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The Influence Of Bacteriocin On The Soft Liner Tensile Strength And Its Inhibitory Effect On *Candida Albicans*

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Abstract

Aim of the study. Many efforts were applied to use new antimicrobial agents due to the recent appearance of bacterial resistance to treatment. One of the antimicrobial agents is the Bacteriocin. Bacteriocins are small molecules produced by probiotics bacteria and have the ability to minimize pathogens colonization. This study performed to evaluate the effect of bacteriocin against *candida albicans* growth, inhibition of the formation of biofilm and tensile strength of the hot soft liner and compare the results with the use of denture cleanser tablets.
Materials and Methods. Two types of specimens were prepared first type to measure the tensile strength for the soft liner and the other type to measure the inhibition of growth and the biofilm of *C. albicans*. Each type was divided into subgroups (15 specimens each) to be immersed in water as control, Bacteriocin and denture cleansing tablets at different intervals. For the tensile strength test, rectangular hot cure soft denture liner specimens prepared with dimensions of 80mm*9mm*3mm length, width and thickness respectively. While for microbiological test, 10mm diameter and 3mm thick discs of heat cured soft lining material were prepared. The specimens used for the tensile strength test were divided into three groups according to the immersion method: 1st group is the control immersed in water, 2nd group immersed in Bacteriocin and 3rd group immersed in denture cleansing tablet (Lacalut DENT Cleaning tabs/Germany). While for the microbiological test the specimens divided into control group, group treated with bacteriocin for 1 day and group treated with bacteriocin for 15 and 30 days. The same thing done for the specimens cleaned by denture cleansing tablets. The immersion was performed by placing the specimens in tube (10ml) filled with bacteriocin or cleansing tablet in water and kept for 15min. Then the specimens were removed from the tube and cleaned with tapped water and stored in distilled water. This was repeated for 15 days for the first group, and 30 days for the other group. The tensile strength was measured by the Instron testing machine, the inhibition of *C.albicans* growth and biofilm formation was tested by co-cultured method.
Results. This study showed that the use of bacteriocin has no effect on the tensile strength when used for 15 days and the reduction in the tensile strength appear in the 30 days of use when compared with the control specimen which cleaned with water and with that cleaned with denture cleansing tab. Also the use of bacteriocin inhibits *C. albicans* growth and the biofilm with MIC recorded at 600µg /ml

and inhibit the biofilm formation on the soft liner after immersing the specimens with the bacteriocin for 1, 15 and 30 days and its results is better than the use of denture cleansing tab. The use of bacteriocin in the cleaning of the specimens for 15 days cause slight reduction in the tensile strength from 1.18pa to 1.06pa which considered as non significant changes (p>0.05) in the tensile strength and, after treatment with Bacteriocin for 30 days it showed reduction in the tensile strength similar to that produced by the denture cleansing tab for the same period of immersion time. The specimens cleaned once daily by immersion in the Bacteriocin or denture cleansing tab for 15 min. The use of bacteriocin in the cleaning of hot soft liner resulted in 70.49 % inhibition in candida biofilm formation after 30 days cleaning of the specimens, and inhibited 42.33% of biofilm formation after 15 day cleaning.

Conclusions. Bacteriocin is an effective cleanser against candida growth; therefore, it can be used as a preventive agent or as a treatment for the candida stomatitis. However, the tensile strength of the soft liner was decreased after the use of bacteriocin for 30 days. Bacteriocin denture liner disinfection should not be used more than 15 days.

Key words. Bacteriocin, Soft liner, *C. albicans*, Tensile strength, Denture cleanser.

Introduction

For more than a century, soft denture lining materials have been used in dentistry. A plasticized polyvinyl resin was developed in 1945 followed by the development of silicons in 1958.[1]

Soft denture liners are aimed to reduce loads by adjusting adaptation of the denture base to the underlying residual ridges, to minimize resorption of the residual ridge and to make the dentures more satisfactory.[2]

High resilience of the soft lining material aid in absorbing the forces of mastication and distributes them over a large area.[3]

One of the problems appear during the use of soft liners is the colonization of the Candida on the impression surface of the denture. Colonization may reduce longevity of the lined dentures.[4]

The microorganisms accumulated on the soft liners may induce denture stomatitis so the disinfection of the denture may be important.[5]

The method of disinfection should be useful without producing adverse consequence on the denture base and lining materials.[6]

