ORIGINAL ARTICLE

Influence of kappa-carrageenan powder addition on staphylococcus epidermidis adhesion on the room temperature vulcanized maxillofacial silicone

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ABSTRACT

Background: Maxillofacial silicone is the most acceptable and mostly used material in maxillofacial prostheses fabrication but still beyond the ideal material. It has some problems with antimicrobial efficiency.
Aim: To add kappa-carrageenan powder to maxillofacial silicone and evaluate its efficacy against *Staphylococcus epidermidis*.
Methods: In this study, VST50 room temperature vulcanized maxillofacial silicone was used to prepare specimens to be tested and the κ-carrageenan powder was added as the antibacterial agent with selected percentages of 1wt.% and 2wt.% according to pilot study and bacterial adhesion test was used to evaluate its antibacterial efficacy by counting the adherent bacterial cells. The data were analyzed using one-way ANOVA tests which was considered statistically significant at a level of $p< 0.05$.
Results: Both experimental groups B (1wt.% κ- carrageenan powder addition) and C (2wt.% κ-carrageenan powder addition) showed a highly significant decrease of adherent bacterial cells compared to that of control group A (without addition). Experimental group C showed the lowest mean value among them (3.73±0.8667) followed by group B (5.30±0.8919) then the control group with value of (62.16±6.1190).
Conclusion: The κ-carrageenan powder is an effective antibacterial agent against *S. epidermidis.* And can be added to VST50 room temperature vulcanized maxillofacial silicone to decrease the adherent bacterial cells on its surface with both percentages, but 2wt.% is more effective than 1wt.%.
Keywords: maxillofacial silicone, kappa carrageenan, plant extract, bacterial adhesion, *Staphylococcus epidermidis,*

INTRODUCTION

Silicone polymers currently considered as the material of choice for maxillofacial prostheses construction[1]. It is largely used yet still require emending since it last only for short usage period and need to be replaced[2]. Also, it has no antimicrobial potential and deterioration by microbial colonization is another shortage, adding an antimicrobial to silicone material bulk is one solution[3]. *S. epidermidis* is a human commensal that can be converted to an infectious form under some circumstances[4]**.** Its pathogenicity is mainly due to the ability to form biofilms on indwelling medical devices[5]**.** The side effects of the antimicrobial drugs and the resistance species emerged by global antibiotic abuse necessitate another line of treatment[6].

Many herbal materials exhibit antioxidant, anticancer, anti-inflammatory, antimicrobial, and antiviral properties[7]. Kappa-carrageenan is one of these materials, it is used previously as an emulsifier and thickener and as antimicrobial and in pharmaceutical applications[8].

This study aimed to evaluate its antimicrobial efficacy against *S. epidermidis* bacteria when added to VST50 room temperature vulcanized maxillofacial silicone.

MATERIALS AND METHODS

Microbiological Aspect of the Study: Sterile swabs used to take four bacterial specimens from infected area in patients wearing maxillofacial silicone avoiding the necrotic parts [(9)], then, inoculated on blood agar and mannitol salt agar medium, in an aerobic condition at 37 °C for 48 hour [(10)]. *S. epidermidis* form non hemolytic grey-white round smooth colonies about 1-2 mm in diameter. They were positive in catalyses test and the bacterial species confirmed by VITEK 2 compact identification system.
Mold fabrication: For each mold, 3 clear acrylic sheets with 2±0.05mm thickness were made (matrix, base and cover). The matrix sheet made with 10 mm disk shaped holes and glued to base sheet by sticky material (chloroform) to prevent its movement when pouring the silicone. Designed by computer-aided design program, AutoCAD 2013, and fabricated by the CNC machine [(11, 12)].
Pilot study: It was done to select the most effective percentage of κ-carrageenan powder to be added to silicone material. Four percentages were selected (0.5wt.%, 1wt.%, 1.5wt.%, and 2wt.%) to be compared with control specimens (0wt.%) with 3 specimens for each percentage. (1wt.%) and (2wt.%) were found to be the most effective percentages (best antibacterial effect with least effect on silicone mechanical properties).
Specimens fabrication: According to manufacture instruction, VST50 silicone has to be mixed in (10:1) base to catalyst ratio. κ-carrageenan powder was mixed with the base according to weight percentage (for 10 gm of base modified with 1% κ-carrageenan powder; 0.1 gm of κ-carrageenan powder was mixed with 9.9 gm of base and likewise in 2wt.% percentage addition. The κ-carrageenan firstly mixed with the silicone base for 3 minutes without vacuum to avoid powder suction at (360) rpm speed then for 7 minutes with the vacuum (-10 bar) to get rid of air bubbles then the catalyst added and mixed for another 5

minutes [12, 13]. The homogenous mixture poured into the molds, fixed by screws and nuts and g-clamps, stored in 20-25 ºc and humidity below 60%, retrieved and finished[14,15].

