



Effect Of Chlorhexidine Mouth Wash and Silver Nanoparticles on Bacterial Count in Maxillary Cleft Patients. A Cross-Sectional Study

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ABSTRACT

Aims: To evaluate the effect of chlorhexidine silver nanoparticles on bacterial count in maxillary cleft patients. **Methodology:** Ten patients with cleft palate received three different care modalities; (M1) (Control) Obturator lined by soft liner, (M2) Obturator lined by soft liner and used 0.125% chlorhexidine HCl mouth wash two times daily, while in (M3) Obturator lined by soft liner containing 0.1% silver nano particles (Ag-Nps). For each patient the samples were collected after 4 weeks of treatment with each modality, and the number of Colony Forming Unit (CFU) of (*Streptococcus mutans*) (*Lactobacillus acidophilus*) (*Prevotella intermedia*) was calculated (Five samples each). **Results:** The number of CFU was the least in (M3) followed by (M2) and (M1) Respectively in all organisms tested in this study. **Conclusions:** Addition of 0.1% silver nanoparticles in denture liner serves as a good antimicrobial agent in cleft palate patients.

KEY-WORDS: cleft palate, chlorhexidine mouth wash, silver nanoparticles & soft-liners.

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INTRODUCTION:

Cleft palate (CLP) significantly affects oral functions such as chewing, speech, aesthetics, and psychological well-being (1). In Egypt, the incidence of congenital cleft palate is approximately 0.4 per 1000 births, lower than in European and North American populations (1 per 1000 births) (2).

Surgical repair is the primary treatment for cleft palate (3). Nonetheless, complications such as oronasal fistula (ONF) can occur post-surgery. An irregular junction among the nasal and oral cavities, extending from the uvula up to the alveolar process, is known as ONF (4). It can also result from trauma, tumors, infections, and other conditions (5). Surgical repair is the preferred treatment, but for large ONF, it may not be feasible due to factors like age and cost (6). Following ONF repair, recurrence rates are significant, varying from 0% to 43% (7, 8).

Given the limitations of surgery and the high recurrence rates, obturators are a viable alternative. Obturators should fit comfortably, stabilize well, and not impede speech. They should also be designed and fabricated quickly and accurately (9).

Polymethyl methacrylate (PMMA) is commonly utilized for obturators because of its stability, affordability, minimal density, and appearance (10). Biocompatibility is another critical factor, PMMA materials that have been appropriately cured have high biocompatibility because of their low monomer content (11, 12).

Soft liners act as cushions, distributing masticatory forces and reducing stress on underlying tissues, promoting mucosal healing and patient comfort (13, 14). They also improve denture stability and chewing efficiency (15). However, soft liners are prone to colonization by yeasts and other pathogens (16).

Cleft lip and/or palate patients often have poor oral hygiene and higher concentrations of periodontopathogens in oral biofilms (17-19). Obturator wearers are particularly susceptible to pathogen colonization, which can lead to conditions like denture stomatitis and material degradation (20). Common pathogens in cleft patients include *Prevotella*, *Lactobacillus* and *Streptococcus* species (21-23). Maintaining soft liners and preventing microbial colonization are crucial for oral health (24, 25).

Silver nanoparticles (Ag-NPs) have shown significant antimicrobial properties, making them promising for use in dental materials (26). Silver has long been known for its antimicrobial

effects, which have been scientifically validated (27). Chlorhexidine mouthwash is another effective method for reducing dental plaque and managing gingivitis (28, 29). Chlorhexidine (CHX) is a cationic agent that disrupts bacterial cell walls (30).

The present research aimed to compare the antimicrobial efficacy of denture liners with silver nanoparticles versus chlorhexidine mouthwash in patients with acquired palatal defects. The null hypothesis was that there would be no variation in CFU counts of *Streptococcus mutans*, *Lactobacillus acidophilus* and *Prevotella intermedia* across the three treatment modalities.

SUBJECTS AND METHODS:

Sample size calculation:

A power analysis was done utilizing G*Power software (v3.1.9.2) based on previous studies (31). With a power of 95% and a significance level of 0.05 with an effect size of $f = 1.98$ the required sample size was ten patients.

Study Design:

Ten patients with acquired cleft palate from the Oral and Maxillofacial Department at Al Azhar University, Cairo, Egypt, were enrolled. Laboratory procedures were performed at the Medical Microbiology and Immunology Department, Al Azhar University. Informed consent was obtained from all participants.

Each patient received three treatment modalities: (M1) (Control): Obturator lined with a soft liner, (M2) Obturator lined with a soft liner and 0.125% chlorhexidine HCl mouthwash twice daily and (M3) Obturator lined with a soft liner containing 0.1% silver nanoparticles.

