

IN VITRO ANTIBACTERIAL ACTIVITY OF TEMPORARY SOFT TISSUE LINER COMBINED WITH CHLORHEXIDINE, SILVER NANO PARTICLES AND TEA TREE OIL AGAINST STAPHYLOCOCCUS AUREUS

Sidharth Shukla¹, Rajni A Dable², R.Srinivasa Rao³, Kuldeep⁴, Puneet Mutneja⁵

Post Graduates Student¹, Professor & Head², Professor³, Reader^{4,5}

1-5 - Department of Prosthodontics and crown & Bridge, Teerthanker Mahaveer Dental College and Research Centre, Moradabad

Abstract

Background: Various bacterial microorganisms are normally found on the human oral mucosa that can lead to the pathogenic conditions in the denture wearers. The most common microorganisms found in the oral cavity are *Streptococcus mutans* and *Staphylococcus aureus* which are highly known to accumulate on intaglio surface in the denture. The aim of this study was to compare antibacterial activity of temporary soft tissue liner combined with Chlorhexidine, Silver Nano particles and Tea Tree Oil against *Staphylococcus aureus*.

Materials & Method: Temporary soft tissue liner combined with Chlorhexidine, Silver Nano particles and Tea Tree Oil were tested against *Staphylococcus aureus* using agar core inhibition diameter assay.

Results: Maximum inhibition was seen in the silver nano groups followed by chlorhexidine and the least inhibition was seen in case of tea tree oil group. One-way analysis of variance followed by Tukey's post-hoc test was used to test the intergroup difference. P - Value < 0.05 was considered statistically significant.

Conclusion: This study showed that the incorporation of three different antimicrobial agents in the concentrations of 15 % silver nano-particles, 0.12 % chlorhexidine and 15 % tea tree oil with in temporary liner which was incubated at 37°C for 24 hours. These antimicrobial agents are effective in inhibiting the growth of *Staphylococcus aureus*. Soft tissue liner when mixed with antimicrobial agents showed satisfactory inhibition of *Staphylococcus aureus*.

Keywords: Temporary soft liner, Blood Agar, Silver Nano-particles, Chlorhexidine, Tea Tree Oil.

Introduction

Some data shown by World Oral Health Report, gives a great improvement in oral health in many countries though some oral health problems still persevere mostly among neglected groups in both developed and developing nations! Tooth caries and periodontal problems are one of the most significant among oral health problems globally. Oral and dental diseases badly affect the overall health jeopardizing the quality of life and the working capacity of a human being.²

Various bacterial microorganisms normally found on the human oral mucosa can lead to the pathogenic conditions in the denture wearers. Most of the time the denture remains in place for longer time allowing the food accumulation & then the plaque formation. This ultimately leads to the inflammatory conditions under the dentures.

The most common microorganisms found in the oral cavity are *Streptococcus mutans* and *Staphylococcus aureus* which are highly known to accumulate on intaglio surface of the denture in stomatitis patients.³ It is also seen that in many cases the oral mucosa below dentures undergo deformation.⁴ BR Lytle (1957) has recognized the difference between physiologically acceptable deformation and a pathological one. Impressions of pathologically malformed tissues, may lead to a denture that can bolster the deformities that can aggravate the patient's condition. The traumatizing denture such as, overextended or under extended, poorly fitted or

maloccluded need to be treated by taking each situation into consideration.⁵

Introduction of antibacterial agents can make the soft lining materials work better and bring relief to the traumatized tissues. Several studies have investigated that the addition of antibacterial agents into temporary soft tissue liners reduce development of microorganisms on the surface of material.⁶

The aim of this study was to observe and evaluate the antibacterial activity of temporary soft tissue liner combined with different antibacterial agents Chlorhexidine, Silver Nano particles and Tea Tree Oil.

Material & Methods

A Circular metal mold of dimension 5 mm diameter and 1 mm in thickness (Figure 1) was used to fabricate specimens of temporary soft tissue liner(Visco-gel, Dentsply Clarke Avenue Milford, DE 19963 USA) following the manufacturer's instructions. The temporary soft tissue liner material was manipulated in a disinfected dependish as per manufacturer's instructions. The discs were prepared using this metal mold. After the material was set these discs were placed in the agar petri dish to obtain the *Staphylococcus aureus* culture.

Staphylococcus aureus culture: Suitable blood agar was prepared and poured into a disinfected plate. After blood agar was dried, the plates were inoculated with *Staphylococcus aureus* culture obtained from the