Testing procedure - Fourier transforms infrared spectroscopy (FTIR): It was done to determine if there was any chemical interaction between the silicone and the κ-carrageenan material. Specimens were made as thin flushes measuring (10×10×0.5) mm length, width and thickness respectively and the κ-carrageenan was just enough to coat the device lens.

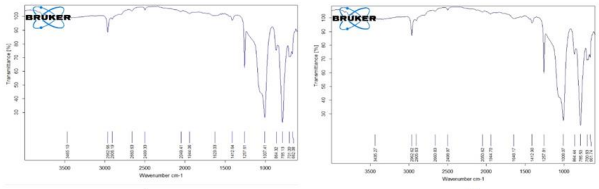
Scanning electron microscope (SEM): It was done to evaluate the homogeneity of the silicone-powder mixture and how the κ-carrageenan particles were distributed.

Bacterial adhesion test: This test used to evaluate the efficacy of κ-carrageenan powder as an antibacterial agent by counting the adhered bacterial cells on the specimen surface under inverted light microscope. Miller broth was used to form the bacterial suspension, Sterilized by autoclaving at 15 pounds (lbs) pressure (121 °C) for 15 minutes. Then a suspension of 10^7 colony forming unit (CFU/ml) (0.5 McFarland standards) was prepared by using a McFarland densitometer [16]. The silicone specimens were sterilized in an autoclave at 121 ◦C for 20 min [17]. Then deposited in sterile plastic bottles containing the prepared bacterial suspension; the specimens were incubated for 1hr. at room temperature then removed from the suspension and rinsed twice by phosphate buffered saline solution (PBS) for one minute and dried by filter paper [18]. Methanol was used to fix the adhered cells and crystal violate to stain them and then examined under inverted light microscope [19, 20].

RESULTS

FTIR: Spectral results showed no chemical interaction between κ-carrageenan powder and VST50 maxillofacial silicone as there is no change in the spectral range of the silicone after the addition of the κ-carrageenan powder.

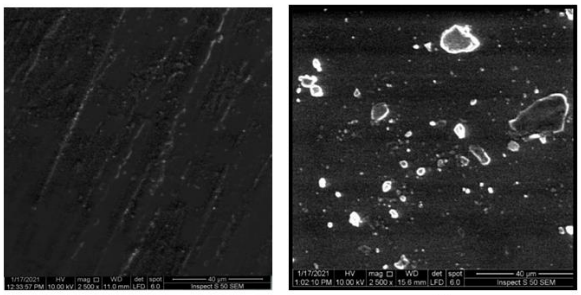
Figure 1: Fourier-transform infrared spectroscopy spectral result of VST50 maxillofacial silicone elastomer, A: without addition of κ-carrageenan powder. B: with addition of κ-carrageenan.



A B

SEM: It shows well dispersion of the κ-carrageenan powder particles within the silicone matrix.

Figure 2: Scanning electrone microscope results of VST50 maxillofacial silicone elastomer. A: without addition of k-carrageenan powder; B: with addition of κ-carrageenan powder



A B

Bacterial adhesion test: The cells appeared as round cells alone or in clusters and the crystal violet stain was retained by the bacterial cell, Statistical analysis of bacterial adhesion test using one-way ANOVA showed a highly significant decrease in the number of adhered bacterial cells of both percentages and Dunnett T3 test was implied (Table 3.4), there was a highly significant difference between group A and B; group A and C as well as between group B and C ($P < 0.01$).

Table 1: Descriptive statistics of bacterial adhesion test among groups

Groups	N	Mean	±SD	±SE	Minimum	Maximum
A (0 wt.%)	10	62.1600	6.1190	1.9350	52.00	70.10
B (1wt.%)	10	5.3000	0.8919	0.2821	4.20	7.00
C (2wt.%)	10	3.7300	0.8667	0.2741	2.20	5.00

Levene test=25.764, p value=0.000[HS], HS=highly significant at p<0.01.

