



Peel Bond Strength and Antifungal Activity of Soft Denture Lining Materials Incorporated with Different Concentrations of Chlorhexidine: An In-Vitro Study

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ABSTRACT:

Aim and background: The purpose of this study was to evaluate and compare the peel bond strength between the PMMA and soft denture lining material when soft liner is incorporated in 1% and 1.5% CHX at different time periods and its antifungal activity.

Materials and methods: A total of 54 samples were used in control group and experimental groups to test the peel bond strength and the modes of failure in UTM and antifungal activity by agar diffusion test.

Results: For peel bond strength ANOVA followed by Post Hoc Tukey test showed that control (14 days) was significantly higher ($p < 0.05$) than all other except control (immediately). 1.5% CHX (immediately) showed significantly lower peel bond strength than all other groups ($p < 0.05$). Modes of failure was analysed by Chi Square followed by Kruskal Wallis and Dunn-Bonferonni test, the values obtained shows group with 100% snap was significant from groups with 50% snap and 50% tear ($p < 0.05$). Antifungal activity was analysed by ANOVA followed by Post Hoc Tukey test shows that control and experimental groups when compared were statistically significant ($p = 0$) but comparison of experimental groups was not significant ($p = 0.198$).

Conclusion: Peel bond strength was more when CHX was not incorporated to the soft liner. Incorporation of 1.5% CHX reduced the peel bond strength when tested immediately. Addition of CHX resulted in antifungal activity. However, concentration of CHX added did not significantly affect antifungal activity.

Clinical significance: The study would enable us to find the efficacy of chlorhexidine concentration and its effect on peel bond strength between denture base material and soft reliners.

Introduction

Denture lining materials are used in the intaglio surface of dentures to aid in proper fit of the denture and tissue healing. Based on its composition they can be acrylic based or silicon based soft liners. They are more prone to deterioration with time and harbour micro-organisms. Hence to overcome this, there are various treatment options.

[1] One of the treatment techniques involves incorporation of antimicrobials in the denture liners as a form of drug

delivery system. Candida albicans is the causative agent of oral candidiasis accounting for upto 95% of the cases. Ill-fitting dentures or continuous wearing of dentures by the patients cause fungus like Candida albicans to adhere in denture surface and cause denture stomatitis. Denture stomatitis (DS) is seen in upto 75% of denture wearers as erythema of denture bearing palatal mucosa.

Chlorhexidine diacetate (CHX) is an antimicrobial agent that exhibits substantivity property which makes it effective



against fungus like candida albicans and also other wide variety of microorganisms. Due to its substantivity activity it can be studied for its slow and sustained delivery over a period of time. Chlorhexidine has effectiveness against candida species for a longer period of evaluation and hence was chosen in the study to test immediately and after 14 days, which is the treatment period of denture stomatitis.^[2,3]

Denture stomatitis is the most common oral candidiasis found in removable denture wearers. It could be due to various causes, the most common cause being an inflammatory hypersensitivity reaction against *Candida albicans*.^[4] Treatment for denture stomatitis includes systemic and topical antifungal therapies. In topical antifungal therapy, chlorhexidine is not only used in hygiene protocols but also effective as an oral antiseptic.^[5] Geriatric patients are commonly immuno-compromised due to several age-related conditions. Such patients develop oral candidiasis commonly when a removable denture is provided. Incorporation of chlorhexidine into soft liner ensure sustained therapeutic effect for such cases. Still the potential impairment of bonding between soft denture lining material and denture base or at which concentrations it works best as an antifungal agent is not clear.^[3] This study would analyze the activity of chlorhexidine over a prolonged duration so as to simulate the clinical period of up to 14 days which is the treatment period for denture stomatitis. The study would enable us to find the efficacy of chlorhexidine concentration and its effect on peel bond strength between denture base material and soft relining materials.

The current study was therefore designed with an aim to evaluate and compare if there is any change in peel bond strength between the polymethyl methacrylate denture base and soft denture lining material when soft liner is incorporated in 1% and 1.5% concentrations of chlorhexidine and also the antifungal activity of soft denture liners incorporated with 1% and 1.5% concentrations (by weight) of chlorhexidine.

The study began with two null hypotheses:

First hypothesis being, there will be no change in the peel bond strength between polymethyl methacrylate denture resins and soft liners with and without addition of chlorhexidine at different concentrations.