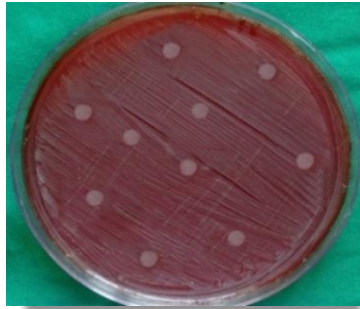


Figure 1: Specimen

laboratory (central microbiology lab Teerthanker Mahaveer Medical College, Moradabad). After immunization, the patri plate was incubated for 24 hours at the temperature of 37°C. The colonies were suspended in 5 ml in volume which is approximately 1 mm were picked up from this culture and spread over the blood agar in the plates. Plates were arranged with Blood agar that was treated with 2% glucose and 0.5 µg/ml methylene blue dye. The organized blood agar was poured into the different plates by the depth of 4 mm thickness. The dishes were permitted to dry for 15 minutes in the incubator chamber. A sterile cotton swab was used to streak the agar plates with the inoculums. One Petri dish was served as the indicator of control group for temporary soft liner which is of *Staphylococcus aureus*. In another 3 plates antimicrobial test disks, i.e. subgroup 1 (AgNano-particles) subgroup 2 (Chlorhexidine) subgroup 3 (Tea Tree Oil) were placed over the plate of agar such a way that the disc is totally merged from all the sides, using sterile tweezers. All the agar dishes were incubated at the temperature of 37°C and were left for 24 hours. Then the ‘zone of inhibition’ was measured to its full diameters along with sample disks by using a metal scale and a conventional divider.



Figure 2: Temporary Soft Liner + 0.12% Chlorhexidine

Results

Table 1 shows result of ANOVA for medicaments used in temporary soft liners as antimicrobial agents, the results showed that there was as highly statistically significant difference ($p=0.001$) between and within groups.

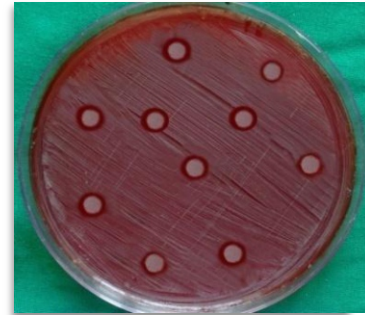


Figure 3: Temporary Soft Liner + 15% Tea Tree Oil



Figure 4: Temporary Soft Liner + 15% Ag Nano-particles

| | Sum of Squares | df | Mean Square | F | Sig. |
|----------------|----------------|----|-------------|--------|-------|
| Between Groups | 64.217 | 2 | 32.108 | 19.872 | 0.001 |
| Within Groups | 43.625 | 27 | 1.616 | | |
| Total | 107.842 | 29 | | | |

$P \leq 0.05$ (Statistically Significant)

Table 1: Application of ANOVA for medicaments used in Temporary Soft Liner as antimicrobial agents.

Table 2 shows result of Tukey Post Hoc in intergroup, the results shows that there was significant difference when Ag Nano was compared with Tea Tree Oil and Chlorhexidine with Mean difference and P value of (Mean Difference =2.20, $P = 0.002$) and (Mean Difference=3.55, $P = 0.000$) respectively while no statistically significant difference was observed when Chlorhexidine was compared with Tea Tree Oil (Mean Difference = -1.35, $P = 0.062$) and Figure 1 shows the comparative antimicrobial activity of the different test materials.

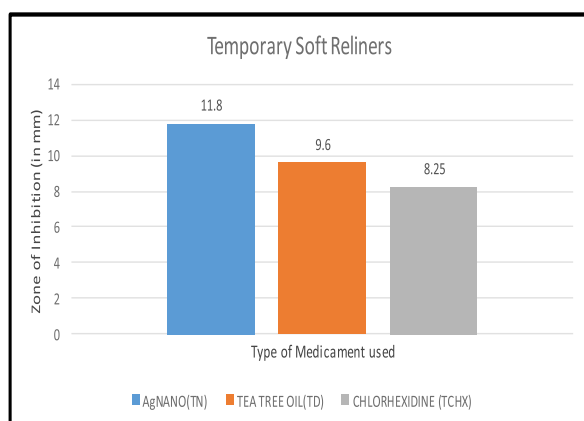
Statistical Analysis

Mean and standard deviation of the zone of inhibition was calculated. One-way analysis of variance followed by Tukey's post-hoc test was used to test the intergroup difference. P - value < 0.05 was considered statistically significant.

| Reference Group | Comparison Group | Mean Difference | Std. Error | P value | 95% Confidence Interval | |
|-----------------|------------------|-----------------|------------|---------|-------------------------|-------------|
| | | | | | Lower Bound | Upper Bound |
| Ag Nano | Tea Tree Oil | 2.20000* | .56846 | .002 | 0.7905 | 3.6095 |
| | Chlorhexidine | 3.55000* | .56846 | .000 | 2.1405 | 4.9595 |
| Chlorhexidine | Tea Tree Oil | -1.35000 | .56846 | .062 | -2.7595 | .0595 |

$P \leq 0.05$ (Statistically Significant)

Table 2: Intergroup comparison of Mean Zone of Inhibition (in mm) for the medicaments used in soft liner as antimicrobial agents using Tukey Post Hoc test



Graph 1: Comparative antimicrobial activity of the soft liner with silver nano, tea tree oil, chlorhexidine

Discussion

When the tissues covering the denture foundation become compromised, these tissues must be allowed to heal before a new denture is prepared. This can be achieved by eliminating the responsible factors and by the resurfacing of old denture with a temporary or permanent soft denture liner that allows the soft tissues to recover to normal state. That's why; these materials are recognized as tissue conditioners. Afterwards, preventive measures are also necessary to avoid relapse to the previous pathologic condition.⁷

Existing literature shows that the dynamic oral environment produces many changes in the cavity which has affect unfavorable to oral hard and soft tissues. Alterations in microbial colonies are primarily responsible for denture stomatitis.

