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# Evaluating the Effect of Copper Oxide Nanoparticles after Added to the Maxillofacial Silicone on the Adherence of *Staphylococcus Epidermidis*

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## ABSTRACT

***Background:*** *Maxillofacial defects may arise from congenital, developmental, traumatic, or surgical procedures. The presence of such defects may adversely affect an individual's appearance and function, resulting in an inability to lead a typical life and impacting their psychological well-being. Surgical reconstruction is often regarded as the primary therapeutic option in such instances. However, it may not be feasible in numerous cases due to various unfavorable conditions. Consequently, the need for maxillofacial prosthesis reconstruction becomes obligatory.* ***Purpose****: This study assesses the antibacterial efficacy of copper oxide nanoparticles at different rates against Staphylococcus epidermidis after incorporation into maxillofacial silicone.* ***Methods****: A pilot study was first conducted in Iraq and copper oxide nanoparticles were added to VST50F silicone elastomer in different five percentages (0.01 wt%, 0.02 wt%, 0.03 wt%, 0.04 wt%, and 0.05 wt%). Thirty specimens were prepared and grouped into six groups: one control group and five experimental groups, and then the best effective two percentages (0.03 wt% and 0.04 wt%) were selected for the main study. Thirty specimens for the main study were divided into three groups: control group (A) and two experimental groups (B and C). A statistical analysis was done with an ANOVA and the Games-Howell multiple comparison test ($P < 0.05$). The data's normal distribution and homogeneity were assessed. Field emission scanning electron microscopy (FES-EM) and Fourier transform infrared spectroscopy (FTIR) were also conducted.* ***Results****: The statistical analysis showed a significant difference between all groups with $P < 0.05$. There was a significant difference between control group A and experimental groups B and C with $P < 0.05$, as well as between experimental groups B and C ($P < 0.05$). FESEM showed that nanoparticles were distributed well within the silicone matrix. FTIR spectra proved no chemical reaction to occurr between the copper oxide nanoparticles and VST50F silicone.* ***Conclusion****: Incorporating copper oxide nanoparticles into VST50F maxillofacial silicone improved their antibacterial efficacy against Staphylococcus epidermidis.*

**Keywords: Maxillofacial Prothesis; Nanoparticles; Silicone Elastomers; *Staphylococcus Epidermidis***

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## INTRUDUCTION

Maxillofacial defects may arise from congenital, developmental, traumatic, or surgical procedures. The presence of such defects may adversely affect an individual's appearance and function, resulting in an inability to lead a typical life and impacting their psychological well-being (1). Surgical reconstruction is often regarded as the primary therapeutic option in such instances. However, it may not be feasible in numerous cases due to various unfavorable conditions. Consequently, the need for maxillofacial prosthesis reconstruction becomes obligatory (2). Polymeric materials are commonly employed in the fabrication of maxillofacial prostheses., the aforementioned materials encompass vinyl chloride polymers and co-polymers, acrylic types such as polymethyl methacrylate, and silicone elastomers, which can be classified into either high temperature vulcanization or room temperature vulcanization. Silicone elastomers have garnered a significant clinical significance due to their notable attributes such as heat and chemical inertness, durability, strength, elasticity, ease of manipulation, and esthetics (2,3).

However, it is worth noting that there is currently no single maxillofacial material, including silicone, that fully satisfies the criteria for an ideal prosthesis. These materials have encountered challenges such as mechanical deterioration, color instability, limited durability, and changes in properties. Various factors induce the issues, which require frequent replacement of the prosthesis (4). Thus, in order to overcome these issues, it is necessary to reinforce the silicone maxillofacial material. Due to the development of nanoscience and nanotechnology, the application of nanoparticles (NPs) in elastomers has been investigated as a means of improving their properties. Numerous NPs have undergone testing, and subsequent studies demonstrate the efficacy of NPs in enhancing the mechanical, physical, and antimicrobial characteristics of silicone elastomers [5,6]. Microbial biofilms have been identified as significant contributors to the degradation of maxillofacial prostheses Biofilms refer to organized communities of microorganisms consisting of a single or multiple species enclosed in a self-produced exopolysaccharide matrix that adheres to a biotic or abiotic surface (7,8). Candida albicans and Staphylococci spp, especially Staphylococcus epidermidis (S. epidermidis) and Staphylococcus aureus (S. aureus), are the essential causative agents of infections related to prosthetic devices. Mixed biofilms may develop from a variety of factors, including the porosity and roughness of the prostheses (9,10).

