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Original Article

Longitudinal Clinical Trial on the Potential of Acrylics as Antimicrobial Power Banks and Their Impact on Oral Health of Patients

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Abstract

Introduction: The most common problems caused by the long-term use of removable partial dentures are periodontitis and caries affecting abutment teeth. The acrylic tooth junction is prone to plaque accumulation and growth of microbial biofilms. Hence, partial dentures need to promote oral self-cleansing not only by their design but also by their chemical nature. Acrylic resins are capable of acting as rechargeable power banks in vivo, and providing sustained concentrations of antimicrobial agents at the tooth-gingiva-denture junction would greatly enhance prognosis. This randomized clinical trial was designed to assess the caries susceptibility and periodontal health in patients who were treated with acrylic removable partial dentures over a 12-month period. The effects of storing dentures in chlorhexidine (CHX), fluoride, and water were analyzed. Salivary pH was taken as a measure of caries susceptibility and the gingival index (GI) and plaque index (PI) were used as measures of periodontal health.

Methods: This randomized clinical trial was conducted according to CONSORT guidelines on 100 patients requiring removable partial dentures. Patients in the control group, CHX group, and F group stored their dentures in water, CHX, and fluoride solutions overnight, respectively. The salivary pH, GI, and PI were recorded: (i) before treatment, (ii) 3 days, (iii) 3 months, (iv) 6 months, and (v) 1 year after the treatment.

Results: The salivary pH decreased, and PI and GI scores increased in all groups three days after insertion. However, a statistical significant increase in salivary pH and reduction in PI and GI scores at 3, 6, 12-month follow-up appointments were seen in the CHX and F groups compared to the control group.

Conclusion: The study shows that acrylics are capable of acting as anti-microbial power banks for improving gingival health and reducing caries susceptibility in patients.

Keywords: Removable partial denture, Chlorhexidine, Fluoride, Salivary pH, Caries susceptibility, Plaque index, Gingival index

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Introduction

It was observed that 59% of the elderly in India are partially edentulous (1). Many of them are rehabilitated with a removable prosthesis. However, literature reveals poor prognosis of removable partial dentures with a high failure rate after a mean follow-up of 4 years (2). The main causes of this problem include inadequate oral hygiene maintenance, debris, and plaque accumulation on the denture and at the tooth-acrylic-gingival junction. This promotes the growth of biofilms which act as reservoirs for bacteria and fungi. Partial dentures may also hinder the natural cleansing action of saliva, cheeks, and tongue on teeth and gingiva. Bacteria in denture biofilms create an acidic microenvironment conducive to demineralization of adjacent tooth structure. Studies show that the common causes of failure of removable partial dentures are increased incidence of caries (3), particularly root caries, deteriorated periodontal health, and loss of abutment

teeth (4,5). Hence, great care should be incorporated into the design of partial dentures to ensure oral self-cleansing. Therefore, making them chemically antimicrobial can greatly enhance prognosis.

The null hypothesis of this study was that patients soaking acrylic partial dentures overnight in water, chlorhexidine (CHX), and fluoride solution have similar caries susceptibility and periodontal health.

CHX is the gold-standard oral antimicrobial and antiplaque agent. It acts by causing cell wall disruption, inhibiting the growth of bacteria and even spores. Being cationic in nature, it binds to mucosa and teeth, enhancing substantivity for up to 12 hours (6).

Fluoride has also been shown to affect the pH of saliva by reducing bacterial acid production (7). Studies have shown that F ion reduces the growth of *Streptococcus mutans* in plaque by inhibiting the bacterial sugar uptake system (8-10). In vitro studies have demonstrated that acrylic resins can uptake CHX (11) and fluoride (12) and release them over an extended duration. However, there are no in vivo studies analysing the potential of acrylics to preserve the oral health of patients wearing partial dentures.

Salivary pH influences the equilibrium of calcium phosphates in the enamel (13). Hence, it can be used as a reliable diagnostic tool for caries activity. Reduced salivary pH describes a reduced buffering capability of saliva, which increases the patient's caries susceptibility (14).

