

## Antimicrobial Efficacy Of Temporary And Permanent Denture Soft Lining Material Modified by Titanium - Dioxide Nanoparticles - An *In-vitro* Study

Research Article

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### Abstract

Tissue conditioners are soft denture liners which are commonly applied to the tissue surface of a denture. Tissue conditioners are soft denture liners which are commonly applied to the fitting surface of a denture. Soft liners absorb the masticatory impact and act as a 'shock absorber'.

**Aim:** The aim of present study is assessment the effect of addition of Titanium-dioxide Nanoparticles to a soft liner on would inhibit the growth of Microbes in Permanent and Temporary Softliners.

**Materials and Methods:** The study done is an in vitro experimental design. Titanium NP are incorporated into Temporary and permanent denture soft liner and these specimen are kept in the Blood Agar media for 3 microorganisms namely *Candida albicans*, *Streptococcus mutans*, *Lactobacillus* and all the culture plates are incubated for 24 hours under Room Temperature and Zone of Inhibition is calculated in the Agar media using measuring tape.

**Results:** The zone of inhibition in Lactobacillus was maximum for permanent soft liner with NP (20.33±1.528) and minimum in Temporary soft liner with NP (10.67±0.577), the mean difference among groups was statistically significant (p<0.05).

**Conclusion:** Permanent and Temporary soft liners with Titanium Nanoparticles showed antimicrobial efficacy against Lactobacilli.

**Keywords:** Denture Liners; Nanoparticles; Titanium Dioxide; Denture Stomatitis.

### Introduction

Relining a complete denture is required less oftenly due to soft tissue changes arising from bone resorption. Tissue conditioners are soft denture liners which are commonly applied to the fitting surface of a denture. Soft liners absorbs the masticatory impact and act as a 'shock absorber'. Some Patients suffer from chronic pain, soreness or discomfort due to prolonged contact between the rigid denture base materials and the underlying tissues; usually in patients with sharp, thin, or heavily resorbed ridges or those with severe undercuts. Denture relining materials absorb masticatory force and distribute to underlying alveolar ridge widely and

enhance patient comfort [1]. Relining a denture is performed directly in the mouth using auto-polymerizing denture relining materials and indirectly in the laboratory by heat-cured denture relining materials [1, 2]. Denture relining materials are used in sensitive patients and help in conditioning tissue and limit the traumatic effect of denture causing cushioning effect [3]. Denture relining material distribute the loads transferred to soft tissues during motion in Immediate denture. Soft lining materials are contaminated in the oral environment and are not possible to clean (or) brush effectively [4]. Denture relining materials are hard to maintain and failure of cleaning between denture base and liners creates an environment which renders bacterial colonization and also com-

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promise the durability of the soft liner. A number of problems associated with the use of reliners have been reported, such as porosity, loss of softness, debonding from the denture base, rough surface, color changes, colonization by microorganisms and poor tear strength [5].

Denture disinfectant agents are Chlorhexidine gluconate, Sodium hypochlorite, Hydrogen peroxide can be used against accumulation of microbes [6]. Incorporation of Nanoparticles into Denture Softliner and gives positive results on *In vitro* growth inhibition of Microorganisms [7].

Antifungal activity of Denture soft liner modified using silver Nanoparticles should be capable of preventing colonisation of *Candida albicans* and denture Soft Linings [7-10]. Antifungal Efficacy and the Mechanical properties of softliner against *Candida* and Incorporation of Garlic and Neem concluded that Neem and Garlic added to Softliner had an Inhibitory effect on *Candida albicans*.

There are numerous studies assessing Antimicrobial activity of Denture Soft Lining Material, and there are few studies regarding the effect of Titanium Nanoparticles in dentistry that too on Temporary and Permanent Denture Softliner [10, 11]. A study says that Direct contact with friable tissue when added upon by Silver Nanoparticles shows change of bacterial growth over Soft Lining Material and there is remarkable amount of Microbial reduction [12, 13].

Micro porosities and roughness in the denture surface will lead to adherence of *Candida albicans* and formation of the colonies on the fitting surface of the denture. These microorganisms have to be removed by mechanical or chemical cleansing; however in some situations, there cannot be completely removed from the denture surfaces Polymethylmethacrylate (PMMA) is the most common material used in the fabrication of removable complete or Partial denture prosthesis [14].

The aim of present study is assessment the effect of addition of Titanium-dioxide Nanoparticles to a soft liner on would inhibit the growth of Microbes in Permanent and Temporary Softliners.

## Materials and Methods

The study done was an *in vitro*, experimental design.

### Preparation of Nanoparticles

100 millimolar of Titanium-dioxide Nanoparticles in 100 ml of Serratia nematodiphiles broth and kept in shaker for 24 hours and purified by centrifugation and Dried.

