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# A COMPARATIVE ANALYSIS OF THE ANTIMICROBIAL EFFICACY OF SILVER AND TITANIUM NANOPARTICLE-INFUSED TISSUE CONDITIONERS

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## ABSTRACT

**Background:** Silver nanoparticles and titanium nanoparticles is a potential antimicrobial that is widely used in several field of medicine. It is a well known agent used in dentistry also to eliminate the oral flora by adding with dental substitute.

**Aims & objectives:** Aim of this study was to identify the in-vitro antimicrobial activity of the tissue conditioner containing silver nanoparticles and titanium nanoparticles on microbial strains, *staphylococcus aureus, streptococcus mutants and candida albicans.*

**Materials & Methods:** Experimental disc samples were placed on culture dish and microbial suspensions (100uL) of tested strains will be inoculated and then were incubated at 37 degree Celsius. Microbial growth was verified at 24 hours and 72 hours and the antimicrobial effects of samples were evaluated as a percentage reduction of cells in withdrawn suspension. Data were recorded as a means of three colony forming unit (CFU) numerations and borderline of the antimicrobial effect determine at 0.1% viable cells.

**Results:** A 0.1% silver and titanium nanoparticles combined with tissue conditioner displayed bactericidal effect against staphylococcus aureus, Streotococcus mutants and Candida albicans . In comparison titanium nanoparticles showed more antimicrobial effect.

**Conclusion:** Within the limitation of this in vitro study, the result suggest that the tissue conditioner containing silver and titanium nanoparticles could be an antimicrobial dental material in denture plaque control.

**Keywords:** Silver nanparticles; Titanium nanoparticles; Tissue conditioner; Antimicrobial effect.

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## INTRODUCTION

Prosthodontics is mostly involved with the interference and treatment of chronic soreness from dentures and with the preservation of the supporting structures. The problem of managing abused

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and compromised tissue in patients with congenital or acquired anatomic abnormalities, systemic deficiencies, detrimental psychologic factors (bruxism), faulty prosthesis, or a mixture of those issues with onerous and rigid polymers was once terribly tough, if impracticable to resolve. With the advent of elastomer polymers, the management of these issues has greatly improved. The softness and suppleness of those materials, as results of their physical and chemical composition, afford the chance to shield the supporting tissues from functional and parafunctional occulsal stresses. There are two general applications of this material: as tissue conditioner and as resilient liners.

For practical purposes, denture base materials are fabricated of rigid materials. Dentist must recognize that prolonged contact of those bases with underlying tissues is absolute to elicit changes within the tissues. Tissue conditioners are commonly accustomed enhance the recovery of denture bearing tissues from trauma, damage or residual ridge resorption usually caused by ill fitting dentures.[1-3]

In 1967, Kydd and Mandley expressed that tissue lining materials allow wider dispersion of forces and hence aids to decrease the force per unit area which it transmits to the supporting tissues.

However, these liners have several drawbacks that seem throughout their clinical use and have an effect an adversely on their serviceableness by neutering their structure and properties,[4] one among the foremost important disadvantages of these liners, the colonization of such materials by pathological microorganisms particularly *C. albicans* which may adhere and head to penetrate within the fabricating material causing denture induced stomatitis or denture sore mouth.[5] Microbial growth results from the adherence of microbial cells which are promoted by rough surface, from adhesive interaction between fungal species and oral microorganism *(candida albicans, oral streptococci, staphylococcus aureus).* These microorganism produce to pharyngeal and respiratory tract infection which have been isolated from denture and the oral cavity in geriatric patient with decreased immunological activity.[6]

Maintenance of tissue conditioners and interference of accumulation of microorganism on such materials is done by mechanical (brushing with dental brush), chemical (5% sodium hypochlorite) and ultrasonic with UV methods. But these methods can cause considerable damage to tissue conditioners.[2,7]

Now nanotechnology is widely employed in our day to day life including its use as a medicine using nanotechnology, it is simple to analyze and manipulate atoms, chemical bonds and molecules present between various compounds. Nanotechnolgy is used in dental field as nano-medicine. Nanostructures are imployed in innovations or diagnosis of dentistry. Some nanoparticles are used for oral disease preventive drugs, prostheses and for the teeth implantation like silver nanoparticles, carbon nanoparticles, hydroxyapatite (HAp) and tittanium nanoparticles etc.