Denture liners are a type of soft relining materials which have been clinically used during the post surgical treatment and wound healing period. Microbial ingress into the lining material results due to poor hygiene of denture. In addition these materials also experience wear and tear after long term usage which leads to ill fitting dentures.⁸

Microbes responsible for denture stomatitis are generally a mixture of various species of *Candida* and *Staphylococcus*. *Staph aureus* is the microbe which is commonly isolated from the surfaces of lots of dental materials placed into the oral cavity. Their growth has been also a contributing factor in the inflammation of soft tissues leading to the development of stomatitis.⁸

Denture induced stomatitis can be treated by various treatment modalities directed toward the denture base and tissue liners. (CJ Thomas, 1978) Amphotericin B or Nystatin, Chlorhexidine are common topical antimicrobial agents which are used for stomatitis, some other antimicrobial agents such as Fluconazole and Ketoconazole are also available for systemic antifungal treatment.⁴

The antibacterial properties of soft denture lining material have not been evaluated so far. It's an established fact that materials such as Visco-gel and Mollosil are commonly colonized and penetrated by microorganisms.⁹ Studies have shown that incorporating certain chemicals may alter viability of microbes. Several studies have confirmed that AgNP have an adverse effect on pathological micro-organism such as a *Staph aureus*, *Streptococcus mutans* and *Candida albicans*.¹⁰ A study by Kvittek et al. state that if it is used in limited concentration AgNPs do not generate any harm full effect on oral tissues and also inhibit the growth of microorganisms.¹¹

AgNPs have the disadvantage of discoloring the material. So to overcome this synthetic or herbal antimicrobial agent were added into the denture liners. Therapeutic herbs have gained attention for being bactericidal/ bacteriostatic, safe and affordable. These qualities are of importance for the developing nations.

A study by Groppo FC et al (2002) which is based on the comparison of the antimicrobial activity of Garlic, Tea tree oil, and Chlorhexidine against oral micro organisms showed that the garlic and chlorhexidine showed good anti microbial effect against *Staphylococcus aureus* and *Streptococcus mutans* compared to other oral micro organisms, and tea tree oil showed significant antimicrobial effect against *Staphylococcus aureus*, *Streptococcus mutans* and other oral micro organisms.¹² They also conclude that the Tea tree oil and Garlic are effective anti microbial agents and may be used as an alternative to chlorhexidine. Similar results were seen in the present study that Tea tree oil and Chlorhexidine showed significant antimicrobial activity against *Staphylococcus aureus*, and the present study also showed that Tea tree oil was more effective antimicrobial agent than chlorhexidine.

Study by A.N.B. Ellepola et al. (2000) stated that Chlorhexidine exhibits broad spectrum antimicrobial activity including *Candida albicans* and is widely used in mouth washes. Several studies, both in vivo and in vitro, have reported the antimicrobial activity of chlorhexidine. It is believed that chlorhexidine inhibits germ tube formation in *Candida albicans* there by suppressing the candidal activity.¹³ The present study has reported that Chlorhexidine showed significant antimicrobial activity against *Staphylococcus aureus*.

A study was carried by Koteswara Rao Pachava et al (2015) they concluded that denture soft liners incorporated with TTO have shown antifungal efficacy up to 60 days and they suggested that the possibility of this essential oil for therapeutic use against denture stomatitis and other oral infections.¹⁴ The present study also showed similar results.

Existing literature shows that chlorhexidine, clotrimazole, fluconazole, tea tree oil, and silver nano particles can be used with tissue conditioner,⁵ some studies signifying the growth inhibition of *Staph aureus* by these agents. The present study also shows that, silver nano particles, tea tree oil, and chlorhexidine inhibit the growth of *Staph aureus* in the agar culture medium.

This study showed that the incorporation of three different antimicrobial agents in the concentrations of 15 %silver nano-particles, 0.12 % chlorhexidine and 15 %tea tree oil with in temporary liner which was incubated at 37°C for 24 hours. These antimicrobial have effective in inhibiting the growth of *Staphylococcus aureus*.

The data obtained from present study shows that all values obtained for the temporary soft liner group were in the range of acceptance as compared with previous studies. The result is justified with the help of readings obtained from microbiology laboratory. Comparative analysis of antimicrobial property among three antimicrobial agents silver nano-particles, chlorhexidine and tea tree oil incorporated with temporary liner.

The study shows that silver nano particles incorporated with temporary liner has maximum zone of inhibition comparatively with chlorhexidine and tea tree oil. The highest anti microbial property is of silver nano then chlorhexidine and tea tree oil.

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Corresponding Author

Dr. Sidharth Shukla
PG Student
Department of Prothodontics & Crown and Bridge
TMDCRC, Moradabad
Email: dr.siddarthshukla@gmail.com

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