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Antibacterial Efficacy of Microcrystalline Cellulose-Infused Maxillofacial Silicone

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Abstract

Background: Maxillofacial prosthetics frequently tackle with the challenge of bacterial colonization, particularly by Staphylococcus epidermidis, causing risks of material failure and infections. Recent relevance has grown in using antimicrobial potential of microcrystalline cellulose as a possible solution, markedly recognized in different dental uses. Aims: This study aimed to determine the antibacterial efficacy of incorporating microcrystalline cellulose into silicone while focusing on its capacity to reduce Staphylococcus epidermidis adherence. Furthermore, the study also examined the influence on some mechanical properties of silicone. Materials and methods: The investigation employed 120 room temperature vulcanized silicone specimens. Microcrystalline cellulose was integrated as an antibacterial agent at concentrations of 1wt.% and 2wt.%, chosen based on preliminary pilot study results. Then, antibacterial properties were assessed using a bacterial adhesion test, determining bacterial adherence to silicone. Mechanical properties were assessed, including tear strength and Shore A hardness, with each test involving 10 specimens for each concentration and control groups. Statistical analysis involved one-way ANOVA tests with significance level set at $p < 0.05$. Results: There was a substantial decrease in adherent bacterial cells compared to the control group. The most significant reduction in colonization appeared in the 2wt.% microcrystalline cellulose addition group, followed by the 1wt.% group, both varying significantly from the control group. Tear strength exhibited a significant decrease, with the 2wt.% addition group presenting the lowest value, followed by the 1wt.% group and the control group. Conversely, hardness revealed significant increases, with the highest value in the 2wt.% group. Conclusion: Microcrystalline cellulose demonstrates effectiveness against Staphylococcus epidermidis. It confirms promise as an additive to silicone, decreasing bacterial adhesion effectively at both 1wt.% and 2wt.% percentages, with the latter showing superior antibacterial performance. Importantly, cellulose incorporation leads to a statistically significant reduction in tear strength, alongside an increase in hardness that remains within acceptable limits.

Introduction:

In the realm of maxillofacial prosthetics, the primary objective is to rehabilitate patients afflicted with facial defects and deformities. This rehabilitation serves to not only restore their natural appearance but also enhance their self-image, ultimately contributing to improved psychological well-being (1). The construction of maxillofacial prostheses involves a spectrum of polymeric materials, including polyvinyl chloride, polyurethanes, poly (methyl methacrylate), chlorinated polyethylene, and silicones (2). Silicone, in particular, has emerged as the material of choice due to its advantageous properties, encompassing accepted mechanical strength, ease of manipulation, chemical inertness, durability, and heightened patient comfort in comparison to earlier alternatives. Nevertheless, silicone's mechanical properties still fall short of ideal requirements (3).

One prevalent challenge associated with maxillofacial silicone prostheses is the deterioration of their properties owing to microbial colonization, necessitating prosthesis replacement (4). Addressing this issue, the incorporation of antimicrobial agents into silicone matrices stands as a potential solution. The human body hosts a diverse spectrum of microorganisms that maintain a delicate biological equilibrium in tandem with their living environments. However, uncontrolled, and rapid microbial proliferation can cause a variety of hazardous consequences (5). Staphylococcus epidermidis S. epidermidis warrants special consideration. Typically, it is perceived as a benign commensal bacterium, abundantly inhabiting the human skin. However, under specific predisposing conditions, S. epidermidis can undergo a transformation from a benign colonizer to an invasive pathogen. This shift in behavior is closely linked to the bacterium's remarkable capability to form biofilms, a characteristic that endows it with opportunistic pathogenic potential (6). Conventional antimicrobial agents, such as antibiotics, serve as potent tools for combatting bacterial infections within the human body. Nevertheless, these agents are not without their limitations, as they may cause adverse effects. Furthermore, the unregulated access to antibiotics without the need for a prescription from retail pharmacies contributes substantially to the global challenge of antimicrobial resistance (7). In response to these multifaceted challenges, considerable attention has been directed towards materials sourced from various plants, which are renowned for their medicinal attributes. Many of these herbal compounds exhibit a diverse array of beneficial properties, including antioxidative, anticancer, anti-inflammatory, antimicrobial, and antiviral activities (8). These bioactive molecules possess the potential to effectively manage and inhibit pathogenic microorganisms while minimizing adverse effects on host cells. Consequently, they have emerged as promising candidates for the development of novel antimicrobial agents, with a focus on mitigating the limitations associated with conventional antibiotic therapies (9).

