguis	10^4	10^3	**10^1**	**10^1**	**10^3**	10^4	10^2	**10^1**	**0**	**10^4**	10^3	10^1	**10^1**	**0**	**10^4**
P. vulga	10^3	**10^2**	**10^1**	**0**	**10^3**	10^3	**10^2**	**10^1**	**0**	**10^4**	10^2	**10^1**	**10^1**	**0**	**10^4**
C. pseudotuberculosis	**10^3**	**10^2**	**10^1**	**0**	**10^4**	**10^1**	**10^1**	**10^1**	**0**	**10^5**	**10^1**	**10^1**	**0**	**0**	**10^5**
E. coli	10^4	**10^3**	**10^3**	**10^1**	**10^4**	10^4	**10^3**	**10^2**	**10^1**	**10^5**	10^3	**10^3**	**10^1**	**10^1**	**10^5**
C. tropicalis	**10^4**	**10^2**	**10^1**	**0**	**10^5**	**10^3**	**10^1**	**0**	**0**	**10^5**	**10^2**	**0**	**0**	**0**	**10^6**
S. aureus	**10^4**	**10^3**	**10^3**	**0**	**10^5**	**10^4**	**10^3**	**10^3**	**0**	**10^5**	**10^3**	**10^3**	**10^2**	**0**	**10^6**

PHMB: Polyhexanide; C: Control; h: Hours. Numbers with bold indicate statistically significant difference in comparison to control ($p<0.01$).

Among the adherent cell numbers of studied microorganisms on denture base and soft lining specimens, after rinsing with 1 mL of phosphate-buffered saline, no significant difference was detected between the different concentrations of PHMB suspensions and the control ($p > 0.05$).

4. DISCUSSION

In the present study, the null hypothesis was partially accepted due to the PHMB of various concentrations affecting only the viable cell number of different microorganisms on the denture base and soft lining materials. According to our findings, the number of adherent microorganisms was not responsive to PHMB.

In this in vitro study, to determine the viable cell numbers after the incubation period and treatment with different PHMB suspensions, the culture method (CFU/mL) was appointed. This method is sensitive enough to allow for exclusive detection of surviving microorganisms under favorable conditions (26). Budtz-Jorgensen et al. stated that the build-up of denture plaque was initially rapid but then slowed down (27). In their study, the number of bacteria and yeasts were similar in 2-day-old and 7-day-old plaque (27). In the present study, PHMB suspensions were prepared in concentrations of 0.5 to 5%. These amounts were determined according to a study from Koburger and colleagues (24). They indicated that the minimum inhibitory concentration and minimum bactericidal concentration of PHMB varied from 0.5 mg/L to 4 mg/L and from 1 mg/L to 32 mg/L, respectively. They determined the comparable concentrations for the minimum inhibitory concentration (MIC_{48}) and minimum bactericidal concentration (MBC_{24}).

Disinfectant solutions are good options to achieve proper hygiene in denture base and/or reline materials. According to the literature, sodium hypochlorite (10), chlorhexidine digluconate (10), sodium perborate (11), glutaraldehyde (13), and different natural ingredients (12,14) have been tested. Despite the significant reduction in bacteria and *Candida* spp. counts, none of these agents has demonstrated superiority to the others. On the other hand, PHMB has been marketed as a disinfectant solution in western countries for many years (28). Successful outcomes have been achieved using PHMB in wound treatments, mouthwash formulations, and soft lens care solutions (17,19,23). PHMB is also well known for its broad antimicrobial spectrum against gram positive and negative bacteria and yeasts (29,30). However, in 2013, the European Chemicals Agency classified PHMB as ''fatal if inhaled,'' so it should be used with caution (31). In the present study, we did not check the amount of coated and residual PHMB content on the surface of the samples after incubation and subsequent washing, and we consider this as a limitation of the study. To best of our knowledge, however, there is no described safety threshold value for PHMB as a denture disinfectant agent, and future studies are warranted related to this issue. In our study, 6 different species of bacillus (3 gram positive and 3 gram negative), 4 different species of gram positive coccus, a gram negative diplococcus, and 2 different species of yeasts were tested. These microorganisms exist in oral microbiota and play a role in plaque accumulation and pathogenesis of denture stomatitis (32). According to our results, PHMB of various concentrations was able to decrease the number of viable cells only in some of the tested microorganisms that were incubated on different types of denture base and soft lining materials in comparison to the control samples, and any significant difference was observed between the different concentrations of PHMB suspensions. These findings were in accordance with the antibacterial effect of PHMB on different surfaces, objects, and instruments (28,29).