Materials and Methods

Study Design

This randomized clinical trial was conducted on 100 partially edentulous healthy individuals aged 30-70 years (mean age: 48 years) who were referred to the Department of Prosthodontics. The sample size for the study was calculated using G*Power software version 3.1.9.6, considering a power of 95% and a significance level of 5%. Patients were provided with a detailed description of the study purpose, treatment methodology, study duration, and possible side effects. Participants were required to sign an informed consent form before being included in the study. Ethical clearance was obtained from the Institutional Ethics Committee (Pr.003/IEC/GDCH/2024-25 /01). This study followed the CONSORT guidelines for reporting outcomes of clinical trials (15).

Patients with systemic diseases or those using medications that could affect their gingival health were excluded from the study. Patients with symptoms of fluorosis, habits of smoking, tobacco, pan chewing, and active caries, those using mouthwash and fluoridated toothpastes, and patients with low IQ who were unable to comply with instructions were all excluded from the study. This eliminated the effect of confounding factors. The Oral Hygiene Index (OHI) of patients varied from good to fair. A total of 91 subjects attended all recalls until the 1-year follow-up. Of these subjects, 52 were females and 39 were males. Only the data of these patients were statistically analyzed (Figure 1). Salivary pH was measured using a Hanna digital pH meter (Model: Hanna HI98107 New pHep, USA).

Data Collection

All patients underwent complete ultrasonic scaling before participating in the study to ensure uniform baseline oral hygiene at the beginning of the study. All clinical procedures were performed during morning appointments between 09:00 and 12:00. Participants were asked not to eat or drink 1 hour before the procedure. After rinsing their mouth with plain water, they were seated comfortably on the dental chair, with their head tilted forward. Salivary samples were collected using the spitting method and their pH values were recorded (16). After an initial swallow, they were instructed to spit once every 30 seconds and collect saliva into a clean graduated measuring jar provided to them (Figure 2). This was continued until 5 mL of saliva was collected.

The pH of the collected samples was determined using a digital pH meter. The pH meter was calibrated using a buffer solution with a pH of 7 (HI70007P). The probe was rinsed with distilled water before and after each recording. To record the pH value, the electrode was submerged in the sample to be tested. After the reading stabilized on the LCD screen, the value was recorded (Figure 3). In this way, the baseline salivary pH value was recorded before treatment. The pH meter calibration was performed at regular intervals to ensure that the readings were accurate.

Baseline values were recorded by the first investigator. Plaque index (PI) and gingival index (GI) scores were recorded according to Sillness and Loe (17). In accordance with the WHO guidelines for permanent dentition (18), the index was scored using a dental explorer, periodontal probe, and dental mouth mirror under standard operating light. The score interpretation is presented in Table 1. Each dental arch was divided into 3 segments (teeth 8-3, 3-3, and 4-8). Every tooth was examined for plaque and gingival inflammation, and a corresponding score (0 to 3) was assigned. From each segment, the tooth with the highest score was used for calculating the individual's index. The index for each patient was obtained by summing the indices for all six segments and dividing by 6. Intra-examiner reliability was checked from time to time to ensure consistency.

Removable partial dentures were fabricated following the compression moulding technique and a long curing cycle, using DPI heat cure acrylic resin [DPI Heat Cure Acrylic; India]. All the prostheses were fabricated in the laboratory of the department. Good adaptation of acrylic to the gingiva and resin teeth to abutments along guide planes was confirmed to ensure adequate oral self-cleansing. Instructions about placement, removal, and use were given to patients.