Notably, within the context of maxillofacial silicone elastomers, there exists a noteworthy gap in the literature. Specifically, there has been little investigation into the evaluation of the antibacterial effects stemming from the incorporation of microcrystalline cellulose—a biocompatible substance with known antimicrobial properties—into these elastomers (10). Microcrystalline cellulose MCC is a purified, white, odorless, fine, non-fibrous, and partially depolymerized crystalline powder. It is typically derived from cellulose through a hydrolysis process involving the treatment of alpha cellulose with an excess of mineral acid (11). In microbiology, microcrystalline cellulose MCC serves as a viable alternative to carboxymethyl cellulose or agar in plaque assays. It functions as a liquid overlay substrate, effectively preventing the random spread of viral infections through mechanical or convectional flow during viral propagation (12). In dentistry, MCC is utilized as a key ingredient in toothpaste formulations. Its main function lies in improving plaque removal while decreasing abrasion effects

on the dental surfaces. This can be completed by decreasing the relative proportion of abrasive elements inside the toothpaste, thus reducing the general abrasiveness (13). This represents a new avenue that can promise for improving the antimicrobial strength of prosthetic materials. This study involved the incorporation of MCC into silicone, with the aim to develop polydimethylsiloxane PDMS and microcrystalline cellulose (MCC) composites with improved mechanical and antimicrobial properties. incorporating silver nanoparticles (AgNPs) within MCC using chemical methods, different AgNPs concentrations on cellulose surface, and linking MCC/AgNPs with PDMS, the study successfully constructed multifunctional materials. These composites displayed improved strength and the ability to prevent microorganism growth, making them appropriate for uses where both mechanical performance and antimicrobial activity are necessary, such as therapeutic devices and food packaging (14). This study aimed to estimate the antibacterial efficiency of microcrystalline cellulose in silicone against Staphylococcus epidermidis and investigate its impact on silicone's mechanical properties.

Materials and methods

Materials

1. Microcrystalline cellulose powder (Sigma-Aldrich, USA).
2. Room vulcanized maxillofacial silicone (VST-50, Factor II, USA).

A. Sample grouping

In the course of this in-vitro experimental study, three distinct experiments were conducted, with each experiment encompassing the investigation of three distinct groups. These groups were determined on the basis of a predetermined percentages of microcrystalline cellulose (MCC) powder derived from preliminary pilot studies. Specifically, the groups were classified as follows: Group A served as the control; denoting specimens devoid of any MCC addition (0.0 wt.% MCC). In contrast, Group B constituted the experimental group characterized by the inclusion of 1 wt.% MCC powder, while Group C represented another experimental cohort, incorporating 2 wt.% MCC powder. Notably, each of these groups comprised of ten samples, maintaining consistency throughout the experimentation process.

B. Isolation and identification of S. epidermidis

S. epidermidis isolation involved swabbing infected areas of maxillofacial prosthesis-wearing patients at Ghazi al-Hariri Hospital. Swabs (AFCO, Jordan) were meticulously applied to the infected skin while avoiding necrotic tissue. Specimens were then cultured on blood agar and mannitol salt agar, following a 48-hour aerobic incubation at 37°C (15-16). For S. epidermidis identification, colonies appeared as 1–2mm diameter, greyish white, raised, circular, and smooth with a glistening and slightly translucent to opaque texture after overnight incubation on blood agar. They exhibited a non-hemolytic pattern on the blood agar medium (15-17) (Figure 1).

A positive catalase test confirmed their staphylococcal nature, while negative results ruled out streptococci. The VITEK 2 compact identification system was then employed following the manufacturer's guidelines. Isolates were transferred to glass tubes containing 3 mL of distilled water to measure and adjust turbidity, ensuring a bacterial cell count equivalent to the 0.5 Macfarlane standard. These bacterial suspensions were subsequently loaded into cassettes using negative pressure and introduced into the VITEK 2 compact system for a 12-hour biochemical reaction. The interpretation of results was carried out utilizing specialized software within the VITEK 2 compact system, facilitating the precise identification of bacterial species and strains (18).