Silver (Ag) and Titanium (Ti) are recognized for their antimicrobial properties and longstanding use in pharmaceuticals, offering well-tolerated tissue responses and lower toxicity compared to many other substances. Alternative metals against a broad spectrum of sessile microoganism and fungi that colonize on plastic surface.[2-7]

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Silver containing material is already utilised in prosthesis such as in central venous catheter, vascular graft and wound dressing. Silver inorganic particles with its sustained silver cations release are more simpler means of prophylaxis than microsized silver powder which shows lower antimicrobial activity attributable to limited surface.[2]

Titanium nanoparticles are widely utilized as a self cleansing and self disinfecting material for surface coating in many applications. itanium Oxide plays a significant role in environmental purification due to its nanotoxicity, photo-induced superhydrophobicity, and antifogging properties. These properties are being applied for removing bacteria, harmful organic material from water and air.[3]

Several "in vitro and in vivo" studies have shown the beneficial effect of antimicrobial agents like Silver and Titanium nanoparticles combined with tissue conditioners.[2-7]

Thus this study was undertaken to check for the antimicrobial efficacy of silver and titanium nanoparticles incorporated with tissue conditioner.

## MATERIALS AND METHODS

### 1. Preparation of silver and titanium nano particle

Aqueous silver and titanium sols were prepared using the following procedure: 10.0 ml of analytical grade silver and titanium were dissolved in distilled water, with 2.0% Polyvinyl Pyrrolidone (PVP) added as a stabilizer. The resulting solution was then deaerated by bubbling argon gas through it for one hour to eliminate all air bubbles.

### 2. Sample fabrication (Ag-tissue conditioner and Ti-tissue conditioner)

The selected tissue conditioner, D-Soft-Liner, was supplied in powder and liquid forms. Specific doses of silver (Ag) and titanium (Ti) were added to the conditioner liquid. Colloidal silver (Ag) and titanium (Ti) were initially mixed and homogenized with the conditioner liquid in a sterile glass beaker at a concentration of 0.1% (vol/vol: colloidal Ag and Ti to conditioner liquid). Immediately after homogenization, the conditioner powder was added and stirred for 30 seconds, adhering to the manufacturer's recommended powder-to-liquid ratio.

To create uniformly shaped samples with a smooth surface, the mixed conditioner paste was poured into a brass culture plate mold with holes measuring 20 mm in diameter and 3.0 mm in depth. The paste was pressed between glass slides and allowed to solidify under humid conditions. A total of 75 samples were prepared for each concentration of incorporated Ag and Ti. Prior to microbial testing, all samples were sterilized using ethylene oxide gas for 24 hours to ensure initial sterility.

### 3. Microorganism

Three standard microbial strains—*Staphylococcus aureus*, *Streptococcus mutans*, and *Candida albicans*—were employed in the study. Microbial suspensions were prepared by isolating single colonies from agar plates and culturing them overnight in the appropriate broth at 37°C. After incubation, the optical density (OD) of each suspension was adjusted to 1.0 at 600 nm using a spectrophotometer. The suspensions were then diluted 1:100 with phosphate-buffered saline (pH 7.4) to obtain a final concentration of $1.0 \times 10^7$ cells/mL.

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**4. Antimicrobial Assay**

Each disc sample of Ag- and Ti-incorporated tissue conditioner, along with control samples, was placed at the bottom of individual wells in a 60-well cell culture plate (well diameter: 22.1 mm). A 100 µL aliquot of the prepared microbial suspension was added to 1.0 mL of Sabouraud broth in each well, followed by incubation at 37°C. After incubation periods of 24 and 72 hours to evaluate the effects of extended contact, 100 µL of the suspension was carefully collected from each well. The number of viable cells (CFU: Colony Forming Units) was determined using the spread plate method, with a detection limit of 500 CFU per plate, achieved through serial dilution.