Oral Hygiene Instructions

Patients were educated about the importance of oral

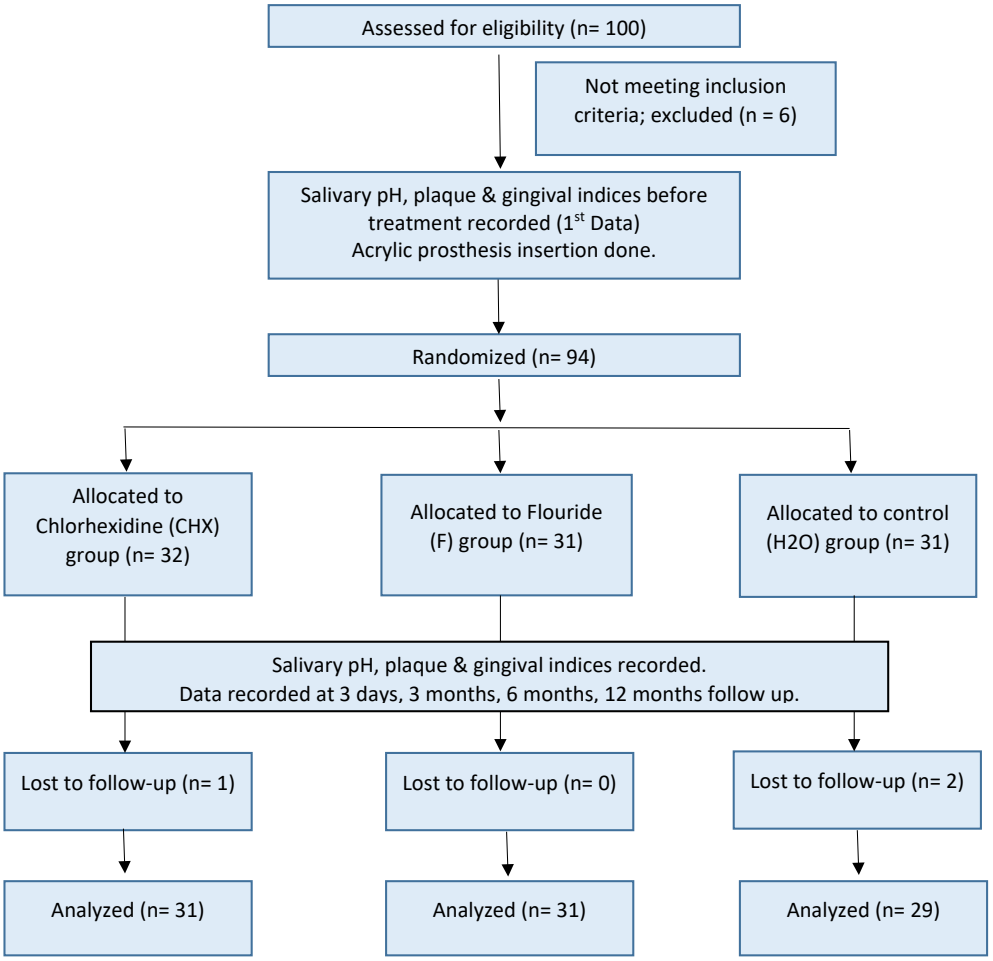

Figure 1. CONSORT Flow Diagram of the Study

Figure 2. Collection of Salivary Samples Using the Spitting Method for Analysis of Salivary pH

Figure 3. Digital pH Meter Used for Analyzing Salivary pH

hygiene maintenance. They were instructed to use non-fluoridated toothpaste for tooth brushing. All subjects were given detailed instructions about techniques of brushing, rinsing, the use of interproximal brushes, flossing, and cleaning the prosthesis. Instructions were reinforced during each follow-up appointment.

Intervention

The participants were required to pick up sealed envelopes, thus allocating them to one of the 3 groups in a ratio of 1:1:1 by simple randomization. This was implemented to mitigate selection bias and to diminish the impact of age and gender disparities on the outcomes among patients in the groups.

1. Group CHX: Patients who kept their dentures in CHX solution overnight

Table 1. Criteria for Scoring Plaque Index (PI) and Gingival Index (GI)

Scores	PI	GI
0	No plaque	Normal gingiva, no inflammation, discoloration, or bleeding
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth was observed. The plaque may be seen in situ only after the application of disclosing solution or use of the probe on the tooth surface.	Mild inflammation, slight colour change, mild alteration of gingival surface, no bleeding on pressure
2	Moderate accumulation of soft deposits within the gingival pocket, or the tooth and gingival margin which can be seen with the naked eye	Moderate inflammation, erythema and swelling, bleeding on pressure
3	Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin	Severe inflammation, erythema and swelling, tendency to spontaneous bleeding, ulceration