C. Acrylic mold fabrication

The molds were created through a process involving computer-aided design (CAD) software, specifically AutoCAD 2019. This software was utilized to precisely define the measurements and specifications for each of the test specimens. Subsequently, the individual components of the molds were fabricated

using computer numerical control (CNC) technology. This procedure ensured the accurate replication of the intended mold designs, thereby facilitating the consistent and precise fabrication of the test specimens (Figure 2).

D. Mixing and pouring

Following the manufacturer's instructions, VST-50 silicone used a 10:1 ratio of base to catalyst. For Group B, involved mixing 0.1 grams of MCC powder with 9.9 grams of base. In Group C, used 0.2 grams of MCC powder with 9.8 grams of base. These modified base formulations, totaling ten grams each, were combined with one gram of catalyst, following manufacturer instructions. MCC powder was weighed using an electronic scale (China). Silicone base was then added to prevent powder dispersion (Figure 3). The silicone base and MCC powder were mixed with a vacuum mixer for 10 minutes, employing a vacuum to eliminate air bubbles for 7 minutes at 360 rpm and -10 bar. The catalyst was added by weight and mixed with the modified silicone base for 5 minutes using a vacuum mixer to achieve a homogeneous, bubble-free mixture.

The mixture was poured into the acrylic mold with caution to minimize air bubble formation on the specimen's surface when the cover was applied. Subsequently, the cover was secured with screws and nuts, tightened using G-clamps. Constant pressure was maintained throughout the material's curing process by applying a 1 kg load (CLW-A, Rex Gauge Company, Inc., Buffalo, USA) to the center of the cover for 8-12 hours under standard conditions. After polymerization, specimens were removed from the molds and finished.

E. Bacterial adhesion test

Miller broth HIMEDIA, India served as the medium for creating bacterial suspension, essential for assessing the adhesion ability of S. epidermidis on the silicone specimens. This broth was prepared by dissolving 25 grams of the medium in 1000 ml of distilled water and sterilizing it through autoclaving at 121°C under 15 pounds (lbs) pressure for 15 minutes. Subsequently, a bacterial suspension of 10^7 colony-forming units per milliliter (CFU/ml) was established using a McFarland densitometer, aligning with 0.5 McFarland standards (19).

The silicone specimens underwent sterilization in an autoclave at 121°C for 20 minutes (20). These sterile silicone specimens were then immersed in sterile plastic containers containing the prepared bacterial suspension and left to incubate for one hour at room temperature. Upon completion of the incubation period, the specimens were withdrawn from the suspension, rinsed twice with phosphate-buffered saline solution (PBS) for one minute each, using gentle agitation to remove non-adherent bacterial cells. Subsequently, they were dried using filter paper (21).

To fix the adherent bacterial cells on the silicone specimens, methanol (CARLO ERBA, France) was employed. Following this fixation step, the specimens were stained with crystal violet HIMEDIA, India for 60 seconds, rinsed again with PBS solution for 30 seconds, and dried using filter paper. Examination was carried out under an inverted light microscope (22). The adherent cells were counted, and the mean was calculated.

F. Tear strength

Specimens were fabricated and evaluated in accordance with ISO 34-1 (2015) (23) standards. The thickness of each specimen was determined using a digital vernier caliper, measuring at three positions across the width and averaging the measurements. Subsequently, specimens were affixed in the clamps of a computerized Instron universal testing machine, ensuring symmetrical and axial alignment for testing (Figure 3).

G. Shore A hardness

Hardness testing, adhering to ISO 7619-1 (2010) (24) standards, was conducted using a digital shore A durometer with a 1.25mm blunt indenter. Within each specimen, five points were marked, with a 6mm separation between them and the lateral borders, and the average of these five readings was recorded as the hardness value (Figure 4).