Bacteriocin are proteins or complex proteins produced by bacteria to reduce the process of growing of closely related or non - related bacterial strain without harming the producing bacteria.[7] Many studies have identified and purified bacteriocins for several applications such as food preservation , food technology , cancer therapy , antimicrobial and human health maintenance .[8] Bacteriocin is ribosomally biosynthesized antibacterial peptide produced by each of Gram-negative and Gram-positive bacteria [9] . Coding of genes for lactic acid bacteria (LAB) bacteriocins are situated on chromosomal DNA and plasmide[10], production can present at bacterial growth stages under different environmental parameters. Bacteriocins produced by LAB have narrow and broad spectrum effect against different strains such as *Escherichia coli* and *Campylobacter jejuni*.[9]

Candida albicans is the most frequent pathogen resulting in infections varying from superficial mucosal to the dangerous systemic infections**.** This opportunistic pathogen is portion of the common human flora that form colonies in several sites of the human**.**[11] Under states of immune suppression or any disturbances to the host environment, *C. albicans* can convert into a pathogenic yeast rapidly, causing infections.[12] The Candida pathogenicity is attributed to many factors, like the ability to evade the host defenses, biofilm formation, adherence and the production of enzymes such as phospholipases ,haemolysin and proteases which damage the tissue.[13] Because the colonization of the pathogens

became more resistant to the traditional treatment this study aimed to use the Bacteriocin in the cleansing of the soft liner and tested its effect on the inhibition of *C. albicans* and tensile strength of the material.

Material and Method

Experimental design:

Acrylic based soft denture liner material (Vertex/soft liner heat polymerized/ Netherland) was used in the specimens' preparation. Two solutions were used in the cleaning process of the specimens, Bacteriocine (400 µg/ml) and the second solution was denture cleanser tablet dissolved in 250ml distilled water (Lacalut DENT Cleaning tabs/Germany).

Grouping of the specimens:

Two specimens' groups were used in this study. The first group used to test the tensile strength of the soft denture lining while the second group used to investigate the inhibition of the *C. albicans* growth and biofilm formation. Each group subdivided into 2 subgroups according to the type of the cleanser agent used. One group cleaned with bacteriocin and the other cleaned with denture cleansing tablet.

Preparation of specimens for tensile strength test:

Hot cure soft denture liner specimens were prepared using plastic pattern with dimensions of 80mm*9mm*3mm length, width and thickness respectively.[14] The samples were prepared according to the manufacturer instructions.

Preparation of specimens for microbiological test

10mm diameter and 3mm thick discs of wax pattern were used for the preparation of heat cured soft lining material. The laboratory steps of samples preparation were applied according to the manufacturer instructions.

Treatment of the specimens:

The samples then were kept in distilled water. The prepared specimens were divided into subgroups according to the type of treatment. For the tensile strength test the specimens divided into: the control group G I, second group G II cleaned with testing solution for 15 days and the last group G III cleaned with testing solution for 30 days. The control group G I was kept in distilled water, G II cleaned with bacteriocin once a day for 15 minutes [(15)] by placing the specimen in a tube (10ml) filled with bacteriocin to ensure full coverage of the specimens, then the specimens were removed from the bacteriocin, cleaned with tapped water and kept in distilled water to the next day of cleaning. This process was repeated every day for 15 days. Another group completed this procedure for another 15 days to be cleaned for 30 days. This is also done for the other group which cleaned with denture cleansing tab to be tested at zero time, 15 days and 30 days. The control group for the microbiological test was immersed in water while the other specimens immersed in the bacteriocin for 1,15 and 30 days. This was repeated for the specimens which cleaned by the use of denture cleansing tablets dissolved in distilled water. The rectangular specimens were tested for tensile strength and the disc shaped specimens were tested for microbiological test.

Testing procedure for the tensile strength:
After the immersion of all specimens in the testing solutions for the given time interval, the specimens were tested with Instron testing machine with JIANQIAO (figure 2). The tensile strength was calculated according to the formula (ASTM specification 1986):[16]
Tensile strength=F (maximum force before failure in newton)/A (cross sectional area in mm^2)

Testing procedure for the candida biofilm formation

***Candida albicans* isolate and purified Bacteriocin**

Candida albicans were isolated from oral thrush (obtained from College of Science /Biology Department/ Mustansiriyah University) , subjected to the cultural, microscopical and biochemical [17] as well as Vitek 2 system .
Bacteriocin was purified from *Leuconostoc mesenteroides* ssp. *cremoris* obtained from College of Science /Biology Department/ Mustansiriyah University /Iraq/Baghdad. This study was approved by the scientific committee of the Department of Prosthodontics, College of Dentistry, Mustansiriyah University.