2. Group F: Patients who kept their dentures in fluoride solution overnight
3. Control group: Patients who kept their dentures in distilled water overnight

Briefly, 42 g of sodium fluoride (NaF) powder (Labogens; Vatva; Ahmedabad; Gujarat) was weighed out and transferred into a 1-L volumetric flask. Distilled water was added up to the 1L mark and stirred to prepare 1 M NaF solution. Pure samples were required for standardization; hence, root canal disinfectant was utilized as it comprised solely 2% CHX (Neelkanth Ortho Dent Pvt. Ltd; Rajasthan) without any colour additives (Figure 4).

Double blinding was used to prevent bias. Neither the patient nor the investigator who recorded the data was aware of which group the patient belonged to. The second investigator, after checking patients' envelopes, recorded the group to which they belonged. Then, 500 mL bottles of fluoride solution were handed over to patients in the F group, CHX solution to patients in the CHX group, and distilled water to patients in the control group. The patients were instructed to store their dentures in their respective solutions overnight and to daily replace the solution in the denture storage container with new solution from the bottle (Figure 5).

Figure 4. Reagents Used to Formulate Fluoride and Chlorhexidine Solutions

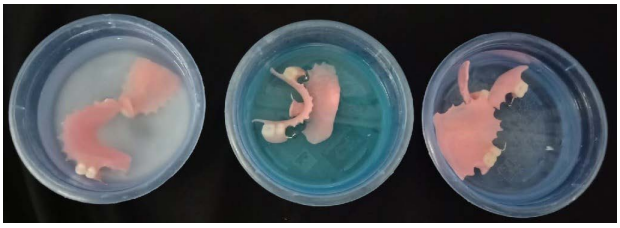

Figure 5. Removable Partial Dentures of 3 Groups Being Stored in (1) Fluoride Solution, (2) Chlorhexidine Solution, and (3) Plain Water Overnight

Monitoring

Patients were recalled at the following time intervals:

1. at the beginning of treatment
2. 3 days after insertion of the prosthesis
3. 3-month follow-up
4. 6-month follow-up
5. 12-month follow-up

Oral hygiene instructions were reinforced, and salivary pH, PI, and GI were recorded at each recall appointment by the first investigator. Continuous recalls and reinforcement of instructions ensured patient compliance to the study protocol.

Statistical Analysis

The obtained data were tabulated and statistically analyzed using SPSS version 26.0. Since the study involved comparison of mean scores of the same subjects at multiple time intervals, repeated measures ANOVA was the most suitable statistical test, assuming normality, sphericity, and homogeneity of variances. Inter-group changes were compared by one-way ANOVA. P value < 0.05 was considered statistically significant. Descriptive statistics are presented as mean ± standard deviation (SD).

Results

Mean pH scores are shown in Figure 6. The results of repeated measures ANOVA showed that salivary pH values significantly reduced ($P < 0.015$) 3 days after placement of the prosthesis in all the groups compared with the baseline values. The salivary pH further reduced at the 3, 6, and 12-month follow-up appointments in the CHX group. On the other hand, salivary pH increased progressively at the 3, 6, and 12-month follow-up appointments in the CHX and F groups. The results of the paired t test showed a significant reduction in salivary pH of the control group 12 months after the use of the prosthesis ($P = 0.013$). On the other hand, salivary pH significantly increased in the CHX and F groups ($P = 0.021$) compared to the mean pH before treatment.