H. Field emission scanning electron microscope

FESEM (INSPECT F50, FEI company, Netherland) was used to examine the distribution of MCC powder inside the silicone matrix. Two specimens were scanned (Control and 2% wt. MCC).

I. Statistical analysis

Statistical analysis was performed using SPSS version 21. The analysis included descriptive statistics and one-way ANOVA, followed by post hoc tests using Dunnett's test to determine intergroup significance.

Statistical significance levels were set at $P > 0.05$ for non-significant NS, $P \leq 0.05$ for significant S, and $P \leq 0.01$ for highly significant HS findings.

Results

1. Bacterial adherence test

Both experimental groups, B and C, exhibited significantly lower mean counts of adherent bacterial cells compared to group A. Group C displayed the lowest mean count, with a value of 13.49, followed by group B with a value of 21.41. In contrast, the control group exhibited the highest mean count of adherent bacterial cells, with a value of 80.71 (Table 1).

Using an inverted light microscope, adherent cells were quantified for each specimen, adhering to the prescribed device scale for precise measurements. Subsequently, the mean cell count was determined, consistent with the device's specified scale calibration for accurate data analysis (Figure 5).

2. Tear strength

There was a decrease in tear strength for groups B and C respectively compared to group A. ANOVA test was significant indicating a high difference between the groups. Dunnett's test revealed significant differences between group A and groups B and C, while non-significant difference was found between group B and C (Table 2).

3. Shore A hardness

In both experimental groups B and C, the mean values notably surpassed those of the control group A, with group C showing the highest mean value among all groups. Statistical analysis, including a one-way ANOVA, indicated a highly significant difference. Further post-hoc Tuckey's test revealed a significant difference only between group A and C (Table 3).

4. Field emission scanning electron microscope

Scanning electron microscope revealed random distribution of MCC inside the silicone matrix with some agglomeration (Figure 6).

Discussion

The significant decrease in S. epidermidis counts following the addition of microcrystalline cellulose (MCC) powder to the silicone elastomer can be attributed to various factors. Firstly, MCC possesses inherent antimicrobial properties owing to its physical structure and chemical composition, including tiny crystalline particles that can create an unfavorable environment for bacteria (25).

MCC's high surface area and porous structure may disrupt bacterial cell walls and membranes, potentially leading to cell damage or death. Furthermore, MCC act as a mechanical barrier, physically preventing the adhesion of bacteria and colonization on the surface of silicone. This barrier interferes with the initial attachment of bacteria, making it difficult for them to maintain biofilms (26). In addition, MCC's hygroscopic nature allows it to absorb and retain moisture, limiting water availability that is required for the growth of bacteria (27). Moreover, the integration of MCC can alter the surface properties of the material, potentially increasing surface roughness or reducing surface energy, making the surface less favorable for bacterial attachment and formation of biofilm (28). Chemical interactions between MCC and bacteria or their metabolic products can disrupt bacterial functions or inhibit bacterial growth, although these

mechanisms would require further investigation. In addition, MCC particles may occupy bacterial attachment sites on the elastomer surface, reducing available sites for the adhesion of bacteria, and possibly interfering with nutrient diffusion, limiting bacterial proliferation (29). Lastly, the presence of MCC may disrupt established bacterial biofilms, facilitating the detachment and removal of adherent bacteria. These combined factors contribute to the observed decrease in S. epidermidis counts upon the addition of MCC to the silicone elastomer.

The decrease in tear strength of silicone elastomer upon the addition of 1% and 2% microcrystalline cellulose (MCC) can be explained through several mechanisms. Silicone elastomers are inherently cross-linked polymers, which provide them with their characteristic elasticity and tear resistance. The introduction of MCC, a solid particulate material, disrupts the uniform cross-linking structure within the elastomer. MCC particles act as physical obstacles, hindering the formation of strong cross-links between polymer chains, thereby weakening the overall structure and reducing tear strength (30). The interfering that was caused by MCC weakens the cohesive forces between polymer chains, making them more susceptible to separation when subjected to tensile forces which further contributes to the reduced tear strength. Additionally, MCC, especially at higher concentrations such as 2%, can generate stress concentration points inside the elastomer matrix, which, when subjected to external forces, can initiate and propagate cracks, ultimately reducing tear strength (31).