The second hypothesis was that there will be no difference in the antifungal activity when different concentrations of chlorhexidine were added to soft denture relining materials.

Materials and methodology

The present invitro study was conducted to evaluate the peel bond strength of soft denture lining material when added with 1% and 1.5% concentration of chlorhexidine diacetate with the control group immediately and after 14 days and also to evaluate the antifungal activity of 1% and 1.5% chlorhexidine diacetate with the control group by agar diffusion test to test against candida albicans.

Specimen fabrication

1. Peel bond strength

75x10x3mm thickness mould were placed in the flask and heat polymerized acrylic denture resin were packed in the mould space and cured. The flasks were then bench cooled and kept under running water for 15 mins and the specimens were taken out finished and polished.^[6] Few specimens were kept in distilled water at 37°C and few had to be tested immediately. These specimens were polished with silicone carbide paper and this side is kept facing outside in a mould measuring 75x10x6mm. out of that 75 mm, only 10mm was bonded with the soft denture liner and the remaining 65mm is separated by a polyester strip from bonding to denture lining material. The mould space with remaining 3mm thickness is filled with denture soft relining material without chlorhexidine (control) and with 1% and 1.5% chlorhexidine added to the powder of the soft denture powder and the liquid of the soft-liner is added according to the manufacturer's instructions and then poured into the mould space

2. Anti-fungal activity test

Disc shaped mould is prepared by placing the disk in vinylpolysiloxane silicone type II non reversible duplicating material with 3mm thickness and 10mm diameter and the mould space is filled with soft denture lining material without chlorhexidine (control) and with 1% and 1.5% chlorhexidine added to powder of soft-liner and then mixed with the liquid according to the manufacturer's instruction then poured. It was made sure that the disc has no irregularities. Laminar air-flow chamber was used to make these samples to avoid contamination. For additional sterilization UV light was used for 30 mins on either side.

Peel bond strength

To perform the peel bond strength immediately and after 14days, universal testing machine was used. The portion of acrylic based soft denture lining material not bonded to the heat cure acrylic denture base material (65mm) was folded upwards and fixed onto the upper fixture of the equipment. The lower fixture of the equipment holds the unlined heat cure acrylic denture base material at the same distance (20mm) from the adhesive area. (fig 1) These specimens were then subjected to tension at 10mm per minute speed to



find the peeling that happens between the heat cure acrylic and soft denture liner which was incorporated with chlorhexidine and the force (N) at which it peels, snaps or tears were noted. The failure modes were as follows, 'snap' is when the debonding occurs in the soft-liner away from the bonded area, 'peel' is when debonding occurs between the soft lining material and denture base material interface and 'tear' is when the debonding occurs in the soft lining material within the bonded area. (graph 1)

Antifungal activity test

For the antifungal test to be performed, commercially available *Candida albicans* stains were activated by following the ATCC guidelines. 20ml sterile BHI was used to incubate the colonies at 37°C for about 48h. To achieve optimal growth, two of the isolated colonies were incubated for other 48hrs in 20ml of sterile liquid culture of BHI at 37°C.

A 3 mL aliquot was analysed with a 625 nm (A625) spectrometer to measure the colony forming units (CFU), taking as a basis the interval between A625 0.08 and 0.14, which corresponds to 1.5×10^8 CFU/mL³³).

Petri plates with 10ml of brain heart infusion agar was spread over with the activated strains of 100 µL inoculum (1×10^8 CFU/mL) of candida albicans and sterile punches were made to hold the soft liner specimens of three groups (without chlorhexidine and with 1% and 1.5% of chlorhexidine) and the plate were then incubated for 48 hours at 37°C after which the diameter of the inhibition zones were measured by digital vernier calliper and reflected light. (fig 2) Three measurements were made for each specimen with the calliper and the disc diameter was subtracted and the average was calculated.