All experiments were performed independently in triplicate, and the results were expressed as means with standard deviations. According to established standards, an antimicrobial effect was defined as a reduction in viable cells to 0.1%, corresponding to a 99.9% decrease in colony-forming units (CFUs), which is considered the minimum bactericidal concentration (MBC) for antibiotics. Data were statistically analyzed using one-way ANOVA and Student's t-test at a significance level of 0.05.

## RESULTS

**Table – 1: Comparison of *streptococcus mutans.* Mean Growth Restriction between the Tissue Conditioner Materials**

| Time | Material | Streptococcus mutans | | t-value | p-value |
|---|---|---|---|---|---|
| | | Mean | SD | | |
| 24 Hrs | Silver nanoparticles | 40.96 | 4.80 | -12.71 | **<0.001** |
| | Titanium nanoparticles | 57.56 | 4.43 | | |
| 72 Hrs | Silver nanoparticles | 53.92 | 4.58 | -13.35 | **<0.001** |
| | Titanium nanoparticles | 75.88 | 6.83 | | |

At 24 hrs, for S. mutans the mean CFU for the silver nanoparticles was 40.96±4.80 units while the mean CFU for the titanium nanoparticles was 57.56±4.43 units. So the mean CFU of titanium nanoparticles was greater than the silver nanoparticles and the difference was highly significant ($p<0.001$).

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At 72 hrs, the mean CFU for the silver nanoparticles was 53.92±4.58 units while the mean CFU for the titanium nanoparticles was 75.88±6.83 units. So the mean CFU of titanium nanoparticles was greater than the silver nanoparticles and the difference was highly significant ($p<0.001$).

So it is to be concluded that titanium nanoparticles was much efficient than the silver nanoparticles in restricting the growth of Streptococcus mutans.

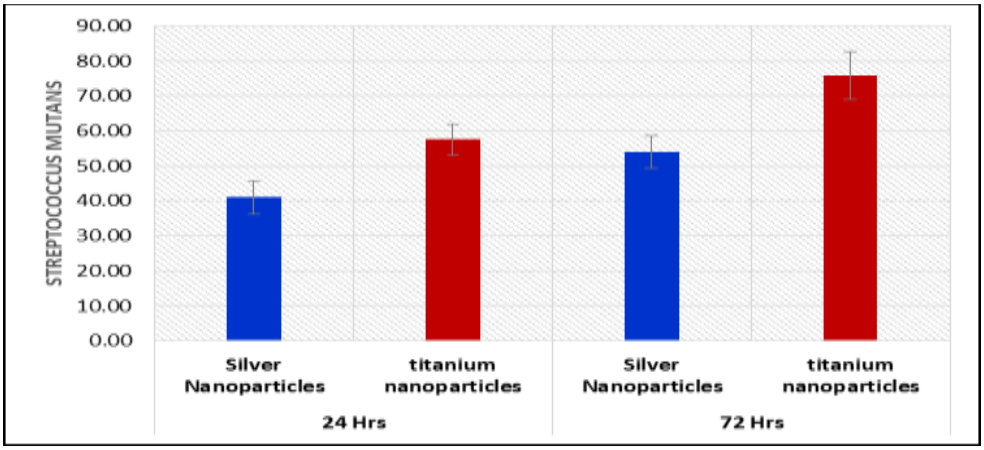


**Table – 2: Comparison of *streptococcus mutans* Growth Restriction Proportion between the Tissue Conditioner Materials**

