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Research Article

The Staining Effect of Mouthwash Containing Silver Nitrate Mouthwash on Dental Enamel: A Comparative Study

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ABSTRACT
This in-vitro study evaluated and compared the enamel staining potential of a 0.011% w/v silver nitrate solution and an ionic silver based mouthwash (SilvOral® supplied by ALPINIA pharmaceuticals) on extracted human premolars. Forty teeth without caries were randomly allocated to three groups: silver nitrate (n=20), ionic silver mouthwash (n=20) and distilled water control (n=20). Colorimetric measurements (ΔE, L, C, H*) were taken by Vita Easyshade® spectrophotometer at baseline (T1), after 24h immersion (T2) and after brushing (T3). Results found that the silver nitrate group was significantly discolored with the mean ΔE value of 10.9 in T2, with a great variation towards dark shades (C3-C4), a decrease in lightness (mean L*=-1.2). In contrast, in the mouthwash group, lighter Vita shades (A3-A4) were maintained, higher lightness values (mean L* = +8.4) were achieved and lower perceptible change of color was registered (mean ΔE = 6.3). It was found that while the stain caused by silver nitrate was largely permanent, the mouthwash group was able to partially reverse the stain (ΔE of 4.8) after brushing. Analysis of variance (ANOVA, $p < 0.05$) confirmed the color stability was significantly better with the mouthwash. These results indicate that the ionic silver mouthwash has lower and greater reversible enamel discoloration than silver nitrate and support it to have a safer esthetic profile for clinical application.

Keywords: Silver Nitrate, Ionic Silver Mouthwash, Dental Enamel, Color Change (ΔE), Staining Potential, Tooth Discoloration.

INTRODUCTION

Aesthetics of the teeth has become a major issue among both the patients and the clinicians. Intrinsic or extrinsic teeth discoloration compromises beauty, self-respect, and tends to affect the acceptability and satisfaction of dental care [1]. Extrinsic staining on the surfaces of enamels can be induced by a variety of agents, including: dietary chromogenics, tobacco, antiseptic agents, restorative materials, etc. Mouthwashes are also popular in preventive dentistry due to their anti-microbial, anti-plaque and caries-preventive properties, but some of them sorely impact on tooth color as a cause of dissatisfaction in patients [2].

The use of silver compounds in dentistry is not a new discovery since they have been known to possess the potent antimicrobial effect and ability to arrest carious lesions. Historically, caries control was done using silver nitrate, especially the solution of Howe before giving way to fluoride agents. Recently, protocols involving silver nitrate plus fluxoride varnish have been of renewed interest to halt caries in the populations with low dental care access due to their simplicity, and low cost, and effectiveness. A significant weakness, though, which makes them less acceptable, is staining of the teeth, particularly when the treatment is done in exposed parts of the dentition [3].

The process through which silver compounds stain the enamel and the dentin is quite well comprehended. Silver ions (Ag +) liberated in solutions (i. e. silver nitrate) can react with enamel surfaces, penetrating microporosities or demineralized enamel to a depth of up to 25 um (or more) in other instances. Ag+ can be reduced to metallic silver on exposure to reducing agents (organic material, light, etc.), or are formed as silver phosphate or silver oxide. These formed silver species are reduced or precipitated and have a dark, usually grey to black colour, which may be visible and as long-lived [4].

Although silver diamine fluoride (SDF) has now come to be more extensively researched regarding its caries-arresting property, as well as its esthetic limitations (that is, black staining of lesions), much of the research on

silver compounds has been done on lesions, demineralization, and remineralization, and the limited studies on silver compounds have measured color change in sound non-lesioned enamel surfaces [5].

Nano-silver and silver nanoparticles (AgNPs) have also been studied as a constituent of dental materials (e.g., restorative resins, adhesive) and mouthrinses. Some studies reveal that when AgNPs are incorporated in resins or mouthwashes, there is a concentration dependent effect on the color change of enamel, with higher concentrations resulting in higher ΔE (color differences) which are perceptible and probably unacceptable. For example, in a case of orthodontic resin modified with different concentrations of AgNPs (0.05%-1% wt) the group with 1% presented significant ΔE_0 values in comparison with control after storage, which means visible color change [6].

Nano silvers and silver nanoparticles (AgNPs) have been examined both in dental products (e.g., restorative resin, adhesive) and as an additive in mouthrinses. Other studies indicate that the addition of AgNPs in the form of resins or even mouthwashes results in an effect of concentration on the color change on enamel, with increasing concentrations producing increasing ΔE (color differences) which were both noticeable and perhaps unacceptable. An example is a case study on orthodontic resin where its percentage of AgNPs (0.05%-1% wt) was changed to 1 percent, where the visible color change occurred in the 1 percent group but not in the control group [7].

Additionally, direct evaluation of enamel discoloration in in vitro studies of nanoparticles in mouthwashes has been directly measured. In one of these studies, premolars which were in colloidal solutions of nano Ag displayed pronounced ΔE after 24 hours but the intensity and orientation of color change was also dependent on the size of the particle, concentration and the type of silver compound. Discoloration was reduced but not removed completely with brushing [8].

Thus, the purpose of the current study is to compare the staining effect of a silver nitrate solution and an ionic silver mouthwash (both at 0.011% w/v silver nitrate equivalent) on extracted human premolars in order to define (1) the extent to which color change appears after immersion in each solution compared to the control, (2) whether the brushing prevents the appearance of the color change, and (3) whether the color change is significantly lesser when the samples are immersed in an ionic silver mouthwash than when the samples are immersed.