*S. epidermidis*, referred to as a gram-positive staphylococcus with coagulase-negative properties, is a highly prevalent bacterium that colonizes the skin of healthy individuals. It can be found in various skin microenvironments, such as dry, moist, sebaceous, and foot regions. It can either aid or harm the skin. S. epidermidis plays a beneficial role in maintaining the equilibrium of microorganisms on the epithelial surfaces of humans by controlling the proliferation of harmful pathogens, especially S. aureus. Conversely, S. epidermidis is recognized as a significant etiological agent responsible for nosocomial infections in humans, which can cause severe infection (11,12). S. epidermidis is commonly implicated in infections associated with the use of indwelling medical devices, implants, and prosthetic devices following adhesion to surfaces and the formation of micro-colonies (13). Coagulase-negative staphylococcal species exhibit a proclivity for invading the human body through prosthetic devices, wherein a restricted population of bacteria migrates along the prosthetic device and gains access to the bloodstream. Bacteria possess the capacity to form biofilms as a means of protecting themselves against immune responses from the host or antimicrobial agents (14). The conventional cleaning procedures employed in the regular maintenance of prostheses are insufficient to eliminate all microorganisms present on the surface (15). Commonly used disinfectants cause deterioration in color, hardening, and significant variations in the surface roughness of silicone prostheses. Therefore, there is a requirement for further developed silicone materials that exhibit a greater resistance to microbial proliferation (16).

The development of microbial resistance to traditional antimicrobial agents has become a prominent issue in contemporary times. The emergence of multidrug-resistant (MDR) infectious microorganisms presents a formidable obstacle to the healthcare system. The utilization of nanomaterials as a viable option for anti-infective purposes has gained credibility owing to their distinctive chemical properties and high surface area to volume ratio. Metal and metal oxide NPs with antimicrobial characteristics are a promising approach for suppressing MDR strains among nanomaterials (17,18).

Copper oxide (CuO) is a chemical compound comprised of copper and oxygen. CuO NPs have the appearance of a brownish-black powder. CuO NPs are photocatalytic, stable, and inexpensive, and they have the potential to be used as anti-infective agents due to their unique crystal morphologies and extremely large surface areas [19]. CuO NPs have a wide range of microbicidal activity against fungi, viruses, and bacteria, including MDR bacteria [20,21]. CuO NPs have been widely investigated in various biomaterials. Furthermore, several studies have provided evidence that the incorporation of CuO NPs into various dental materials yields a notable decrease in microbial biofilm formation and potentially enhances the materials' physical and chemical characteristics [22]. Nonetheless, there is a lack of data regarding their incorporation into maxillofacial silicone. The objective of this investigation was to assess the impact of CuO NPs on the adhesion of *S. epidermidis* biofilm subsequent to their incorporation into VST 50F maxillofacial silicone at different weight percentages.

## METHODS

CuO NPS (40 nm) (Sky Spring Nanomaterials, USA) and VST 50F room temperature vulcanized maxillofacial silicone (FactorII Inc., USA) were used. A preliminary study was conducted to determine two optimal percentages to be added to VST 50F silicone elastomer in the main study. CuO NPs were added to VST 50F silicone elastomer in five different percentages (0.01 wt%, 0.02 wt%, 0.03 wt%, 0.04 wt%, and 0.05 wt%). Thirty specimens were meticulously prepared and subsequently categorized into six groups: one control group and five experimental groups, with five specimens for each group. A bacterial adherence test was done for all the six groups. According to the result of the pilot study, two percentages of CuO NPs additives (0.03 wt% and 0.04 wt%) were selected in addition to the control group.