| Time | Material | Streptococcus mutans | | | | | | chi sq | p-value |
|---|---|---|---|---|---|---|---|---|---|
| | | 26 - 50% | | 51 - 75% | | 76 - 100% | | | |
| | | No. | % | No. | % | No. | % | | |
| 24 Hr | Silver nanoparticles | 25 | 100.0% | 0 | 0.0% | 0 | 0.0% | 50.00 | **<0.001** |
| | Titanium nanoparticles | 0 | 0.0% | 25 | 100.0% | 0 | 0.0% | | |
| 72 Hr | Silver nanoparticles | 5 | 20.0% | 20 | 80.0% | 0 | 0.0% | 13.24 | **0.001** |
| | Titanium nanoparticles | 0 | 0.0% | 17 | 68.0% | 8 | 32.0% | | |

At 24 hrs, for *S. mutans* the CFU for the silver nanoparticles was lying between 26-50% for all the 25 (100%) samples while for titanium nanoparticles the CFU was lying between 51-75% for all the 25 (100%) samples. So the proportion difference in CFU between the two materials was highly significant ($p<0.001$). Higher CFU level was seen more in titanium nanoparticles compared to the silver nanoparticles.

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At 72 hrs, for *S. mutans* the CFU for the silver nanoparticles was lying between 26-50% in 5 (20%) samples and between 51-75% in rest 20 (80%) samples, while for titanium nanoparticles the CFU was lying between 51-75% in 17 (68%) cases and between 76-100% in 8 (32%) samples. So the proportion difference in CFU between the two materials was highly significant (p<0.001). Higher CFU level was seen more in titanium nanoparticles compared to the silver nanoparticles.

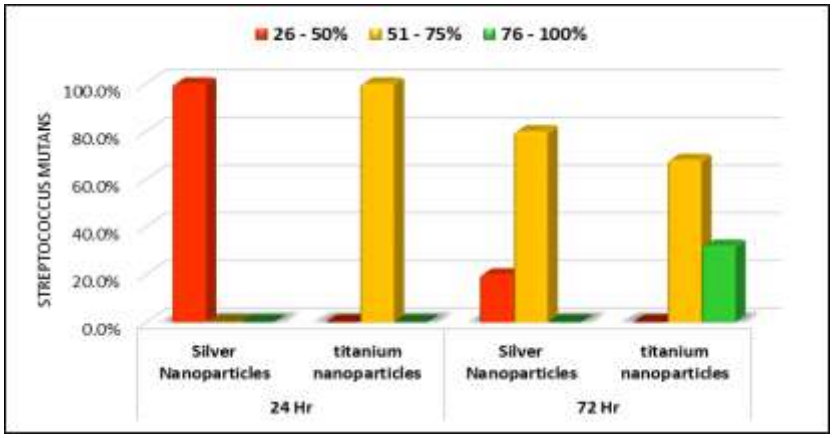


**Table – 3 : Comparison of *streptococcus aureus* Mean Growth Restriction between the Tissue Conditioner Materials**

| Time | Material | *Streptococcus aureus* | | t-value | p-value |
|---|---|---|---|---|---|
| | | Mean | SD | | |
| 24 Hrs | Silver nanoparticles | 31.96 | 5.52 | -7.05 | **<0.001** |
| | Titanium nanoparticles | 42.16 | 4.67 | | |
| 72 Hrs | Silver nanoparticles | 60.60 | 6.12 | 2.73 | **0.009** |
| | Titanium nanoparticles | 56.64 | 3.89 | | |

At 24 hrs, for *S. aureus* the mean CFU for the silver nanoparticles was 31.96±5.52 units while the mean CFU for the titanium nanoparticles was 42.16±4.67 units. So the mean CFU of titanium nanoparticles was greater than the silver nanoparticles and the difference was highly significant (p<0.001).

At 72 hrs, the mean CFU for the silver nanoparticles was 60.60±6.12 units while the mean CFU for the titanium nanoparticles was 56.64±3.89 units. So the mean CFU of titanium nanoparticles was greater than the silver nanoparticles and the difference was highly significant (p=0.009).