Minimum Inhibitory Concentration (MIC) for Bacteriocin

The MIC of bacteriocin determined by the micro dilution method in the culture broth with modification. Further dilutions were prepared to concentrations ranging from (600-50)µg/ml. All the wells of the 96-well microplate with sabouraud dextrose broth were inoculated with 2.5 µl of an overnight culture of *Candida albicans* isolates(1.5×10^8 CFU/ ml). Microtiter plates were covered and incubated at 37 °C for 24h. The minimum inhibitory concentration was determined at a concentration which no evident growth could be observed after sub culturing on sabouraud dextrose agar.[18]

Inhibition of candida biofilm in acrylic denture soft liner:

The effect of bacteriocin and denture cleansing on biofilm formation of *C.albicans* was studied using acrylic denture soft linear specimens according to the procedure described by Salman,2018[19] with modification , all experiments were done in triplicate . The acrylic denture resin specimens kept for 24h in bacteriocin and denture cleansing solutions separately at sub MIC concentration , a control acrylic specimens kept in water. After that, the solution of specimens was removed and drying at room temperature. The dried specimens were immersed in YPD broth that inoculated with *C. albicans* suspension compared to 0.5 McFarland , then they incubated at 37°C for(24,48,72) h. ,then the broth was taken out and the specimens washed two times with sterile phosphate buffer solution (PBS) to remove the unattached *C. albicans* cells and left to dry at room temperature for 15 min. , then add crystal violet to the specimens for 20 min. After removing the crystal violet solution , specimens were washed three times, with PBS (pH 7.2) to remove unbound dye and left to dry at room temperature ,finally adding of acetone : ethanol mixture (20 : 80 v/v) to each specimen , waiting for 10 min then, acetone : ethanol mixture was collected for measuring the absorbance of each specimen at 450 nm by an Elisa reader.[20] Inhibition of biofilm formation percentage calculated as equation described by Chevalier *et al*.,(2012) .[21]

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

* The inhibition of *Candida albicans* biofilm by specimens immersed in water was zero.

Statistical analysis:
The t-test statistical analysis was used in this study to determine if there is significant differences between the mean of each two groups.

Results

Tensile strength test
The results showed that there was decrease in the tensile strength after cleaning with bacteriocin and tablet cleanser and this reduction in the tensile strength increase with the number of times of cleaning. The mean value of tensile strength for the control group G I was 1.18pa, for the group cleaned by bacteriocin for 15 days G II was 1.06pa, the group cleaned by bacteriocin for 30 days G III was 1.1pa, the group cleaned by cleansing tablets for 15 days G IV was 0.93pa and the group cleaned by cleansing tablets for 30 days G V was 0.9pa (figure 1).
The readings taken for the tensile bond strength from the Instron testing machine were subjected to t-test statistical analysis (figure 2). After the collection of readings the statistical analysis performed and t-test used to make a comparison between the results of each group with the other. Each group of the soft liner cleaned with bacteriocin for 15 and 30 days compared with the control and then compared with each other. The same is done for those cleaned with the cleansing tablets (table 1).
The results showed non significant reduction (p value=1) in the tensile strength between the samples cleaned by Bacteriocin for 15 days (G II) and the control groups G I while there was significant reduction in the tensile strength between group cleaned by Bacteriocin for 30 days G III and the control group G I (p value=0.02). This reduction in the tensile strength after cleaning by Bacteriocin for 30 days is similar to the reduction in the tensile strength after cleaning by denture cleansing tab which is also significant (table 1). There was non significant difference (p value=0.8) between G IV (specimens cleaned by cleansing tablets for 15 days) and G I (control group) while there was significant difference (p value=0.005) between G V (specimens cleaned by cleansing tablets for 30 days) and G I (control group).

Inhibition of candida biofilm:
The antifungal activity of bacteriocin was determined on the basis MIC values . MIC at concentration 600µg /ml was determined for *C. albicans* isolates.
The inhibition percentage of candidal biofilm formation on denture soft liner specimens was also calculated ,the high inhibition percentage for biofilm formation after 30 days was 70.49% and 42.33% for the groups cleaned with bacteriocin and cleansing tab respectively, while the lowest inhibition percentage was 33.65 % for the group treated with bacteriocin for 1 day. The control group (specimens stored in water) show no effect in the inhibition of the biofilm formation (Figure 3).