A total of thirty specimens for the main study were prepared and categorized into three groups: one control group (A) and two experimental groups, group B (0.03 wt% CuO additive) and group C (0.04 wt% CuO additive). The shapes and dimensions of the test specimen molds were generated through the utilization of computer-aided software, specifically AutoCAD 2013 (Autodesk, USA). The template was subsequently constructed via a laser engraving cutting machine. (CNC) (JL-1612, Jinan Link Manufacture and Trading Co., Ltd., China). Three clear acrylic sheets (PT. Margacipta Wirasentosa, Indonesia) (the matrix, bottom, and cover) with 2±0.05 mm thickness were used. The matrix sheet was fabricated with circular perforations measuring 10 mm in diameter and was fixed to the bottom using chloroform (Weld-On, USA) as an adhesive to prevent movement during the pouring of silicone (Fig 1A). G-clamps, nuts, and screws were also utilized to fix the cover and provide further tightening (23).

According to the manufacturer's instructions, VST 50F silicone should be mixed at a 10:1 base to catalyst ratio. The CuO NPs powder was initially weighed in a mixing bowl using a 4-digit electronic scale (Denver Instrument, USA). Subsequently, a predetermined amount of silicone base was added to prevent the dispersion of the volatile NPs. The NPs were blended with the silicone base to produce the modified base, which was mixed for 10 minutes using a vacuum mixer (Dentaurum Airvac, Germany). To prevent the suction of NPs, the vacuum was switched off for the initial three-minute period. Subsequently,

the vacuum was activated for a duration of seven minutes, operating at a speed of 360 rpm and a vacuum pressure of (10-1) bar, with the intention of eliminating any air bubbles (24). The mixture was allowed to cool down for 2 minutes before adding catalyst. This delay was necessary due to the heat generated by the rotational motion of the mixer, which has the effect of reducing the material's available working time. The addition of the catalyst was performed based on weight and subsequently mixed with the modified silicone base for a duration of 5 minutes using a vacuum mixer. This process yielded a uniform and devoid-of-bubble mixture.25 The material was poured into the mold under standard conditions, which included a restricted temperature of 23±2°C and a humidity level of roughly 50±10% (25). The matrix cover was affixed over the poured material by positioning the margin on one side and gradually lowering it at the opposite end. The matrix was covered slowly and carefully to allow excess material to exit the mold. Then, a one-kilogram weight was placed on the mold cover's center. Nuts and G-clamps secured the mold until the specimens hardened (5). In accordance with the manufacturer's recommendations, the mixture was allowed to set for 2-4 hours, after which the specimens were retrieved, finished, and stored for 16 hours 20±25°C, 50 ±10% humidity according to ISO 23529 (26).

Bacterial adherence test Bacteria were collected from four male patients aged 50–55 years at Ghazi al-Hariri hospital in Baghdad's medical city using sterilized cotton swabs. To avert necrotic tissue, the swab was rotated across the infected area.27 The samples were inoculated into blood agar and mannitol salt agar (Oxoid, UK) that had been prepared following the manufacturer's instructions and then incubated aerobically at 37°C for 48 hours (Fig 1B) (28). S. epidermidis exhibits a rapid growth rate on blood agar, leading to the formation of non-hemolytic, cohesive colonies that are white in color and measure approximately 1-2 mm in diameter (29). While producing small pink, red, or colorless colonies with no color change to the medium after overnight incubation in mannitol salt agar (30). The positive catalase test indicated the presence of staphylococci, which was subsequently confirmed utilizing the VITEK 2 compact system (Biomerieux, France) pursuant to the guidelines provided by the manufacturer. A microscopical examination was also performed, and S. epidermidis appeared as cocci in grape-like clusters.

Brain heart infusion broth (Mast Group, UK) was used to prepare the bacterial suspension that would be utilized to test S. epidermidis' bacterial adhesion capacity on silicone specimens. It was prepared through the suspension of 34.5 g in 1000 ml of distilled water, followed by thorough mixing until complete dissolution was achieved. The sterilization process was carried out through autoclaving at a pressure of 15 pounds (lbs) and a temperature of 121°C for a duration of 15 minutes, according to the manufacturer's instructions. A suspension with a concentration of 107 colony forming units per milliliter (CFU/ml) was prepared at 0.5 McFarland standards using a McFarland densitometer (Biomerieux, France).