The PI, GI scores are described in Tables 2 and 3. The PI and GI scores showed a statistically significant temporary increase 3 days after the insertion in all the groups compared with baseline scores. The results of repeated measures ANOVA showed a significant increase ($P < 0.01$) in the PI and GI scores of the control group

Table 2. Comparison of the Mean Plaque Index (PI) Scores of the Chlorhexidine (CHX), Fluoride (F), and Control Groups at Different Time Intervals Using ANOVA

Time Intervals	Groups (mean ± SD)		
	CHX	F	Control
Baseline	0.82 ± 0.33	0.88 ± 0.24	0.87 ± 0.45
3 days	0.89 ± 0.36	0.99 ± 0.26	1.21 ± 0.32
3 months	0.85 ± 0.34	0.85 + 0.27	1.11 ± 0.35
6 months	0.74 ± 0.35	0.75 + 0.26	1.37 ± 0.31
12 months	0.72 ± 0.34	0.73 + 0.26	1.37 ± 0.25
P value*	< 0.01*	< 0.01*	< 0.01*

**P* value was calculated for the change over time using repeated measures analysis of variance (RM-ANOVA).

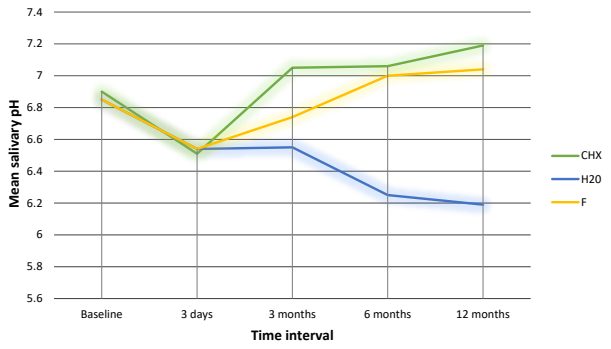

Figure 6. Mean Salivary pH Values of the Groups at Different Time Intervals

in the subsequent follow-ups (3, 6, and 12 months). On the other hand, PI and GI scores of CHX and F groups showed a gradual but significant reduction ($P < 0.01$). The GI scores showed similar trends, correlating with the PI scores.

Based on the results of one-way ANOVA, CHX and F groups had significantly lower PI and GI scores than the control group at 12 months ($P < 0.001$, $P < 0.001$), but the difference between CHX and F groups was not significant ($P = 0.89$, $P = 0.40$). One-way ANOVA showed a significant acidic shift in salivary pH of the control group compared to the CHX and F groups at 12 months ($P < 0.01$).

Discussion

A healthy pH level for saliva lies between 6.2 and 7.6 (19). This is the measure of H^+ion concentration in saliva, the sources of which are secretion by the salivary glands, production by the oral microbiota, or acquisition through food and drink. As pH decreases below 5.9 (i.e., the critical pH), demineralization of tooth structure and initiation of caries occur (20). Patients with a resting salivary pH of around 7.0 have lower caries activity than those with a pH below 6.31 (14,21). In a study, salivary pH was determined by a pH meter, and an inverse relationship between DMFS index and salivary pH was found (22). Hence, salivary pH was used as an indicator of caries susceptibility of the patient in this study.

Table 3. Comparison of the Mean Gingival Index (GI) Scores of the Chlorhexidine (CHX), Fluoride (F), and Control Groups at Different Time Intervals Using ANOVA

Time Intervals	Groups (mean + SD)		
	CHX	F	Control
Baseline	0.65 + 0.37	0.68 + 0.23	0.68 + 0.45
3 days	0.76 + 0.38	0.95 + 0.30	1.03 + 0.42
3 months	0.74 + 0.41	0.73 + 0.26	0.94 + 0.34
6 months	0.56 + 0.37	0.61 + 0.26	0.98 + 0.34
12 months	0.50 + 0.36	0.58 + 0.26	1.03 + 0.32
P value*	< 0.01*	< 0.01*	< 0.01*

**P*-value was calculated for the change over time using repeated measures analysis of variance (RM-ANOVA).

In the present study, it was found that salivary pH reduced 3 days after the use of the prosthesis (Figure 6) The reduction was not significant in the F and CHX groups but a significant fall in pH was seen in the control group. This may be attributed to inadequate maintenance of oral hygiene and plaque build-up. In an in vivo study, Fibryanto and Widyastuti analyzed salivary pH and found that removable partial dentures can cause saliva to become acidic, especially in the morning (23). However, another study on salivary pH in complete denture patients showed no change in pH over 1 week (24). In a study by Muddugangadhar et al, it was noted that the placement of complete dentures increased the salivary pH, resulting in an increased flow rate (25).