The increase in hardness values microcrystalline cellulose additions can be related to the fact that MCC acts as filler material, thus increasing the volume fraction of solid particles inside the matrix. This effect can decrease the spaces between chains which results in a denser material (32).

In addition, MCC particles promote improved cross-linking between polymer chains, increasing the network density. This increased cross-linking contributes significantly to the stiffness and hardness of the material (33). Furthermore, the incorporation of microcrystalline cellulose has the ability to limit the movements of polymer chains, reducing ability to move and resulting in a harder material (34). Lastly, the surface of MCC can form strong bonds with the matrix which leads to an increased interfacial bonding, which further reinforces the structure and hardness of the material.

Conclusions

In conclusion, the observed significant reduction in the counts of Staphylococcus epidermidis can be related to multiple mechanisms, including MCC's antimicrobial properties, mechanical barrier effects, moisture absorption, surface properties alterations, and potential chemical interactions. However, it's important to note that the incorporation of MCC led to a reduction in tear strength and an increase in hardness of the silicone elastomer, which may limit its applicability in certain contexts where mechanical properties are critical.

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Future scope

Future research could search for methods to limit the adverse effects of MCC on mechanical properties while maximizing the antibacterial properties. Additionally, investigating the long-term durability and biocompatibility of MCC-modified silicone in relevant clinical settings would be valuable for its potential use in maxillofacial prosthetics.

Figure 1: S. epidermidis appearance on blood agar.

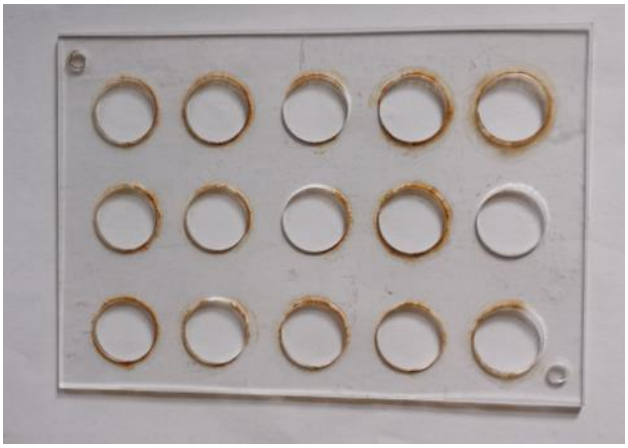

Figure 2: Acrylic mold for bacterial adherence test.

Figure 3: MCC powder added first to be mixed with silicone base.

Figure 3: Tear strength test.

Figure 4: Shore A hardness testing.

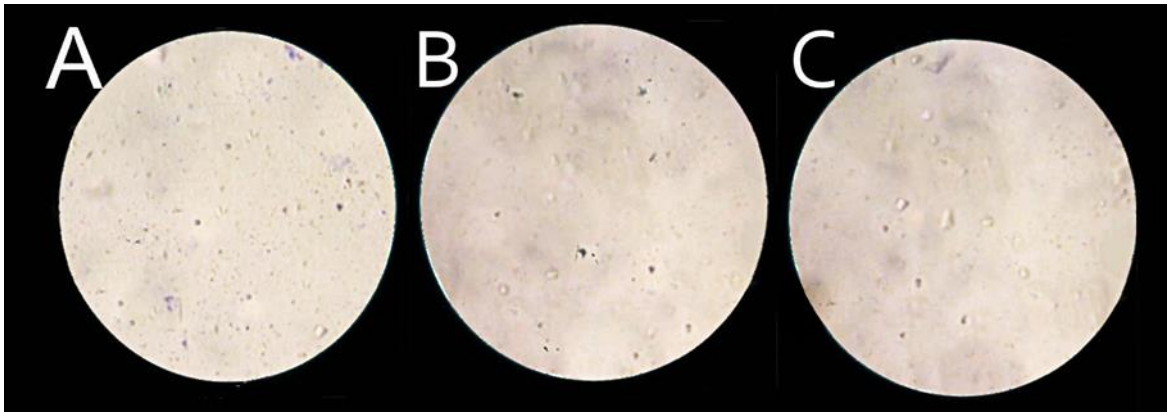

Figure 5: Inverted light microscope, Groups A, B, and C.