In the present study, the remaining adherent cell number of the studied microorganisms on the denture base and

soft lining materials did not demonstrate any significant difference. Adhesion of microorganisms depends on the type of material. Increased surface roughness, free surface energy, and wettability are influential factors of bacterial adhesion (5,6). For instance, bacteria strains with a high free surface energy, such as *Streptococcus mutans*, can adhere preferentially to hydrophilic substrates that exhibit high free surface energy (6). These indicators may explain the diverse number of viable cells on tested materials with different concentrations of PHMB. In this study, these factors were not tested, and this can be considered a limitation of our study. In line with present outcomes, PHMB seems to be a potential disinfecting agent for removable prostheses; however, well-designed clinical studies that consider the possible effects of saliva, intra-oral pH, and temperature as well as the material characteristics are warranted.

5. CONCLUSION

Within the limits of our study, PHMB suspension, of various concentrations, can reduce some species of bacterial and yeast cells. Any significant difference was observed between the different concentrations of PHMB suspensions regarding their antimicrobial effect. Clinical application, optimal level of concentration, and oral tissue response of PHMB need to be tested with further studies.

Acknowledgement

Authors thank to Dr. Ahmet Alim for technical support in laboratory analyses.

REFERENCES

[1] Kawala M, Smardz J, Adamczyk L, Grychowska N, Wieckiewicz M. Selected applications for current polymers in prosthetic dentistry-state of the art. Curr Med Chem 2018; 25: 6002-6012.

[2] Coulthwaite L, Verran J. Potential pathogenic aspects of denture plaque. Br J Biomed Sci 2007; 64: 180-189.

[3] Kang SH, Lee HJ, Hong SH, Kim KH, Kwon TY. Influence of surface characteristics on the adhesion of Candida albicans to various denture lining materials. Acta Odontol Scand 2013; 71: 241-248.

[4] Gomes AS, Sampaio-Maia B, Vasconcelos M, Fonesca PA, Figueiral H. In situ evaluation of the microbial adhesion on a hard acrylic resin and a soft liner used in removable prostheses. Int J Prosthodont 2015; 28: 65-71.

[5] Teughels W, Van Assche N, Sliepen I, Quirynen M. Effect of material characteristics and/or surface topography on biofilm development. Clin Oral Implants Res 2006; 17: 68-81.

[6] Busscher HJ, Weerkamp AH, van der Mei HC, van Pelt AW, de Jong HP, Arends J. Measurement of the surface free energy of bacterial cell surfaces and its relevance for adhesion. Appl Environ Microbiol 1984; 48: 980-983.

[7] Gendreau L, Loewy ZG. Epidemiology and etiology of denture stomatitis. J Prosthodont 2011; 20: 251-260.

[8] Pellizzaro D, Polyzois G, Machado AL, Giampaolo ET, Sanitá PV, Vergani CE. Effectiveness of mechanical brushing with different denture cleansing agents in reducing in vitro Candida albicans biofilm viability. Braz Dent J 2012; 23: 547-554.

[9] Budtz-Jørgensen E. Materials and methods for cleaning dentures. J Prosthet Dent 1979; 42: 619-623.

[10] Altieri KT, Sanitá PV, Machado AL, Giampaolo ET, Pavarina AC, Vergani CE. Effectiveness of two disinfectant solutions and microwave irradiation in disinfecting complete dentures contaminated with methicillin-resistant Staphylococcus aureus. J Am Dent Assoc 2012; 143: 270-277.

[11] Duyck J, Vandamme K, Muller P, Teughels W. Overnight storage of removable dentures in alkaline peroxide-based tablets affects biofilm mass and composition. J Dent 2013; 41: 1281-1289.

[12] Mota AC, de Castro RD, de Araújo Oliveira J, de Oliveira Lima E. Antifungal activity of apple cider vinegar on Candida species involved in denture stomatitis. J Prosthodont 2015; 24: 296-302.

[13] Andonissamy L, Karthigeyan S, Ali SA, Felix JW. Effect of Chemical denture disinfectants and tree extracts on biofilm-forming Staphylococcus aureus and viridans Streptococcus species isolated from complete denture. J Contemp Dent Pract 2019; 20: 1307-1314.

[14] Soto AF, Mendes EM, Arthur RA, Negrini TC, Lamers ML, Mengatto CM. Antimicrobial effect and cytotoxic activity of vinegar-hydrogen peroxide mixture: A possible alternative for denture disinfection. J Prosthet Dent 2019; 121: 966.e1-966.e6.