It should be noted that multiple borders and tooth-acrylic crevices in partial denture patients make maintenance of oral hygiene more difficult compared to complete denture patients. The salivary flow rate shoots up immediately after denture insertion but gradually reduces within a few days after insertion. Both these phenomena together can be attributed to the reduced pH level observed 3 days after the insertion in the present study. This is supported by another study where salivary pH in children with plaque and without plaque was compared; in other words, it was seen that salivary pH was significantly lower in the group with plaque accumulation (26). It was also noted that oral clearance of sucrose was delayed in the plaque group when compared to the non-plaque group.

PI and GI are reliable markers of the periodontal health of a patient. Periodontal condition is influenced by hygiene maintenance at the junction of the prosthesis and marginal gingiva. In many patients wearing partial dentures, food debris and plaque tend to accumulate at this junction. The tongue and cheek muscles during movement have an important cleansing action on the dentogingival junction. However, some parts of the prosthesis, like the clasp and borders of the denture near the marginal gingiva, sometimes hinder this action. Numerous studies have described the negative effect of removable prosthesis on the periodontal status of adjacent teeth (2-4). PI and GI scores of all groups at the 3-day follow-up in the present study are on par with these studies demonstrating increased plaque accumulation

and gingival inflammation. However, the increase was more profound in the control group compared to the CHX and F groups.

In this clinical trial, the effect of recharging acrylics was analyzed by storing them in fluoride and CHX. CHX is the most recommended disinfectant due to its non-toxic and non-polluting properties, absence of gas emissions, and non-irritating effects on skin and mucosa. It is a cationic chlorophenyl bisbiguanide that destroys the cell membrane of negatively charged microbial cells. It has a broad spectrum of antimicrobial activity. At low concentrations (0.02%-0.06%), CHX causes displacement of $Ca^{2+}$and Mg^{2+} and loss of K^{+} from bacterial cell wall, resulting in a bacteriostatic effect. CHX can also inactivate enveloped viruses, such as herpes simplex virus, which are associated with cold sores. CHX shows antifungal activity by preventing biofilm formation on tooth and denture surfaces by species such as Candida (5,26-27). A study on the effect of low concentrations of disinfecting agents on heat-cured acrylic resins identified 0.2% CHX as a fast-acting antimicrobial agent (28).

Acrylic resins have the inherent capacity to absorb water and various chemical components from the oral environment and subsequently release them into the surrounding milieu (29). Preethy Chandran et al conducted a study by soaking acrylic discs in 2% CHX over 24 hours. High-performance liquid chromatography demonstrated that CHX-treated acrylics were capable of releasing CHX into a test solution in concentrations capable of exhibiting antimicrobial activity (11). Hence, the same concentration of CHX (2%) was used to ensure good recharge and release of CHX in the present study. Moreover, soaking in CHX reduces the development of biofilms on denture surfaces (5). These findings are consistent with the results of the present study. Acidic salivary pH values and lower PI and GI scores were recorded at 3, 6, and 12-month follow-up appointments in patients soaking their acrylic dentures in CHX solution overnight. This can be attributed to the interference of CHX with the attachment of biofilms to the tooth and denture surface, thus reducing bacterial biofilms and hence their acid production.

Fluoride ion at concentrations as low as 1 to 2 p.p.m. has a detectable inhibitory effect on salivary acid production and concentrations of 5 to 10 p.p.m. have a marked effect (9). Fluoride has also been proposed to reduce the growth of *S. mutans* in plaque. The effects of fluoride on streptococcal cells are partly ascribed to the inhibition of enolase, decreasing the intracellular level of phosphoenolpyruvate (PEP) and reducing bacterial sugar uptake via the PEP-dependent phosphotransferase system (PEP-PTS) (6-8).