The silicone specimens underwent sterilization by autoclaving at 121°C for 20 minutes. Then the sterilized silicone specimens were deposited in sterilized plastic petri dishes containing the prepared bacterial suspension; the specimens were incubated for 1 hour at room temperature.22 Following the incubation period, the specimens were removed from the bacterial broth, rinsed with phosphate buffered saline for one minute with gentle rocking to remove all non-adhered bacterial cells, and dried with filter paper.22 The specimens then underwent staining with 1% crystal violet (HIMEDIA, India) for 15 minutes, and rinsed until no crystal violet residue remained in the washing solution, and dried with filter paper. Subsequently, the specimens were immersed in ethanol (96%) (Microm Microtech, France) for 20 minutes to solubilize the crystal violet [31-33].

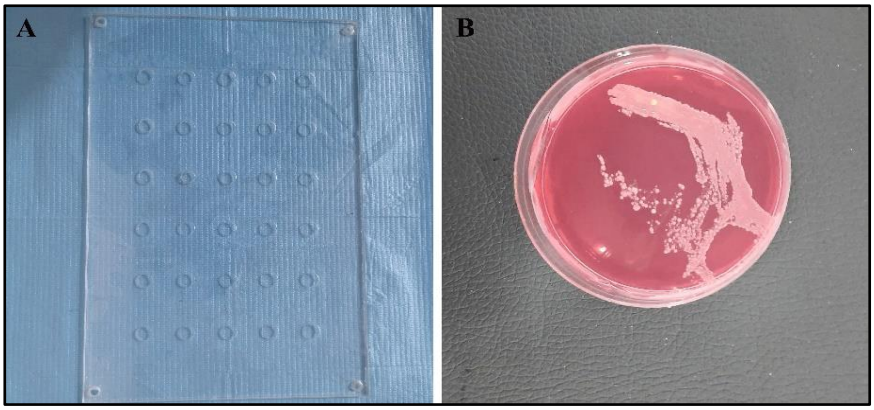


**Figure 1. A: Specimens mold; B: Staphylococcus epidermidis on mannitol salt agar**

## RESULTS

***Field emission scanning electron microscope (FE-SEM)*** A FE-SEM (Inspect F50, FEI, USA) was used to examine the topography and morphology of CuO NPs within the matrix of RTV silicone elastomer (VST 50F) specimens after the addition of CuO NPs. Silicone specimens were coated with metal or gold by a thin (1nm) metal sputter coated film by a sputter coated device, allowing electrons from FE-SEM to react with the specimens as directed by the company. In the FE-SEM pictures of the VST 50F silicone matrix, the CuO NPs were uniformly dispersed with some slight agglomeration, as seen in Fig 2 (A, B, and C).

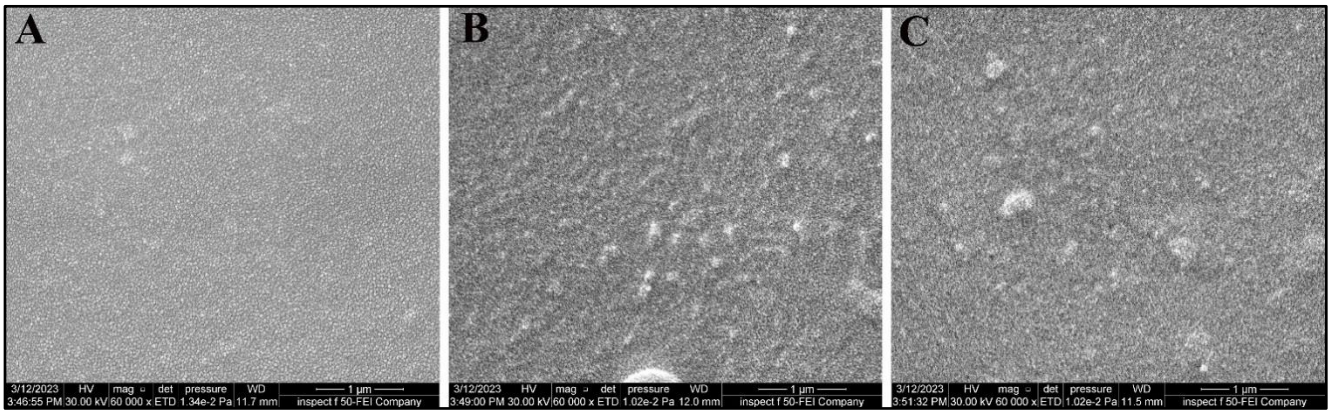


**Figure 2. Images of field emission scanning electron mi croscope images at 60000 X magnifications (1 µm) showing an evenly distributed filler with a slight agglomerate: A: control specimen; B group B specimen C: group C specimen**