### Specimen Preparation

**Preparation of Temporary soft liner:** Soft liner models are prepared using the wax sheet which is cut into 1x1 cm and mould is prepared and thin section of soft liner with 1cm thickness is fabricated and are separated GC soft liner (GC soft liner, GC

Figure 1. TEM image of TiO<sub>2</sub> nanoparticles.

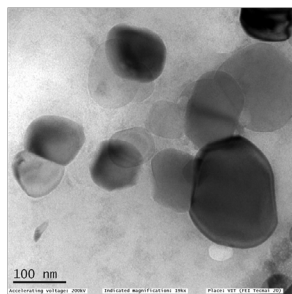


Figure 2. Temporary soft liner with NP towards left and Temporary soft liner without NP.

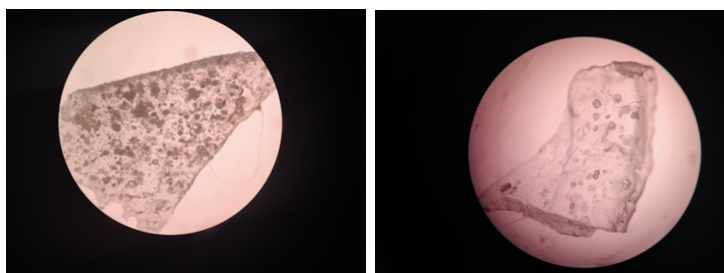
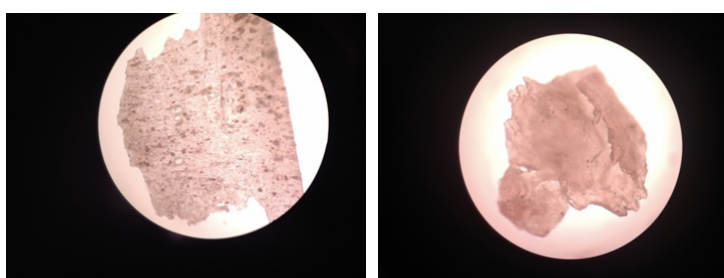


Figure 3. Permanent soft liner with NP towards left and Permanent soft liner without NP.



Corporation, Tokyo, Japan).

**Preparation of Permanent Softliner:** Permanent soft liner are prepared using Permanent soft liner by Molloplast (DETAX GmbH & Co. KG Carl-Zeiss-Str. 476275 Ettlingen Germany) An index of the wax sheet measuring 1x1 cm and thickness of 1cm was made using polyvinyl siloxane materials on which soft liner material was added. Three part dental Flask was used for Flasking the fabricated soft liner using dental stone. Reverse flaking technique was followed by wax elimination. Square shaped mould space was created where Heat-activated Permanent Denture Soft Lining Material and cured according to manufacturer's Instructions to obtain soft liner discs.

**Adding of Titanium Nanoparticles:** For Temporary soft liner for 2.2g/1.8g liquid GC soft liner and 1mg of Titanium dioxide Nanoparticles is added and allow to Heatcuring according to manufacturer's instructions and Titanium dioxide Impregnated Permanent soft liner is removed. Impregnated Nanoparticles were checked under microscope.

**Sterilization of Specimen:** The specimens were handled carefully which includes the utilization of latex gloves for all phases of Investing dewaxing, packing and retrieval of specimens. All the specimens are kept in UV chamber for 2 hours.

**Preparation of AgarMedium:** The specimens were prepared in a petri dish for culture media are prepared under aseptic conditions. After preparing they are kept in UV chamber for sterilization under Aseptic conditions for one hour. Blood Agar preparation was done for 3 microorganisms namely *Candida albicans*, *Streptococcus mutans*, *Lactobacillus* and kept in Laminar air flow chamber for sterilization.

#### Antimicrobial Test

The prepared Titanium Nanoparticles are incorporated into soft-liner are inoculated into Agar plates under aseptic conditions and

grouped into 3 groups and 4 samples in each group.

Each group consists of one microbe namely *Streptococcus mutans*, *Lactobacillus* and *Candida albicans* and each group is having 4 samples namely 1) Temporary soft liner without Nanop.

articles 2) Temporary soft liner with Nanoparticles 3) Permanent soft liner without Nanoparticles 4) Permanent soft liner with Nanoparticles same test was performed 3 times to eliminate bias. All the culture plates are incubated for 24 hours under Room Temperature and Zone of Inhibition is calculated using measuring tape.

#### Statistical Analysis

Descriptive statistics was used to evaluate zone of inhibition of different microbes in the culture media. One Way Anova and Tukey HSD Post hoc test was performed and the p value was determined to evaluate the significance of the variables. The correlation between different groups about zone of inhibition is evaluated and statistics were carried using SPSS Software version 23.0 by IBM India. The results were obtained in the form of tables and graphs.