In an in vivo study, Patekar et al treated acrylics with NaF gel and analyzed the uptake and release of fluoride using an ion-selective electrode. They concluded that acrylics pre-treated with fluoride showed a constant release of fluoride into the test solution over a period of 24 hours (12). Consequently, 1 M NaF was employed to evaluate the fluoride recharge of acrylics in the current study. Several studies in the literature compared salivary pH in children before and after using fluoridated toothpaste. They reported a significant increase in salivary pH after using fluoridated toothpastes when compared to non-fluoridated toothpastes (6,9). The average pH of saliva before brushing was 7.1 but it increased to 7.59 after brushing with fluoridated toothpaste (30).

The decreased PI and GI scores of the F group can be comprehended in the light of the antibacterial efficacy of F ions released by fluoride-recharged acrylics. This explains why the salivary pH of the fluoride group was alkaline compared to the control group during the 3, 6, and 12-month follow-up periods.

Hence, the acrylic component of dentures absorbed CHX/fluoride during overnight soaking and gradually released it into the oral environment at concentrations capable of creating a bacteriostatic effect as proven in in vitro studies. The concentrations of CHX and fluoride being released intraorally are clinically safe as they are far below cytotoxic levels (31).

The shift in salivary pH in all groups towards acidity at the 3-day follow-up demonstrates that debris, plaque accumulation, sugar breakdown, and acid release in patients increased after wearing the partial denture. This process continued in the control group. The falling pH scores in long-term follow-up indicate that these patients may become more susceptible to caries when other risk factors are present. The progressively increasing PI and GI scores in the control group demonstrate the deteriorating gingival health (Tables 2 and 3). CHX and F taken up by acrylic overnight were gradually released during use, showing a sustained antimicrobial effect against plaque and cariogenic bacteria. The combined effect of reinforcing oral hygiene instructions and soaking dentures in test solutions could be the reason behind the increase in pH and decrease in PI and GI scores observed in CHX and F groups during the follow-up appointments.

Studies on the long-term use of most disinfectants on acrylics have shown no significant changes in hardness, flexural strength, or roughness of the samples (32-34). Particularly, the absence of chemical products such as alcohol or phenyl in CHX and NaF would minimize the risk of resin dissolution or damage to acrylics. As non-tinted CHX was used in the study, staining of acrylics was not observed. Studies on CHX doped acrylic resins showed acceptable clinical properties; however, the release of CHX was seen to reduce with time (35). Hence, recharging acrylics in CHX solution would have better results than doping with CHX. However, there are certain limitations to our study. The results have to be validated by studies conducted on larger population. Further high-performance liquid chromatography and colorimetric analysis of saliva are needed to investigate the concentration and rate of chemicals released from recharged acrylics into saliva. The effects of these released

chemicals on oral microbiome need to be studied by microbiological cultures for further evidence. The external validity of the study also needs to be analyzed given the demographic variability among patients.

Conclusion

To improve oral health in removable partial denture patients, special attention should be given to its design to ensure mechanical self-cleansability. This effect can be enhanced by making it also chemically self-cleanable. In this study, the effect of CHX and F on pH and plaque was not observed at the 3-day follow-up but it was seen in subsequent long-term follow-ups. Hence, it can be said that the effect of these agents is cumulative and is best observed when combined with reinforced oral hygiene instructions.

Authors' Contribution

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Funding Acquisition: D. Sudha Madhuri, Reddy Chaithanya.
Investigation: Reddy Chaithanya, Gasthi Anjaneyulu, M. Sarala Kumari.
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Resources: D. Sudha Madhuri.
Software: M. Sarala Kumari.
Supervision: Gujjalapudi Mahalakshmi.
Validation: Mulam Sai Swetha.
Visualization: Reddy Chaithanya.
Writing–Original Draft: Reddy Chaithanya, Gasthi Anjaneyulu, Mulam Sai Swetha.
Writing–Review & Editing: D. Sudha Madhuri, Gujjalapudi Mahalakshmi.

Competing Interests

The authors declare that they have no conflict of interests.

Ethical Approval

This study was approved by the Institutional Ethics Committee of Government Dental College, Kadapa (Pr.003/IEC/GDCH/2024-25 /01). After explaining the risks and benefits, only patients who were willing to participate were included in the study. Additionally, the present study followed the Declaration of Helsinki (ethical principles for medical research involving human subjects).

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