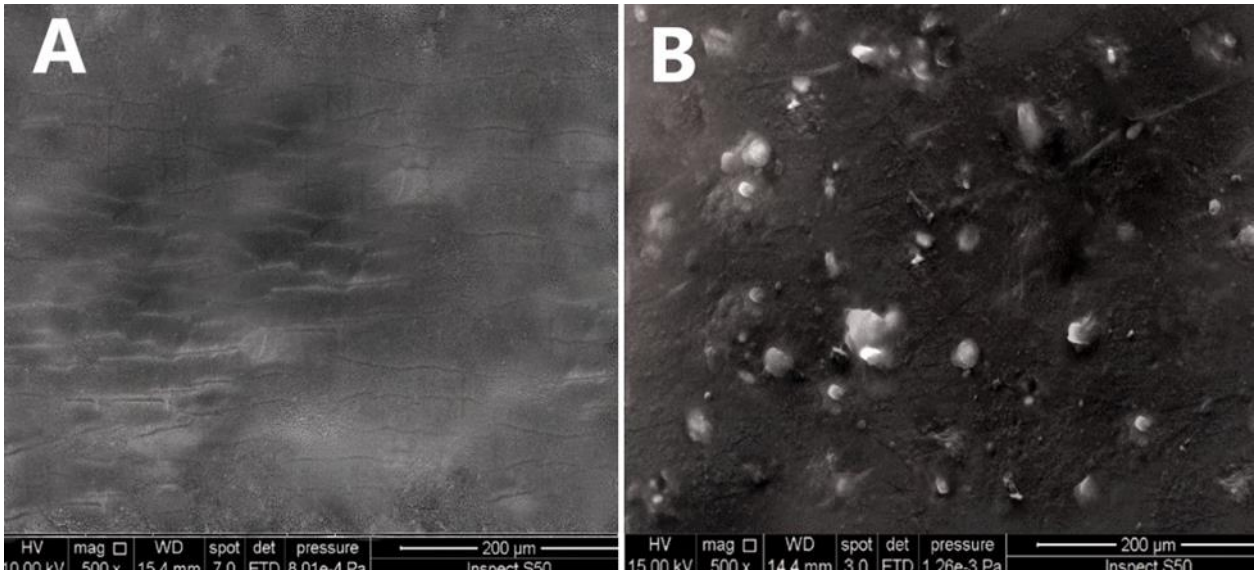

Figure 6: Field emission scanning electron microscope images, A. Control specimen (Group A), B. Silicone after addition of 2% MCC (Group C).

Table 1: Descriptive statistics, one way ANOVA, and Dunnett's post hoc test for bacterial adherence test.

Bacterial adherence test			ANOVA		Dunnett's post hoc	
Group	**Mean**	**±SD**	**F**	**P value**	**Groups**	**P value**
Group A	80.7100	1.78057	7905.179	.000	A B	0.000
Group B	21.4150	1.34434			A C	0.000
Group C	13.4900	.37918			B C	0.000
Levene statistics=10.059, p value=0. .001 HS						

Table 2: Descriptive statistics, one way ANOVA, and Dunnett's post hoc test for tear strength.

Tear strength			ANOVA		Dunnett's post hoc	
Group	**Mean**	**±SD**	**F**	**P value**	**Groups**	**P value**
Group A	30.8800	1.38287	10.218	.000	A B	0.022
Group B	27.8900	2.68730			A C	0.000
Group C	26.6200	2.21600			B C	0.588
Levene statistics=3.737, p value=0.037 S						

Table 3: Descriptive statistics, one way ANOVA, and Tuckey's post hoc test for shore A hardness.

Shore A hardness			ANOVA		Tuckey's post hoc	
Group	**Mean**	**±SD**	**F**	**P value**	**Groups**	**P value**
Group A	34.9500	3.33974	5.576	.009	A B	0.059
Group B	37.5800	1.82562			A C	0.009
Group C	38.4700	1.88093			B C	0.699
Levene statistics=2.700, p value=0.085 NS						

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