After 4 weeks of each treatment, fifteen swabs were collected from each patient (five per organism: *Streptococcus mutans*, *Lactobacillus acidophilus* and *Prevotella intermedia*).

Obturator Construction:

Preliminary impressions were made using alginate, and diagnostic casts were created. A special tray was fabricated, and low-fusing impression compound (Hiflex, Prevest, India) was utilized for peripheral tracing of upper arch followed by the tracing of defect area (32).

The participant was asked to move his head in a circular motion, extend it forward and downward, followed by talk. These motions were used for recording the faulty region. After trimming off any extra material, the motions were repeated till consistent contact was achieved.

Lastly, light body condensation silicone impression material (Alphasil Muller, Omicron, Germany) was used to create a wash impression. A modified master cast composed of type III dental stone was acquired followed by wax-up and casing of Co-Cr framework which was tried in in the patient. Facebow and jaw relationship records were acquired and transmitted to a semi-adjustable articulator. The teeth were set up and tried in the patient mouth (33). Obturator base was processed using heat-cured acrylic resin (Vertex Regular. Vertex-Dental B.V. Netherland) (Figure 1).

The stability and retention of the denture were assessed at the moment of placement and acrylic soft-liner conditioner was applied (GC soft liner, Tokyo, Japan). To modify the liner with 0.1% V/V of Ag-Nps (Nano streams, Egypt), the nanoparticles are added to the liquid of soft liner before mixing. The shape of nanoparticles used in this study were spherical with size <20 nm. By requesting the patient to perform the functional motions, bulb extension was assessed. Throughout the assessment, the patient did not suffer any tongue hindrance, gagging, or breathing difficulties (34).



Figure (1): Finished Obturator

Sample Collection:

A Sterile swabs were rotated within the deepest part of the cleft, maintaining 0.5 cm around its perimeter then placed in the transport medium, the samples were immersed in a test tube filled with broth media and gently agitated for two minutes using a vortex mixer. The bacterial count was performed utilizing the plate count method, which involves transferring the bacteria from the test tube to Agar media followed by spreading over the plates. The number of Colony-Forming Units (CFU) were then calculated (35) (Figure 2).

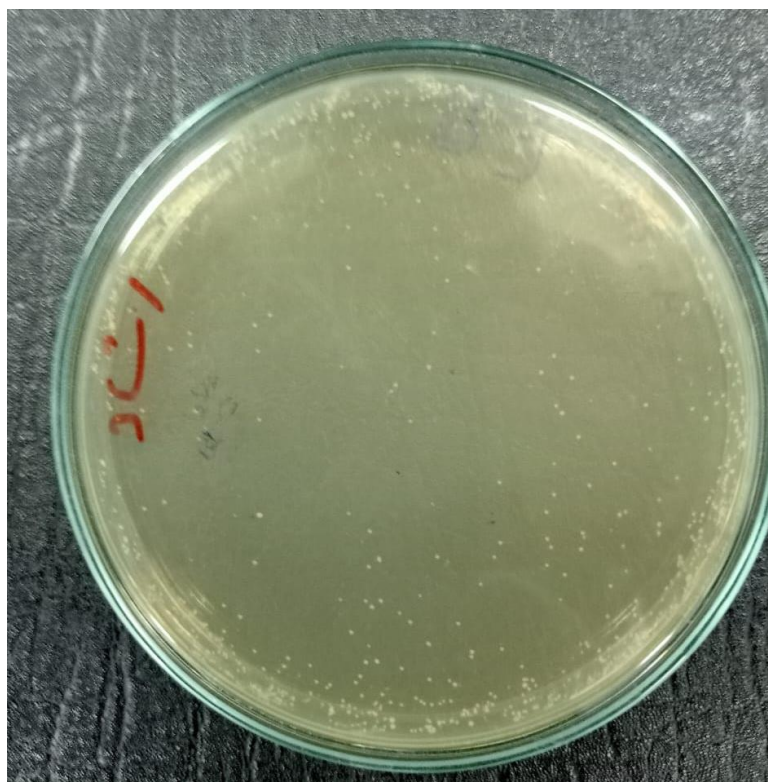


Figure 2: *Streptococcus mutants* colonies

Statistical analysis:

The SPSS© software (Chicago, IL, USA) was used to statistically analyze the data (version 20 for Windows). The Shapiro-Wilk test was used to determine the normality of the data distribution, and the results indicated a normal distribution. One way ANOVA was for done between the three modalities for each organism and pairwise comparison was done using post-hoc Tukey test at ($p < 0.05$).