#### Results

This study shows addition of Titanium Nanoparticles to temporary and permanent soft liner shows antimicrobial action in different culture media after 24 hours of incubation in the petri dishes that are separated and see for changes for the zone of inhibition in streptococcus was maximum for permanent soft liner with NP ( $18.33 \pm 1.528$ ) and minimum in Temporary soft liner with NP ( $10.00 \pm 0$ ), the mean difference among groups was statistically significant ( $p < 0.05$ ) followed by The zone of inhibition in *Lactobacillus* was maximum for permanent soft liner with NP ( $20.33 \pm 1.528$ ) and minimum in Temporary soft liner with NP ( $10.67 \pm 0.577$ ), the mean difference among groups was statistical-

Figure 4. Zone of inhibition seen in Agar by *Streptococcus mutans* in Permanent soft liner.

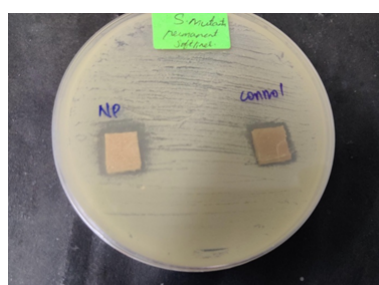
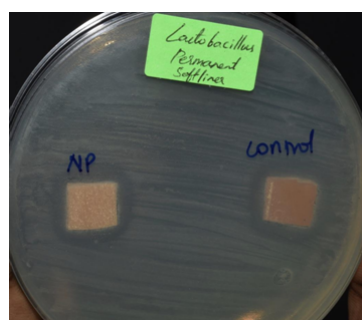


Figure 5. Zone of inhibition seen in Agar by *Lactobacillus* in Permanent soft liner.



**Table 1. Comparison of Zone of inhibition against different microbes among four group 1)Temporary soft liner without Nanoparticles 2)Temporary soft liner with Nanoparticles 3)Permanent soft liner without Nanoparticles 4)Permanent soft liner with Nanoparticles.**

Microbes	Group	Mean ± SD	df	F	p-value
Streptococcus mutans	1)Temporary soft liner without NP	10.67 ± 0.557	3	35.378	0.001*
	2)Temporary soft liner with NP	10.00 ± 0			
	3)Permanent soft liner without NP	14.33 ± 1.528			
	4)Permanent soft liner with NP	18.33 ± 1.528			
Lactobacillus	1)Temporary soft liner without NP	11.33 ± 0.577	3	61.00	0.001*
	2)Temporary soft liner with NP	10.67 ± 0.577			
	3)Permanent soft liner without NP	17.33 ± 1.155			
	4)Permanent soft liner with NP	20.33 ± 1.528			
Candida albicans	1)Temporary soft liner without NP	10.67 ± 0.577	3	0.250	0.859
	2)Temporary soft liner with NP	10.33 ± 0.577			
	3)Permanent soft liner without NP	10.33 ± 0.577			
	4)Permanent soft liner with NP	10.33 ± 0.577			

\*significant p<0.05 p value derived from one way ANOVA test.

**Table 2. Pairwise comparison between four groups namely Temporary soft liner without Nanoparticles, Temporary soft liner with Nanoparticles, Permanent soft liner without Nanoparticles, Permanent soft liner with Nanoparticles based on zone of inhibition on streptococcus and lactobacillus.**

Microbes	Groups	MD	SE	p-value
Streptococcus mutans	Temporary soft liner without NP (vs)Temporary soft liner with NP	0.667	0.913	0.882
	Temporary soft liner without NP (vs)Permanent soft liner without NP	3.667	0.913	0.016*
	Temporary soft liner without NP (vs)Permanent soft liner with NP	7.667	0.913	0.001*
	Temporary soft liner with NP(vs)Permanent soft liner without NP	4.333	0.913	0.006*
	Temporary soft liner with NP (vs)Permanent soft liner with NP	8.333	0.913	0.001*
	Permanent soft liner without NP (vs)Permanent soft liner with NP	4	0.913	0.010*
	Lactobacillus sp	Temporary soft liner without NP (vs)Temporary soft liner with NP	0.667	0.850
Temporary soft liner without NP (vs)Permanent soft liner without NP		6.000	0.850	0.001*
Temporary soft liner without NP (vs)Permanent soft liner with NP		9.000	0.850	0.001*
Temporary soft liner with NP(vs)Permanent soft liner without NP		6.667	0.850	0.001*
Temporary soft liner with NP (vs)Permanent soft liner with NP		9.667	0.850	0.001*
Permanent soft liner without NP (vs)Permanent soft liner with NP		3.000	0.850	0.032*

\* The mean difference is significant at 0.05 level p-value was derived from Tukey HSD Post hoc test significant p<0.05.

ly significant (p<0.05) and in candida the zone of inhibition was maximum for Temporary soft liner without NP (10.67±0.577) and minimum in Temporary soft liner with Nanoparticles, Permanent soft liner without Nanoparticle and Permanent soft liner with Nanoparticle groups (10.33±0.577), the mean difference among groups was statistically insignificant ( p>0.05).