[15] Masetti P, Arbeláez MIA, Pavarina AC, Sanitá PV, Jorge JH. Cytotoxic potential of denture base and reline acrylic resins after immersion in disinfectant solutions. J Prosthet Dent 2018; 120: 155.e1-155.e7.

[16] Chidambaranathan AS, Balasubramanium M. Comprehensive review and comparison of the disinfection techniques currently available in the literature. J Prosthodont 2019; 28: 849-856.

[17] Sowlati-Hashjin S, Carbone P, Karttunen M. Insights into the polyhexamethylene biguanide (PHMB) mechanism of action on bacterial membrane and DNA: A molecular dynamics study. J Phys Chem B 2020; 124: 4487-4497.

[18] Sibbald RG, Coutts P, Woo KY. Reduction of bacterial burden and pain in chronic wounds using a new polyhexamethylene biguanide antimicrobial foam dressing-clinical trial results. Adv Skin Wound Care 2011; 24: 78-84.

[19] Romanowski EG, Yates KA, O'Connor KE, Mah FS, Shanks RM, Kowalski RP. Evaluation of polyhexamethylene biguanide (PHMB) as a disinfectant for adenovirus. JAMA Ophthalmol 2013; 131: 495-498.

[20] Szkołuda P, Karpiński TM. Polyhexanide (PHMB)–properties and applications in medicine. Eur J Biol Res 2020; 10: 225-231.

[21] Hübner NO, Kramer A. Review on the efficacy, safety and clinical applications of polihexanide, a modern wound antiseptic. Skin Pharmacol Physiol 2010; 23: 17-27.

[22] Rosin M, Welk A, Bernhardt O, Ruhnau M, Pitten FA, Kocher T, Kramer A. Effect of a polyhexamethylene biguanide mouthrinse on bacterial counts and plaque. J Clin Periodontol 2001; 28: 1121-1126.

[23] Welk A, Splieth CH, Schmidt-Martens G, Schwahn Ch, Kocher T, Kramer A, Rosin M. The effect of a polyhexamethylene biguanide mouthrinse compared with a triclosan rinse and a

chlorhexidine rinse on bacterial counts and 4-day plaque re-growth. J Clin Periodontol 2005; 32: 499-505.

[24] Koburger T, Hübner NO, Braun M, Siebert J, Kramer A. Standardized comparison of antiseptic efficacy of triclosan, PVP-iodine, octenidine dihydrochloride, polyhexanide and chlorhexidine digluconate. J Antimicrob Chemother 2010; 65: 1712-1719.

[25] Pavan S, dos Santos PH, Filho JN, Spolidorio DM. Colonisation of soft lining materials by micro-organisms. Gerodontology. 2010; 27: 211-216.

[26] Yu X, Jiang W, Shi Y, Ye H, Lin J. Applications of sequencing technology in clinical microbial infection. J Cell Mol Med 2019; 23: 7143-7150.

[27] Budtz-Jörgensen E, Theilade E, Theilade J. Quantitative relationship between yeast and bacteria in denture-induced stomatitis. Scand J Dent Res 1983; 91: 134-142.

[28] Wessels S, Ingmer H. Modes of action of three disinfectant active substances: a review. Regul Toxicol Pharmacol 2013; 67: 456-467.

[29] Yanai R, Ueda K, Nishida T, Toyohara M, Mori O. Effects of ionic and surfactant agents on the antimicrobial activity of polyhexamethylene biguanide. Eye Contact Lens 2011; 37: 85-89.

[30] Ceviker K, Canikoglu M, Tatlıoglu S, Bagdatli Y. Reducing the pathogen burden and promoting healing with polyhexanide in non-healing wounds: a prospective study. J Wound *Care* 2015; 24: 582-586.

[31] European Commission. List of participants/applicants whose dossiers are being examined under the Review Programme for existing active substances used in biocidal products. http://ec.europa.eu/environment/biocides. Accessed on July 3, 2013. EU Commission website.

[32] Morse DJ, Smith A, Wilson MJ, Marsh L, White L, Posso R, Bradshaw DJ, Wei X, Lewis MAO, Williams DW. Molecular community profiling of the bacterial microbiota associated with denture-related stomatitis. Sci Rep 2019; 9: 10228.

How to cite this article: Yilmaz D, Akin H. Antimicrobial Effect of Polyhexanide on Denture Base and Soft Lining Materials. Clin Exp Health Sci 2022; 12: 113-119. DOI: 10.33808/clinexphealthsci.833576