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So it is to be concluded that titanium nanoparticles was much efficient than the silver nanoparticles in restricting the growth of *streptococcus aureus.*

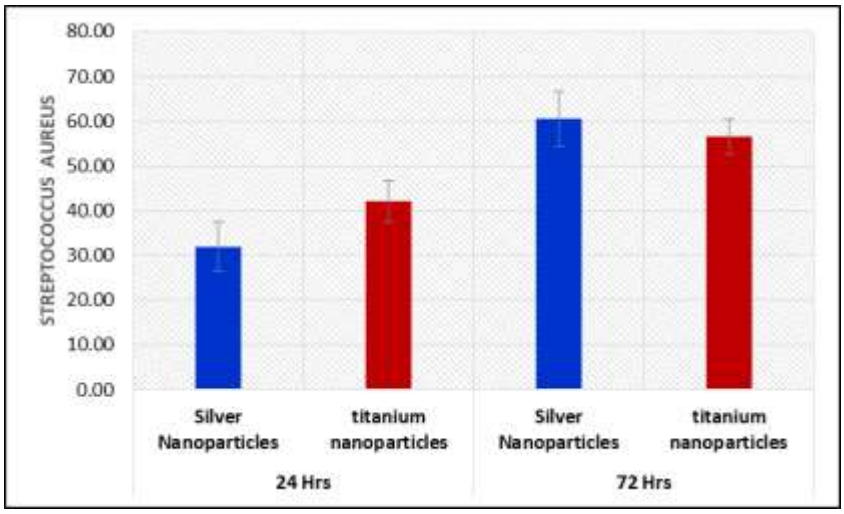


**Table – 4 : Comparison of *streptococcus aureus* Growth Restriction Proportion between the Tissue Conditioner Materials**

| Time | Material | *Streptococcus aureus* | | | | | | chi sq | p-value |
|---|---|---|---|---|---|---|---|---|---|
| | | < 25% | | 26 - 50% | | 51 - 75% | | | |
| | | No. | % | No. | % | No. | % | | |
| 24 Hr | Silver Nanoparticles | 3 | 12.0% | 22 | 88.0% | 0 | 0.0% | **3.19** | **0.074** |
| | titanium nanoparticles | 0 | 0.0% | 25 | 100.0% | 0 | 0.0% | | |
| 72 Hr | Silver Nanoparticles | 0 | 0.0% | 2 | 8.0% | 23 | 92.0% | **2.08** | **0.149** |
| | titanium nanoparticles | 0 | 0.0% | 0 | 0.0% | 25 | 100.0% | | |

At 24 hrs, for *S. aureus* the CFU for the silver nanoparticles was lying below 25% in 3 (12%) samples and between 26-50% in 22 (88%) samples while for titanium nanoparticles the CFU was lying between 26-50% for all the 25 (100%) samples. However the proportion difference in CFU between the two materials was insignificant ($p<0.001$).

At 72 hrs, for *S. aureus* the CFU for the silver nanoparticles was lying between 26-50% in 2 (8%) samples and between 51-75% in rest 23 (92%) samples, while for titanium nanoparticles the CFU was lying between 51-75% in all 25 (100%) samples. However the proportion difference in CFU between the two materials was insignificant ($p=0.149$).