RESULTS:

The mean values and standard deviations of colony forming units of organisms CFU are shown in are presented in Table (1) and graphically represented in figure (3). The mean CFU of *Streptococcus mutans* was (9412583.5 ± 873530.3) for M1, and (406302.5 ± 67681.5) (6659.7 ± 678.1) for M2 and M3 respectively. For *Lactobacillus acidophilus* the mean CFU was (8019014.4 ± 940914.8) for M1, and $(7602149.7 \pm 1035298.8)$ 50051.3 ± 9799.8 for M2 and M3 respectively. For *Prevotella intermedia* the mean CFU was (6816257.1 ± 116155.1) for M1, and $(5801100.7 \pm 2531781.9)$ (71996.3 ± 4042.5) for M2 and M3 respectively. The least colonization was observed in M3 modality followed by M2 and M1 respectively. The results of One-Way ANOVA displayed a statistically significant variation across all modalities in all tested organisms (Table 2) ($p \leq 0.05$).

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Table 1:Mean and SD of CFU of the three organisms used in this study

Modality	M1	M2	M3
Organism	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
<i>Streptococcus mutans</i>	9412583.5 $\pm$ 873530.3	406302.5 $\pm$ 67681.5	6659.7 $\pm$ 678.1
<i>Lactobacillus acidophilus</i>	8019014.4 $\pm$ 940914.8	7602149.7 $\pm$ 1035298.8	50051.3 $\pm$ 9799.8
<i>Prevotella intermedia</i>	6816257.1 $\pm$ 116155.1	5801100.7 $\pm$ 2531781.9	71996.3 $\pm$ 4042.5

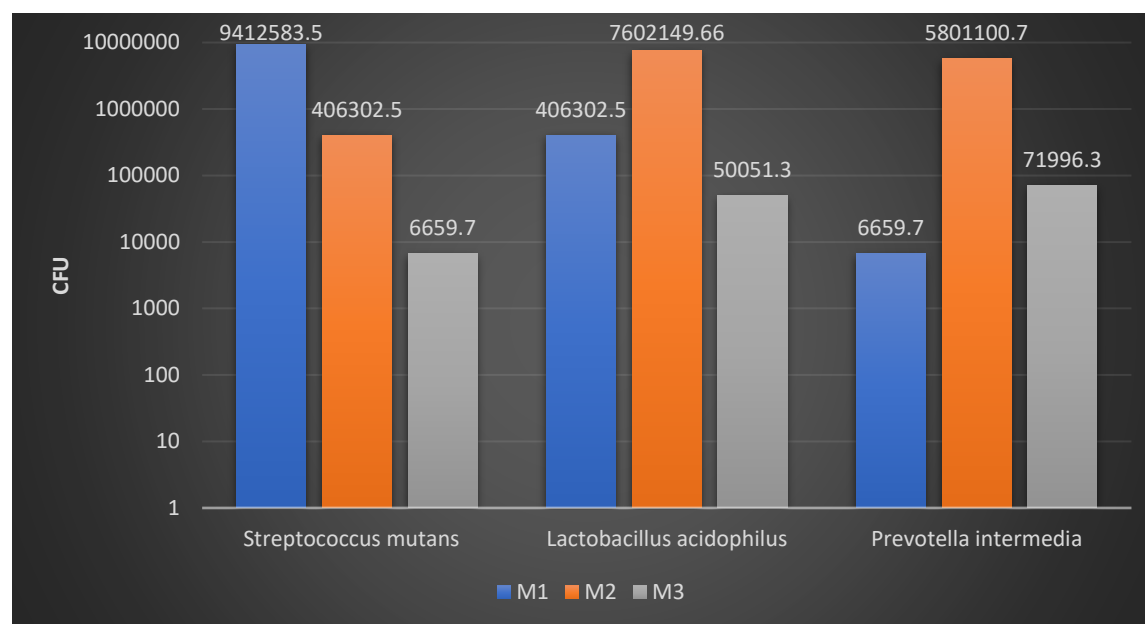


Figure 3:Mean CFU of the three organisms used in this study

Table 2: One way ANOVA with post hoc Tukey test pairwise comparison

ANOVA				
		F	Sig	
<i>Streptococcus mutans</i>		5528.1	.000	
<i>Lactobacillus acidophilus</i>		1541.9	.000	
<i>Prevotella intermedia</i>		308.8	.000	
Post hoc Tukey test				
Organism		<i>Streptococcus mutans</i>	<i>Lactobacillus acidophilus</i>	<i>Prevotella intermedia</i>
Modality		Sig.	Sig	Sig
M1 vs	M2	.000	0.029	0.002
	M3	.000	.000	.000
M2 vs	M3	.000	.000	.000

DISCUSSION:

A soft liner is a robust, soft, elastic substance that makes up all or a portion of a removable denture's soft tissue-contacting surface. According to ISO standards, a short-term liner is one that is administered intraorally for a maximum of thirty days. A liner is considered long-term if it keeps its suppleness and suppleness for longer than thirty days (36).