For pairwise Comparisons the mean difference between all the groups are statistically significant (p<0.05), except between group 1 and 2 (p>0.05) on both the micro organism (*Streptococcus*, *Lactobacillus*).

### Discussion

In the present study, the addition of Titanium Nanoparticles to Tissue conditioner yielded bactericidal and fungicidal properties for three reference strains, *Lactobacillus*, *S. mutans* and *C. albicans* of this *S. mutans* has been associated closely with the pathogenesis of dental caries, which is of limited clinical significance for denture wearers [15]. However, extensive plaque formation on denture might also contribute to the decay of residual natural teeth in over denture patients and also causes inflammation of gingival tissue adjacent to the denture. *Candida albicans* can be regularly isolated, suggesting a pathogenic association between bacteria

and fungi related with denture stomatitis. In recent years, various nanoparticles have been reported to display good biological activities [16]. Amongst them, TiO<sub>2</sub> nanoparticles have oxidative and hydrolysis properties. As a photocatalyst, it can improve the efficiency of electrolytically splitting water into hydrogen and oxygen which can produce electricity in nanoparticles form. When these nanoparticles are exposed to ultraviolet (UV) light, they become increasingly hydrophilic [17]. It has been reported that surface roughness is a vital property because of its impact on microbial adhesion. For this reason, if a Soft Liner Material incorporated with Antifungal drugs (or) Antimicrobials has to be developed to interface on the denture surface to avoid colonization in the Denture [18]. Roughness in the fitting surface of denture base generally promote initial microbial adhesion as the denture plaque matures on the fitting surface it is associated with a protective biofilm, and biofilm-related chronic infections, such as candidiasis, are inherently difficult to treat and fully eradicate with routine therapy. As elderly denture wearer's ability to perform oral self care declines, a demand for a simple, effective and preventive denture care system is required. An agent with a modest antifungal effect, although not biocidal, may even be desirable to decrease the potential side effect of various agents [19]. It is reported that Ag and silver-based compounds are highly toxic to prokaryotic cells showing strong biocidal effects as bacteria species, while Ag showed less effect on eukaryotic cells such as mold and yeasts [20-22]. The microbial investigation conducted on this study confirms that the released concentrations of the Antimicrobial agents from Soft Lining material were able to induce an Antimicrobial effect on Agar culture. These specimens maintain high osteoblastic cell (SaOS-2) viability and induce mild but statistically significant antibacterial activity on *P. gingivalis* and *P. intermedia* strains, which is inline with the low AgNP concentrations detected [23, 24]. Obturator wearers with oro-nasal communication often present with prosthesis-induced stomatitis because of *Candida albicans* colonization on the acrylic nasal surfaces by adding silver Nanoparticles it shows some Anti-microbial feature [25-28]. Urban et al., [29] also studied particle size and distribution of antifungal agents in a plasticized matrix of tissue conditioners. For example, nanoparticles have high surface area and reactivity [30] hence likely to accelerate the drug release.

The advantages of soft liners include greater range of overdenture movement, energy absorption, and equal force distribution to the implants and edentulous ridge [31, 32]. The soft liner encircles the bar and completely obturates the spaces around it. The continuous cleaning of the bar and abutments by soft liners during insertion and removal of the denture prevents plaque accumulation around the bar regardless of oral hygiene practice [33].

## Limitations and Future Scope

This particular study lacks an oral environment furthermore changes in hardness of denture base after incorporation of Titanium Nanoparticle. Further more confirmation with experiments conducted under *in vivo* conditions.

With the basic limitations of the study design, generalizability is a possibility with further accumulation of evidence in this regard. However, within the limitations of the present study it could be concluded that addition of Titanium nanoparticles to softliner, as an adjunct, it is found effective and safe on further research would

be considered as a potential "adjunct" along with the standard care in the to overcome the side effects of synthetic drugs, especially in this era of ever advancing clinical dentistry. It has various clinical significances in implant dentistry, Immediate denture and Myofunctional prosthesis etc., and can make the outcome more advanced and towards the ultimate success.

## Conclusion

Within the limits of this study it is presumed that both Permanent and Temporary both soft liner have there own mild antibacterial activity and Titanium Nanoparticles can show Antimicrobial efficacy in groups *Lactobacillus* and *No* (or) minimum efficacy in *Candida* and *Streptococcus mutans*. Addition of Titanium Nanoparticles as an adjunct, is found to be effective and on further research it would be considered as a potential adjunct along with the standard care in the management of denture stomatitis to overcome side effects of synthetic drugs.

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