Results:

The peel bond strength values obtained shows that the highest peel bond strength was exhibited by the control group (14 days) which was 5.49 ± 0.92 . Followed by control group (immediately) which was 4.37 ± 0.34 . The least peel bond strength value was obtained by 1.5% CHX (immediately) which was 1.96 ± 0.65 . ANOVA followed by Post Hoc Tukey test showed that control (14 days) was significantly higher ($p < 0.05$) than all other except control (immediately). 1.5% CHX (immediately) showed significantly lower peel bond strength than all other groups ($p < 0.05$) (Table I). Modes of failure was analysed by Chi Square followed by Kruskal Wallis and Dunn-Bonferonni test, the values obtained shows 100% snap in 1% CHX (immediately), control tested immediately and after 14 days which was significant from 1% CHX (14 days), 1.5% CHX

tested immediately and after 14 days which shows 50% snap and 50% tear ($p < 0.05$). Antifungal activity was analysed by ANOVA followed by Post Hoc Tukey test shows that the mean value of control group is 0. (Table II) The mean value of 1% and 1.5% CHX was 20.94 ± 1.40 and 21.99 ± 1.02 respectively. Hence control and experimental groups when compared were statistically significant ($p = 0$) but comparison of experimental groups was not significant ($p = 0.198$).^[7]

Discussion:

The first assessment was the peel bond strength of acrylic soft denture liners (with and without chlorhexidine diacetate in 1% and 1.5% concentrations) lining polymethyl methacrylate heat processed acrylic denture base resin.^[8] Since the first two highest values were obtained by the control groups which was made by not incorporating chlorhexidine diacetate it shows that adding chlorhexidine decreases the peel bond strength. The least peel bond strength value was obtained by 1.5% CHX (immediately), which shows higher concentration of CHX decreases the peel bond strength. When the samples tested immediately and after 14 days were compared, the samples tested after 14 days showed higher values which says that immersion increased the peel bond strength.

Therefore, this study shows evidence that, the control group (14 days) had significantly higher peel bond strength and the 1.5% CHX (immediately) had significantly lower peel bond strength. Further, within the limitations of the study it was evident that (1) Addition of chlorhexidine decreases the peel bond strength and (2) testing immediately decreases the peel bond strength when the chlorhexidine concentration was also higher (1.5%).

This could be due to the fact that addition of chlorhexidine affects the physical property of the soft denture lining material.^[1] CHX may damage the polymerized soft lining material within the polymeric matrix.^[1,2] The chlorhexidine particles are bigger in size and irregularly dispersed in the soft acrylic denture material. This irregular distribution pattern of coarse particles of chlorhexidine causes fragility of the soft lining material matrix thus causing porosity which also favours chlorhexidine release.^[9] Addition of chlorhexidine also cause reduction in plasticizer content which in turn cause reduction in disentanglement of the polymer chains causing reduced cushioning effect. This can be explained for hardness attained by the chlorhexidine incorporated soft lining material resulting in a decrease in peel bond strength.^[1,3]



When the samples tested immediately and after 14 days were compared, after 14 days showed higher value in all three groups. However, it was statistically significant only in 1.5% CHX group. Thus, 1.5% CHX (immediately) which had the lowest peel bond strength is significantly lower than 1.5% CHX (14 days). Hence usually the immediately tested samples did not significantly differ much from the samples tested after 14 days. But when the concentration of chlorhexidine was higher the peel bond strength becomes low and if tested immediately it becomes significantly lower.

This could be due to the fact that specimens are softer when immediately fabricated than the specimens immersed in distilled water for 14 days. The stretching of material is favoured by the softness of the material. When the materials are immersed in distilled water for 14 days, plasticizers leach out and the material becomes hard and stiff and lose its softness. The viscoelasticity of the material is lost and the elongation percentage is also decreased which ultimately cause increase in bond strength of the soft lining material which was immersed in distilled water for 14 days.^[1,3] soft lining materials in aqueous environment loses its stability by water sorption or by loss of ethanol and plasticizer components when exposed to water, saliva, denture cleansers and food.^[1] According to 'Sanchez Aliaga A, Farago P V, Michel M D, Sugio C Y C, Neppelenbroek K H, Urban V M', release of chlorhexidine was found to be in higher concentration thus, creating more porosity and hardness that cause increase peel bond strength after immersion over a period of time. ^[4,2,10] Greater water sorption caused by chlorhexidine added soft liner caused swelling of polymer along with drug diffusion. This caused greater chlorhexidine release and also change in the dimensional stability of the material due to its change in volume was observed. ^[4]

The second parameter assessed was the modes of failure between the soft denture lining material and heat processed acrylic resin. The testing was done in the samples along with peel bond strength. The values obtained shows 100% snap in 1% CHX (immediately), control tested immediately and after 14 days. In 1% CHX (14 days), 1.5% CHX tested immediately and after 14 days it shows 50% snap and 50% tear. Hence it can be inferred that in the groups which had 100% snap were stronger than the groups which had 50% tear and 50% snap. This was due to the fact that snap happens away from the bonded area whereas tear happens in the bonded area. There was no peel seen in any specimen. Snap was seen in all the groups which was 100% in 1% CHX (immediately), control tested immediately and after

14 days. Whereas, snap was 50% in 1% CHX (14 days), 1.5% CHX tested immediately and after 14 days, in which other 50% was tear.