The smallest quantity of stress was transferred to the mucosa underneath if the denture base material's thickness was 3 mm and the soft denture liner was 1 mm and 2 mm thick (37). Individuals with considerable bony prominence, obturation-requiring congenital or acquired oral abnormalities, superficial mental nerve, xerostomia, and bruxing tendencies might benefit from soft liner treatment (38).

Soft liners are utilized to treat thin and non-resilient mucosal tissues as well as fragile and persistently irritated tissues due to their capacity for restoring health to deformed and inflamed mucosal tissues (39).

Ag-NPs is used in this study because of their strong antiviral, antifungal, antimicrobial, and anticancer effects, Ag-NPs have a wide range of applications and are regarded as extremely powerful multifunction components (e.g., in nanomedicine, dental field, ecology, the textile industry, cosmetics, etc.) (40-41). Ag-NPs demonstrated antibacterial properties when added to irreversible hydrocolloid imprint materials (42), denture base acrylic resins (43), tissue conditioners (44), and soft lining materials (45).

It has been demonstrated that a greater release of silver nanoparticles (Ag-NPs) from denture liners occurs at a concentration of 0.1%. This concentration remains below the toxic thresholds associated with silver nanoparticles. In instances of low nanoparticle concentration within the denture liner, a significant number of nanoparticles tend to precipitate outside the polymer chains. It appears that insufficient heat impedes the infiltration of nanoparticles into the polymer chains. Additionally, the denture liner absorbs more water compared to the denture base, resulting in the expulsion of a larger quantity of nanoparticle atoms. Conversely, at elevated nanoparticle concentrations, a higher number of atoms become trapped within the polymer chains, leading to a reduced presence of free nanoparticles outside.(46).

Chlorhexidine is used in this study due to their biguanide-structured, broad-spectrum local antibacterial and cationic surfactant's ability to function in both neutral and alkaline conditions. The compound's antifungal qualities are somewhat weaker than its antibacterial ones (47, 48).

A synthesized cationic bisguanide base that is as stable as salt is chlorhexidine. The positively charged chlorhexidine compound is released when chlorhexidine gluconate, which is water soluble in the majority of oral formulations, dissociates easily at physiological pH. On the other hand, over time, diluting in oral cavities causes the amount of chlorhexidine ions to diminish. Secondly, despite chlorhexidine's strong antibacterial properties, the stubborn nature of plaque prevents it from penetrating the deeper layers and eradicating germs there (49).

Our findings imply that the addition of Ag-NPs had a more antibacterial effect than chlorhexidine mouth wash this may be attributed to the silver nanoparticles range in size from 1 to 100 nm, whereas bacteria are measured in microns. Consequently, the likelihood of nanoparticles coming into touch with bacteria increases as Ag-NPs get smaller, so it is the best reducing agent(50-51).

Being a metal nanoparticle, it stays a little diluted in an aqueous environment and becomes mechanically tangled with plaque. Because silver nanoparticles are tiny, they can enter deeper biofilm layers and eventually cause the microbial cells to be destroyed (52). The antibacterial efficiency of silver nanoparticles against chlorhexidine against *E. faecalis* biofilm demonstrated that the former had superior disinfection power and the ability to eradicate both bacteria and bacterial biofilm (53).

Ag-NPs had the highest antibacterial action with similar bacterial suppression at a concentration 25 times less than that of chlorhexidine, according to a comparison of the cytotoxicity of silver and other nanoparticles with chlorhexidine versus the oral pathogenic variety of *S. mutans*. Bacterial viability was just 2% at an Ag-NP concentration of 0.1 mg/ml, but it was 60% using chlorhexidine (54).

When biologically produced silver nanoparticles and chlorhexidine were tested for their antibacterial properties against *S. mutans* and *L. casei*, it was shown that Ag-NPs suppressed both bacteria more effectively than chlorhexidine. When compared to 20 mg/ml of chlorhexidine

gluconate, Ag-NPs at a concentration of 1 mg/ml had a statistically significant antibacterial activity. (55).

Using silver nanoparticles and chlorhexidine for mouthwash against *S. mutans*, they found that the former had a statistically significant antibacterial effect whereas the latter did not (56).

The samples were collected after 4 weeks since long term use of chlorhexidine mouthwash has a negative impact such as staining of teeth, alteration of taste, and irritation on oral mucosa (57)

Regarding the results, the null hypothesis of the research was rejected as there is significant difference in the effect of silver nano particles modified self-cure soft lining material and Chlorhexidine mouthwash on bacterial count in obturator wearers.

CONCLUSIONS:

When compared to chlorhexidine, silver nanoparticles had a noticeably greater bacteriostatic and bactericidal impact against several oral pathogenic microorganisms. These results imply that, at extremely safe biological concentrations, silver nanoparticles could be employed as an efficient antiplaque agent.

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