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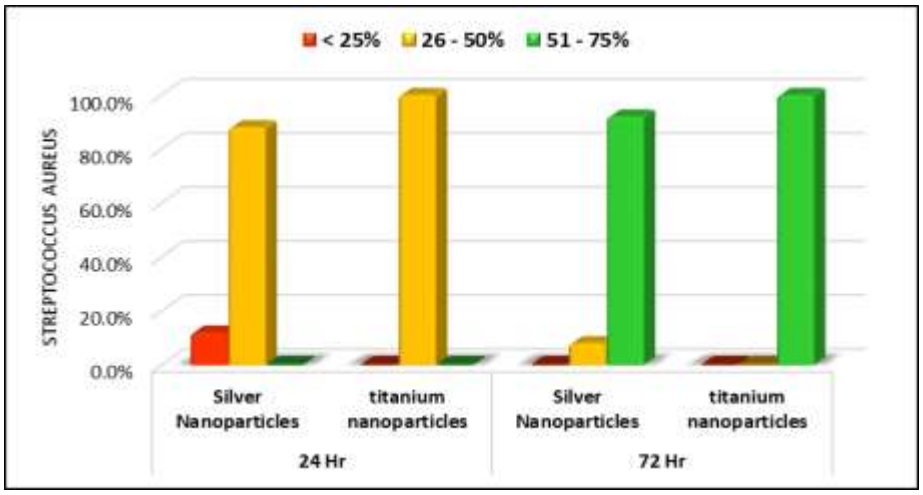


**Table – 5 : Comparison of *Candida albicans* Mean Growth Restriction between the Tissue Conditioner Materials**

| Time | Material | *Candida albicans* | | t-value | p-value |
|---|---|---|---|---|---|
| | | Mean | SD | | |
| 24 Hrs | Silver nanoparticles | 25.72 | 2.78 | **-14.36** | **<0.001** |
| | Titanium nanoparticles | 39.32 | 3.84 | | |
| 72 Hrs | Silver nanoparticles | 40.36 | 5.41 | **-12.97** | **<0.001** |
| | Titanium nanoparticles | 57.64 | 3.89 | | |

At 24 hrs, for *Candida albicans* the mean CFU for the silver nanoparticles was 25.72±2.78 units while the mean CFU for the titanium nanoparticles was 39.32±3.84 units. So the mean CFU of titanium nanoparticles was greater than the silver nanoparticles and the difference was highly significant ($p<0.001$).

At 72 hrs, the mean CFU for the silver nanoparticles was 40.36±5.41 units while the mean CFU for the titanium nanoparticles was 57.64±3.89 units. So the mean CFU of titanium nanoparticles was greater than the silver nanoparticles and the difference was highly significant ($p=0.009$).

So it is to be concluded that titanium nanoparticles was much efficient than the silver nanoparticles in restricting the growth of *Candida albicans.*

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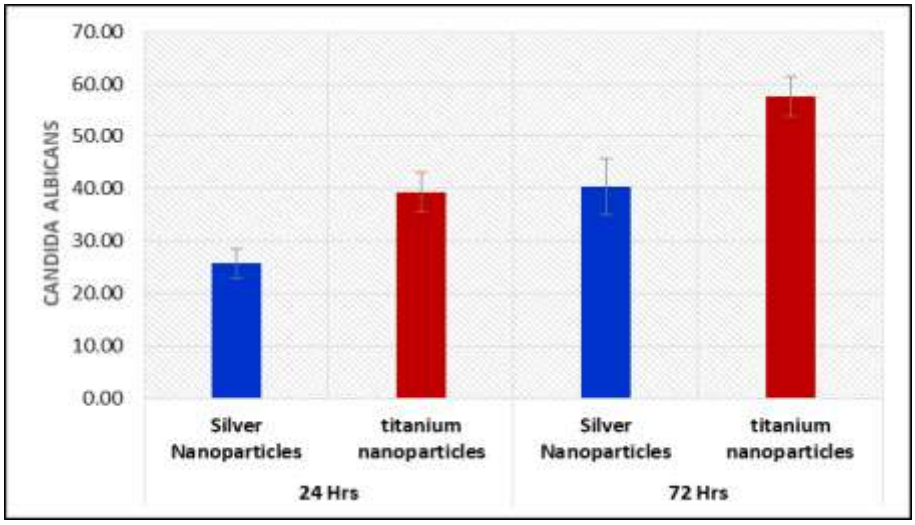