***Fourier transform infrared spectroscopy (FTIR)***

FTIR (Spectrum Two N, PerkinElmer, USA) was used to investigate the potential chemical interaction between the silicone material and CuO NPs. Three specimens, one from each group (A, B, and C), were examined. The silicone specimens were fabricated as thin flushes with dimensions of 10x10x0.5 mm in length, width, and thickness, as per the manufacturer's instructions. FTIR results indicate that the addition of CuO NPs had no effect on the spectra of VST 50F silicone (no chemical interaction), as indicated in Fig 3 (A, B and C).

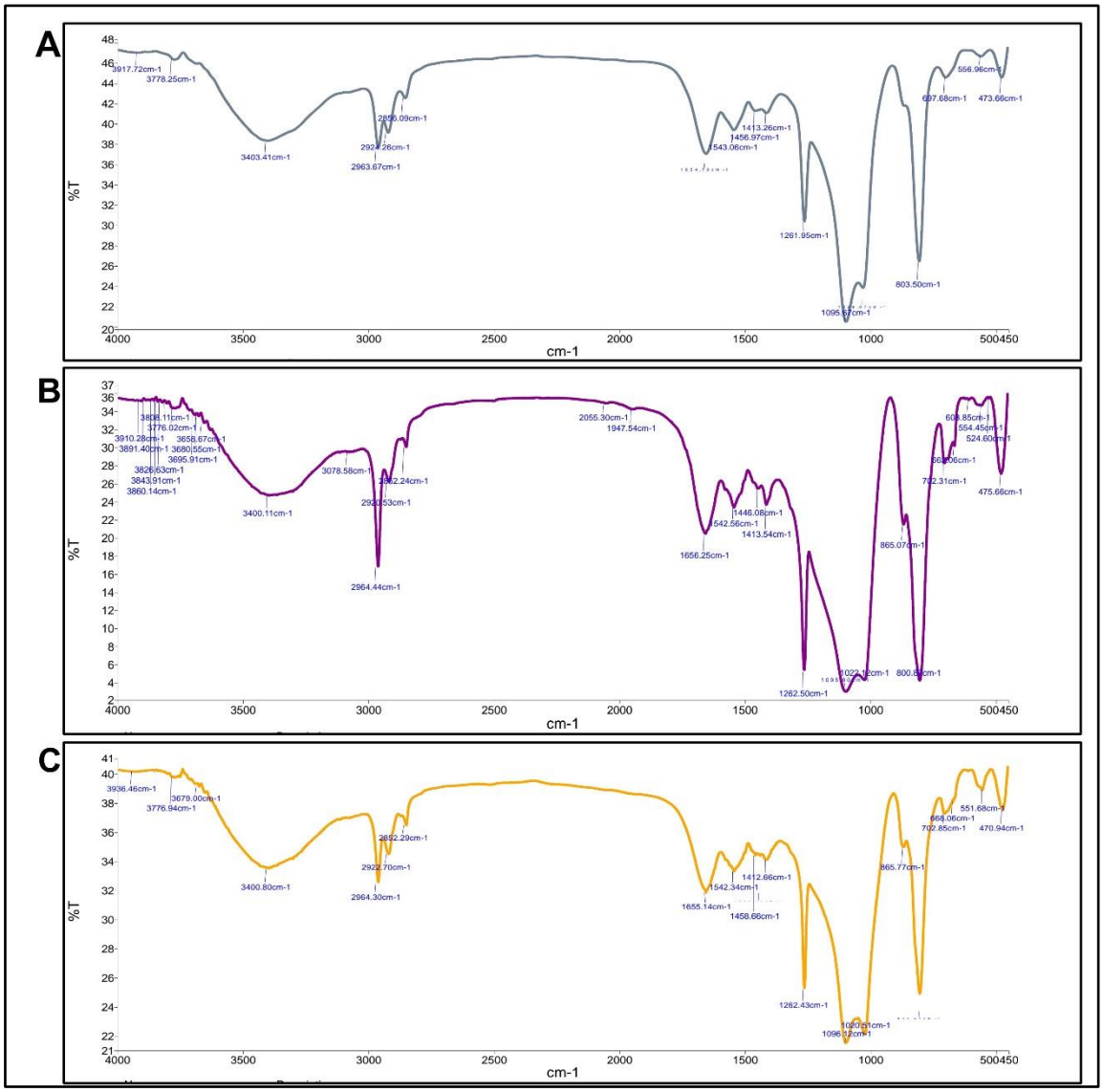


**Figure 3. Fourier transforms infrared spectroscopy: A: control specimen (group A); B: group B specimen; C: group C specimen.**