Discussion
Several denture cleansers are provided in order to develop denture hygiene, however, when the denture cleansers used daily it can influence the physical properties of soft liner and the corresponding denture base.[22]
Damages of the soft liner may be inflicted by brushing so cleansing by chemical agents is suggested for cleaning the soft liners and it is

necessary to choose a proper cleansing agent to reduce the adverse effect from these agents.[15] In this study the effect of bacteriocin was evaluated when the bacteriocin used as a cleaner on the candidal growth and tensile strength of the soft liner and compared with the effects of denture cleansers tablets. It was found that the reduction of tensile strength of the soft liner after cleaning with bacteriocin for 30 days is similar to the reduction with the cleaning by the cleansing tablets while it was less for the cleaning for 15 days. The reduction in the tensile strength due to the loss of plasticizer component of the soft liner to the cleansing solutions and to the water. This reduction in the soft liner tensile strength after cleaning is in agree with Narwal 2015[22] who showed that there is decrease in tensile strength and increase in the hardness with time for both denture cleansers (Clinsodent and VI Clean).

Also the decrease in the tensile strength after cleaning by bacteriocin for 30 days and cleaning by denture cleansing tablet for 30 days was in agree with Brozek et al[23] (2011) who assessed the release of chemical constituents from the soft liners after immersing the samples in various cleansing agents and showed that more constituent were released from all soft liner types after immersion in sodium hypochlorite when compared to Corega immersion.

Biofilms are a main factor in device-associated infections on catheters, denture materials, and other medical implants, as well as on mucosal surfaces in the urogenital track and the oral cavity, caused a significant clinical problem.

The Enterococcus faecalis bacteriocin effective for the reduction of *C. albicans* virulence factors and formation of biofilm.[24] Bacteriocin of *Streptococcus sanguinis* could inhibit *C. albicans* , it could change the cell shape of *C. albicans* that cause increasing of cell membrane permeability.[25] Bacteriocin could not only cause the fungistatic dynamics of *Candida albicans* but also result in the decrease of hydrophobicity in the cell surface, the cell membrane permeability.[26] The mechanism of antifungal agents was the destruction of fungal cell wall, fungal cell membrane, inhibition of protein synthesis and influence on mitochondria ATP synthetase.[27] This explain why there was inhibition in the *candida albicans* biofilm formation after the use of Bacteriocin at different intervals.

Conflicts of Interest: The authors declare no conflicts of interest.

Conclusion

Bacteriocin is an effective agent in the disinfection of the denture and the inhibition of candida growth. It inhibits candida growth better than the conventional denture cleanser for the 15 and 30 days. The bacteriocin is more suitable to be used in soft liner cleansing than the use of denture cleansing tablets since bacteriocin didn't affect the tensile strength at the first 15 days and the reduction in the tensile strength seen at the 30 days of usage.

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Table 1. Summery statistical analysis for tensile strength test in control groups and groups cleaned with Bacteriocin and denture cleansing tablet (Lacalut DENT Cleaning tabs/Germany)

Type	Mean difference	t-test for Equality of Means			
		T	df	P-value	sig
G I &II	0	0	4.88	1	NS
G I&III	-0.25	-3.633	4.24	0.02	S
G III&II	0.26	2.70	4.29	0.05	NS
G I&IV	0.01	0.17	4.91	0.8	NS
G I&V	-0.21	-5.49	5.15	0.005	S
G V&IV	-0.23	-4.28	5.23	0.01	S
G III&V	0.04	0.48	4.57	0.6	NS
G II&IV	0.01	0.16	4.96	0.8	NS

G I = control group
G II= group cleaned by bacteriocin for 15 days
G III= group cleaned by bacteriocin for 30 days
G IV= group cleaned by cleansing tablets for 15 days
G V= group cleaned by cleansing tablets for 30 days