**Table – 6: Comparison of *Candida albicans* Growth Restriction Proportion between the Tissue Conditioner Materials**

| Time | Material | *Candida albicans* | | | | | | chi sq | p-value |
|---|---|---|---|---|---|---|---|---|---|
| | | < 25% | | 26 - 50% | | 51 - 75% | | | |
| | | No. | % | No. | % | No. | % | | |
| 24 Hr | Silver nanoparticles | 8 | 32.0% | 17 | 68.0% | 0 | 0.0% | **9.52** | **0.002** |
| | Titanium nanoparticles | 0 | 0.0% | 25 | 100.0% | 0 | 0.0% | | |
| 72 Hr | Silver nanoparticles | 0 | 0.0% | 23 | 92.0% | 2 | 8.0% | **42.59** | **<0.001** |
| | Titanium nanoparticles | 0 | 0.0% | 0 | 0.0% | 25 | 100.0% | | |

At 24 hrs, for *Candida albicans* the CFU for the silver nanoparticles was lying below 25% in 8 (32%) samples and between 26-50% in rest 17 (68%) samples while for titanium nanoparticles the CFU was lying between 26-50% for all the 25 (100%) samples. So the proportion difference in CFU between the two materials was highly significant (p=0.002). Higher CFU level was seen more in titanium nanoparticles compared to the silver nanoparticles.

At 72 hrs, for *Candida albicans* the CFU for the silver nanoparticles was lying between 26-50% in 23 (92%) samples and between 51-75% in rest 2 (8%) samples, while for titanium nanoparticles the CFU was lying between 51-75% in all 25 (100%) samples. So the proportion

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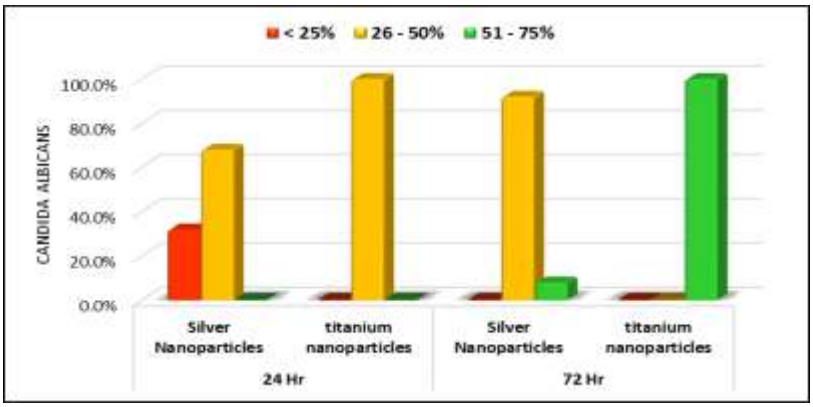
difference in CFU between the two materials was highly significant ($p<0.001$). Higher CFU level was seen more in titanium nanoparticles compared to the silver nanoparticles.




## DISCUSION

This study investigated the antimicrobial and antifungal effect of tissue conditioner containing silver and titanium nanoparticles yielded bactericidal and fungicidal properties for three reference strains, *S. aureus, S. mutans and C. albicans.* These microbial species tested are currently recommended to test antiseptic molecules. *S. aureus*, a pathogen causing respiratory infections, has often been isolated from dentures and the oral cavity and dentures have recently been reported to be a carriage of this pathogen. **Streptococcus mutans* has been closely linked to the pathogenesis of dental caries; however, its relevance is of limited clinical significance for individuals who wear dentures.* However, extensive plaque formation on denture might also contribute to the decay of residual natural teeth and to the inflammation of gingival tissue adjacent to the denture. *C. albicans* can be regularly isolated, suggesting a pathogenic association between bacteria and fungi related with denture stomatitis. [1-3]

Singh K et al,(2010) study about the effect of surface treatment on the long term effectiveness of tissue conditioner and in result they found that application of a coating can significantly reduce the loss of softness and surface integrity of a tissue conditioner.[4]

For estimating the antimicrobial effect in current study there was a few microbial suspension (100 µL) was used. The oral microoganism would seem to be during a stationary phase instead of in growing phase because the nutrition is restricted under the antibodies ; therefore the antimicrobial enzymes existing in the oral cavity. The assays tested with samples immersed during a large volume of microbial suspension couldn't reproduce- in vivo tissue conditioner which closely contacts the gingival mucosa.