From the analysis we can infer that control groups both tested immediately and after 14 days and 1% CHX (immediately) showed significant difference from both 1.5% CHX tested immediately and after 14 days and 1% CHX (14 days). Which concludes that the groups which had only snap was statistically significantly stronger than the groups which had both snap and tear. But the three groups with only snaps were not statistically significant when compared within themselves. The three groups with snap and tear were not statistically significant when compared within themselves.

This could be due to the fact that addition of CHX decreases its physical and mechanical properties as it disturbs the polymeric bonding of acrylic material and hence making it peel easier. ^[1,2] Thus, control groups (tested immediately and after 14 days) were stronger than the 1.5 % CHX tested immediately and after 14 days and 1% CHX (14 days). 1% CHX (immediately) was stronger as it snapped away from the bonded area than the 1.5% CHX tested immediately and after 14 days, as the increased concentration of CHX cause more damage to the polymer chains and cause porosity due to more drug release and the bonding becomes weak. This could be the reason due to which increased concentration of CHX groups tears near the bonded area. 1% CHX (immediately) was stronger than 1% CHX (14 days). This could be due to the fact that the freshly prepared soft liner incorporated with CHX were rich in plasticizers and ethanol components and hence less porosity hence the bonding was good that it snapped away from the bonded area.

Hence the **first hypothesis was partially rejected** as addition of CHX decreased the PBS irrespective of the immersion period and without immersion decreased the PBS when the concentration of CHX was higher (1.5% CHX).

The Third assessment was on the antifungal activity of CHX incorporated in different concentrations. The testing was done in 6 samples each in three groups. The first group being control with no chlorhexidine and the other two experimental groups with 1% and 1.5% CHX added to soft denture liner. The data obtained showed no inhibition zones around the control group and it showed inhibition zones around both the experimental groups. From which it was very evident that chlorhexidine diacetate inhibits the *Candida albicans* when added to soft lining material.



Though there was a significant difference between control and the two experimental groups, there was no significant difference between the experimental groups. Which concludes that addition of chlorhexidine significantly inhibits the *Candida albicans* growth. ^[11,12,13] But the concentration at which it was added (1% and 1.5%CHX) did not make much difference in their inhibition activity.

This could be due to the fact that chlorhexidine is an efficient antifungal agent and have broad spectrum of activity against bacteria and fungus thus, 1% and 1.5% chlorhexidine formed inhibition zones against *Candida albicans*. But control group without chlorhexidine did not form inhibition zones. ^[14,15] There were not much of a difference between 1% and 1.5% chlorhexidine as it is anyway effective in its minimal inhibitory concentrations. ^[2,16] Hence the **second null hypothesis** is accepted as there was no significant difference between the different concentration of CHX added.

Limitations if the study is that, since it is an invitro study the results could be different in oral environment as the composition of distilled water differs from the saliva components. Only one type of soft liner was used, which was acrylic based. **Further research** could be performed in a clinical scenario. Different types of soft liners could also be studied as they were not evaluated in this study. Moreover, newer antifungal agents incorporated into dentures or denture liners have not been evaluated so far. The antifungal activity was studied only against *Candida albicans*.

Conclusion:

Within the limitations of the study, it can be concluded that, peel bond strength was more when CHX was not incorporated to the soft liner. Incorporation of 1.5% CHX reduced the peel bond strength when tested immediately. Incorporation of 1% CHX can be utilised in clinical situations as peel bond strength was not reduced significantly. Addition of CHX resulted in antifungal activity. However, concentration of CHX added did not significantly affect antifungal activity.

Clinical significance: The study would enable us to find the efficacy of chlorhexidine concentration and its effect on peel bond strength between denture base material and soft reliners.

List of Abbreviations:

CHX: Chlorhexidine diacetate.

C. albicans: *Candida albicans*.

PBS: Peel Bond Strength

Reference:

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FIGURE LEGEND:

FIG 1: Peel bond strength testing in UTM

FIG 2: Antifungal activity inhibition zones



FIG 1: Peel bond strength testing in UTM



FIG 2: Antifungal activity inhibition zones