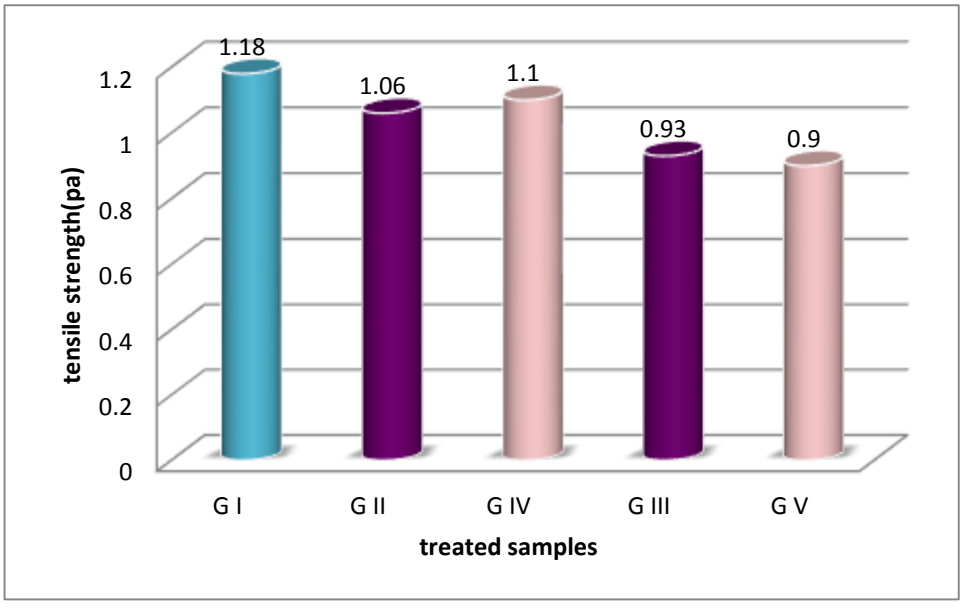

Figure 1. Bar chart for mean values of tensile strength of the specimens after immersion in Bacteriocin and denture cleansing tab

- G I = control group
- G II= group cleaned by bacteriocin for 15 days
- G III= group cleaned by bacteriocin for 30 days
- G IV= group cleaned by cleansing tablets for 15 days
- G V= group cleaned by cleansing tablets for 30 days

Figure 2. The specimen tested for the tensile strength in Instron testing machine

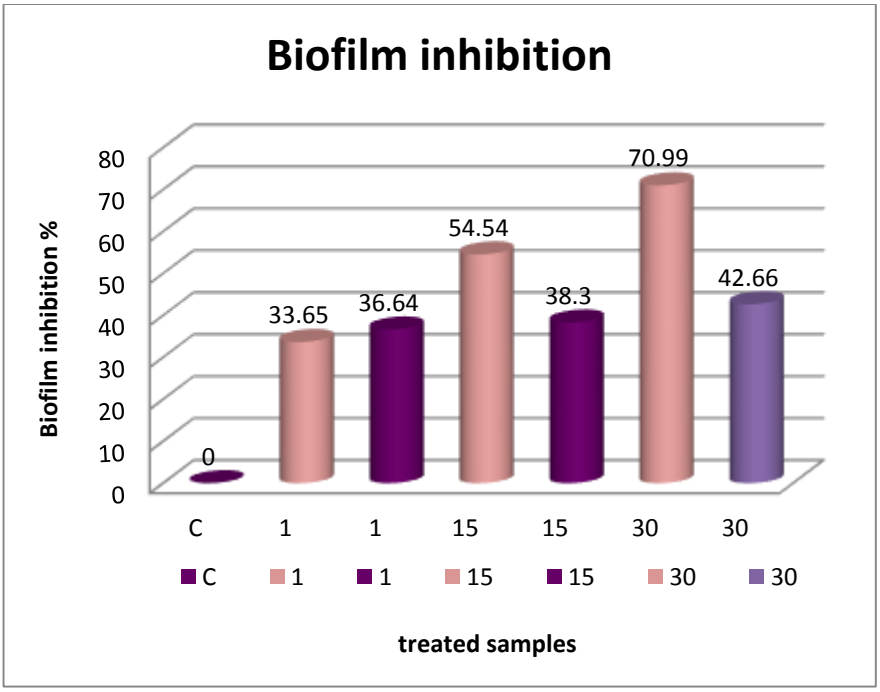

Figure 3. Bar chart for antibiofilm effect of Bacteriocin and denture cleansing tab against *Candida albicans* on denture soft liner

- C control group- samples kept in water.
- 1 samples treated with Bacteriocin for 1 day.
- 1 samples treated with denture cleansing tab for 1 day.
- 15 samples treated with Bacteriocin for 15 days.
- 15 samples treated with denture cleansing tab for 15 days.
- 30 samples treated with Bacteriocin for 30 days.
- 30 samples treated with denture cleansing tab for 30 days.