Microorganisms in suspension (planktonic phase) are sensitive to lower antiseptic concentrations than microbe colonizing surfaces; protected by a biofilm. In present study , the microbes were adjusted to the stationary phase to be deflected in broth ; no criterion of the antimicrobial effect on dental material has been established thus far, the concept of Minimum

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Bactericidal Concentration (MBC) of antibiotics was thus adopted as the antimicrobial concentration at more than 99.9% elimination of the organisms.[5-8]

The current microbial assay confirmed Ag and Ti - based compounds are highly toxic to prokaryotic cell showing strong biocidal effects on as bacteria species, while Ag and Ti showed slighter outcome on eukaryotic cell such as mold and yeasts. The control group displayed no reticence results in opposition to tested strains though tissue conditioner itself possesses antimicrobial effects due to ingredients such as plasticizers and ethanol. Further studies also manifest various antimicrobial effect on *C. albicans and S. aureus*, but these findings were at variance with previously reported findings indicating that such materials have little antimicrobial effect. [14-16]

The results of the antimicrobial study about TiO2 NPs assent with the earlier findings by Akiba et al.,2005 and other researcher that irradiated TiO2 exhibits bactericidal activity ( Desai V S et al.,2009; Hghi M et al.,2012; Shi H et al.,2013; Haghighi F et al.,2013; Sato W et al.,2018; Ahmed AQ et al.,2018; Rezaeil et al.,2019 ) and the efficacy of this disinfection of water using cost effective and reusable photocatalyst. TiO2 particle have earlier access and cause photooxidation of intracellular components thereby accelerating cell death of microoganism ( *E.coli, K. pneumonia, S. aueus, S. mutans and C.albicans* ).[3,9,10-11,19-23]

In previous study Nam KY et al.,(2009) find showed various antimicrobial effects on *S.aureus, S, mutans and C.albicans* . In another study, Pal S et al.(2007) also showed the bactericidal activity of silver nanoparticles, it was shown that the interaction between silver nanoparticles and constituents of the bacterial membrane caused structural changes in and damage to membranes, finally leading to cell death.[6,21-22]

The results of the current study ensnare that Ag and titanium with tissue conditioner might act as a latent antimicrobial material and it could supply the additional advantages of antimicrobial effect even if dentures are worn at night, therefore, could be used as an one of the second therapy for denture stomatitis resistant to conventional treatment and geriatric denture bearing patients under medically compromised status. [25]

When silver and titanium nanoparticles compared, titanium nanoparticle are more significant than the silver nanoparticles because TiNPs shows significant to antimicrobial effect and antifungal effect in the oral environment. However, further studies are still required to clarify the optimal concentration of Ag and Ti, regarding the silver and titanium content, the disruption of oral microflora and toxic effects of silver with excess Ag and titanium mechanical stability should be also considered for the proper and safe clinical application. When dentures are removed, for instance during sleep, the NPs allow for the maintenance of tissue conditioners by eliminating the biolims from the denture. This will be useful in an aging society that requires simple and easy cleaning methods. [7-25]

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## CONCLUSION

With the limitations of this in vitro study it was concluded:

1. Tissue conditioner combined with silver and titanium nanoparticles showed significant antimicrobial effect.
2. Silver and titanium nanoparticles possesed antimicrobial properties against *S. aureus, S. mutans and C. albicans* at 0.1 % of nanoparticles incorporated after a 24 hrs and 72 hrs incubation period.
3. Titanium nanoparticles had highly significant antimicrobial properties against *S. aureus , S. mutans , C. albicans*
4. In comparision of silver and titanium nanoparticles, titanium nanoparticles showed more antimicrobial effect against *S. aureus , S. mutans and C. albicans*

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