***Statistical analysis***

The statistical analysis was done by SPSS (statistical package for social science version 24) (IBM, USA) computer software. A probability "*P*" value of > 0.05 was deemed as non-significant statistically, ≤ 0.05 as significant.All variables in this this study were normally distributed among groups using Shapiro -Wilk test at $P > 0.05$ (Tab 1).

The results of the *S. epidermidis* adherence test showed that both experimental groups B and C had a mean value of OD less than that of control group A. It was observed that group C exhibited the lowest mean value among them and had a value of (0.012), followed by group B (0.029), and then group A with a mean value of (0.161), as shown in Fig. 4 and Tab 2.One-way ANOVA showed a significant difference among groups with a $P < 0.05$ (Tab 3).

Levene's test is used to assess variance homogeneity and, as a result, to determine the type of multiple comparisons post hoc test to use (Tab 4). Post-hoc Games-Howell test showed a significant difference between groups ($P < 0.05$) (Tab 5).

**Table 1. Test of normality.**

| Groups | Statistic | df | *P*-value |
|---|---|---|---|
| Group A | 0.944 | 10 | 0.593* |
| Group B | 0.937 | 10 | 0.523* |
| Group C | 0.968 | 10 | 0.872* |

*= Non-significant at $P > 0.05$.

**Table 2. Descriptive statistics of bacterial adherence test (optical density).**

| Groups | N | Mean | Std. Deviation | Std. Error | Minimum | Maximum |
|---|---|---|---|---|---|---|
| Group A | 10 | 0.1612 | 0.020275 | 0.006411 | 0.133 | 0.190 |
| Group B | 10 | 0.0292 | 0.004566 | 0.001444 | 0.020 | 0.035 |
| Group C | 10 | 0.0127 | 0.002497 | 0.000790 | 0.009 | 0.017 |
| Total | 30 | 0.0677 | 0.068591 | 0.012523 | 0.009 | 0.190 |

**Table 3. One-way ANOVA analysis of variance among groups.**

| | Sum of Squares | df | Mean Square | F | *P*-value |
|---|---|---|---|---|---|
| Between Groups | 0.132 | 2 | 0.066 | 453.600 | 0.000** |
| Within Groups | 0.004 | 27 | 0.000 | | |
| Total | 0.136 | 29 | | | |

**= Significant at $P < 0.05$.

**Table 4. Levene's test (homogeneity of variance).**

| | Levene Statistic | df1 | df2 | *P*-value |
|---|---|---|---|---|
| Based on Mean | 18.749 | 2 | 27 | 0.000** |
| Based on Median | 17.756 | 2 | 27 | 0.000** |
| Based on Median and with adjusted df | 17.756 | 2 | 10.824 | 0.000** |
| Based on trimmed mean | 18.742 | 2 | 27 | 0.000** |

**= Significant at $P < 0.05$.

**Table 5. Multiple comparisons of bacterial adherence test between groups using Games-Howell test.**

| (I) Groups | (J) Groups | Mean Difference (I-J) | Std. Error | *P*- value |
|---|---|---|---|---|
| Group A | Group B | 0.132000 | 0.006572 | 0.000** |
| | Group C | 0.148500 | 0.006460 | 0.000** |
| Group B | Group C | 0.016500 | 0.001646 | 0.000** |

**= Significant at $P < 0.05$.

## CONCLUSION

Based on the findings of this study, the addition of CuO NPs to the maxillofacial silicone displayed antibacterial activity *in vitro* against *S. epidermidis*. CuO NPs in small percentages seem to significantly inhibit bacterial adhesion to the surface of VST 50F silicone elastomer and can serve as a basis for future long-term investigations regarding antimicrobial effectiveness.

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