Table 2: Statistical test of bacterial adhesion test among groups using one-way analysis of variance (ANOVA)

	Sum of Squares	Df	Mean Square	F
Between Groups	22165.298	2	11082.649	852.742
Within Groups	350.905	27	12.996	
Total	22516.203	29		

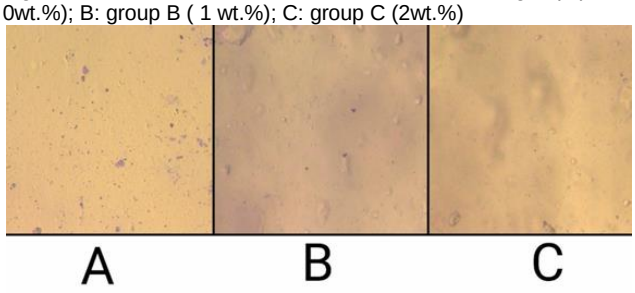
P value 0.000 HS

Table 3: Multiple comparisons of bacterial adhesion test between groups using Dunnett T3

(I) Groups	(J) Groups	Mean Difference (I-J)	p value
A	B	56.8600	0.0000 [HS]
	C	58.4300	0.0000[HS]
B	C	1.5700	0.0025 [HS]

HS=highly significant at $p<0.01$

Figure 3: Results of bacterial adhesion test; A: control group (0wt.%); B: group B (1 wt.%); C: group C (2wt.%)



DISCUSSION

Long-term use of maxillofacial prostheses leads to colonization of microorganisms on the silicone surface and promotes infection of surrounding tissues[21,22].

The removal of the bacterial accumulation is necessary for an external prosthesis. Moreover, it is important to find an efficient method for cleaning that not only prevents infections but also causes no deterioration of the silicone prosthesis [23, 24]. The use of any antimicrobial agents must be limited due to their possible toxic or harmful effects. In recent years, due to previous antibiotics' lesser side effects, the use of herbal materials instead of synthetic or chemical drugs is increasing. Herbal materials are found in medicines. Herbs can be used in the form of plant extracts or as their active components. Furthermore, most of the world's populations used herbal materials due to their strong antimicrobial properties and primary healthcare benefits[7].

The κ-carrageenan largely used previously as antimicrobial based films in many studies such as Choi et al[25], who investigated as antimicrobial agent, through the use of oligosaccharides from κ-carrageenan and found that it has antibacterial activities against *E. coli, S. aureus, S. cere, P. citr* and Mucor sp.[26]. Confirming this fact when using κ-carrageenan as antibacterial agent against *S.aureus*[27]. As well as κ-carrageenan acted as antifungal agent against *C. albicans* when added to denture soft lining material[8]. Other studies used it to enhance the antimicrobial effect of other material[28]. From the knowledge that was gained from reviewing literature there was no work done regarding evaluation the antibacterial effect through addition of κ-carrageenan to any type of maxillofacial silicone elastomer, so it was selected to evaluate its antibacterial effect against *S. epidermidis* using VST50 room temperature vulcanized maxillofacial silicone.

Bacterial adhesion test was done because it plays a crucial role and consider as the first step of bacterial colonization[29].

In this study, it was found to have an antibacterial effect against the mentioned bacteria since the bacterial cells adhered on the silicone specimens decreased significantly with both percentages compared with the control group as mentioned in Table 1.

Results of this study agreed with[30] as they found an antibacterial effect using plant based antimicrobial solutions against *S.epidermidis* with maxillofacial silicone elastomer, and this gives an indication that the plant based materials can act effectively as antimicrobial agents.

Yao et al., 2014[31] stated that the bacterial enzymes which degrade κ-carrageenan are called κ-carrageenases. They all are endohydrolases that cleave the internal b-(1–4) linkages of carrageenans yielding products of the oligo-carrageenans. Oligo-carrageenan produced by the action of microbial enzymes can be more advantageous than produced by acid hydrolysis because enzymes are highly specific to their substrates. This fact suggested playing the major role in the antibacterial effect of κ-carrageenan.

Another factor that played a role in the decreased bacterial adhesion is the hydrophilic nature of κ-carrageenan since *S. epidermidis* is a relatively hydrophobic bacterium[32], and the silicone material is also hydrophobic[33], and this make it more amenable for *S. epidermidis* adhesion and less desorption as suggested by Boks et al 2008[34], so the presence of κ-carrageenan molecules made the silicone surface less hydrophobic, so less adhesion ability of the hydrophobic bacteria. This difference is suggested to be due to the acid–base interactions between staphylococci and these surfaces[34].

The results also showed that there is a highly significant difference between the mean values of both groups (1wt.% and 2wt.%) of κ-carrageenan powder addition. This may be explained by the increase in the percentage by weight of κ-carrageenan which will result in increasing its effect and this fact agree with study done by Salih and Abdul-Ameer[8] as they used the κ-carrageenan as antifungal against *C.albicans* and found an increased effect by increasing the percentage by weight of κ-carrageenan.

CONCLUSION

It can be concluded under the limitation of this study that adding κ-carrageenan powder to VST50 room temperature vulcanized maxillofacial silicone will reduce effectively the adhesion of *S. epidermidis* adhesion on the silicone surface.

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