MATERIALS AND METHODS

Study Design and Setting

This was an experimental in-vitro experiment in the Research Laboratory, Santosh Dental College and Hospital, Ghaziabad, India, to compare the effect of silver nitrate solution and an ionic silver mouthwash on human dental enamel in terms of staining.

Materials

Silver Nitrate Solution

The test solution was prepared using analytical grade silver nitrate (>63.5% purity; Bangalore Refinery, Bangalore, India). The solution was dissolved in distilled water to reach the concentration of 0.011% w/v. Silver nitrate solution was prepared and sterilized via the process of autoclavation before use to achieve sterility and eliminate microbial contamination in the course of the experiment.

Ionic Silver Mouthwash

The mouthwash used in the present study was SilvOral (Alpinia Biopharma Pvt. Ltd.) that is an ionic silver based patented formulation. The product offers silver ions that are equal to 0.011% w/v silver nitrate, which in turn permits a direct comparison with the obtained silver nitrate solution and reflects a commercially available oral rinse.

Control Solution

In this experiment, distilled water was taken as the negative control solution. This enabled any color change to be evaluated that could only be attributed to immersion and handling processes without the effects of silver compounds.

Tooth Sample Preparation

This study gathered forty human upper premolars that were removed due to orthodontics. To have a uniform quality of enamel, all the teeth chosen were caries-free, uncracked and without restorative materials. The samples were stored in distilled water in the room temperature until use immediately after extraction to avoid dehydration. Before the testing, all the teeth were polished and cleaned with a water-pumice slurry using rubber prophylactic cups to scrape off the surface debris and pellicle. The polished teeth were well rinsed by running tap water then

placed in distilled water at a temperature of 37 degC and left in it until 24 h was passed to bring the enamel surface to an equilibrium state prior to the experimental activities.

Group Allocation

After the preparation of samples, the teeth were randomly placed in three experimental groups. The immersion of group 1 specimens in 0.011% w/v silver nitrate solution (n=20) was done. Group 2 samples were dipped in an ionic silver mouthwash comprising of 0.011 percent w/v silver ions (n = 20). The control Group 3 specimens were immersed in distilled water and under the same conditions (n= 20). Random allocation reduced selection bias and provided comparability of groups.

Color Measurement Procedure

The Vita Easyshade(r) spectrophotometer (Vita Zahnfabrik, Bad Sackingen, Germany) was used to measure the color. The measurements of the buccal enamel surface were made on each of the specimens to standardize location. The values of the baseline color (T1) were measured before immersion into the test or control solutions. The samples were then incubated in their respective solutions of 24 h; incubation was achieved by giving the containers a gentle stir after every three hours to ensure that the solution was well mixed. The teeth were air-dried and rinsed and re-measured the color after immersion (T2).

Afterward, every specimen was standardized to be brushed to imitate oral hygiene. Brushing One-minute brushing was done with an Oral-B Vitality Pro(r) powered toothbrush and a fluoride toothpaste (Sensodyne) at a constant load. The teeth were rinsed, dried and the color contended with again after brushing (T3). The measurements of the colors were made twice on each specimen and the average value of the L, a, and b 3 coordinates were taken to be analyzed.

Calculation of Color Change (ΔE)

Color change between time points was calculated using the CIE L*a*b* formula:

$$\Delta E=(\Delta L)2+(\Delta a)2+(\Delta b)2$$

Where ΔL, Δa, and Δb are differences in lightness and chromaticity between two time points.

Statistical Analysis

All groups and time points had the descriptive statistics computed. Baseline (T1), post immersion (T2), and post brushing (T3) mean values and standard deviations (mean ± SD) of color change (ΔE) were determined in each experimental group.

Paired t-tests were also used in within-group comparisons of immersion and subsequent brushing at the three time points to find out whether there were significant changes in ΔE with immersion and post-brushing to the baseline. One-way analysis of variance (ANOVA) and post-hoc tests, respectively, were used to assess intergroup differences at every time point, and then post-hoc tests were followed by assessing the pairwise differences.

A predetermined level of significance of $p < 0.05$ was used in all the analysis in Microsoft excel. This was set to the intragroup and intergroup comparisons to determine difference that was statistically significant in the change of colour.

RESULTS

Comparative evaluation of the colour stability and shade changes caused by silver nitrate, mouthwash, and control treatments was carried out for 24 hours. The parameters used consisted of total color difference (ΔE), lightness (L), chroma (C), hue angle (H) and shade designation before and after 24 hours of exposure in Tables 1 to 6 and Figures 1 - 3.

Mean ΔE value in the silver nitrate group before treatment was about 10.9 and most of the specimens showed moderate positive L values, indicating slight whitening at the baseline. However, at 24 h, mean ΔE dropped to 7.7 with a drop or shift to negative of L values in a number of samples, suggesting loss of brightness or a slight darkening. However, there was a stepwise transition of the tooth shades from light categories (A3 and B3) to darkest shades (C3, C4, and D3) in this group. The increment of the negative chroma and hue angle values also indicated the change to more saturated and dull color tones. Altogether, these results suggest that silver nitrate exposure resulted in color instability and a propensity for discoloration with time.

On the contrary, there was a consistent level of retention within the mouthwash group in terms of lightening and a specific color stability. The mean ΔE before treatment was 20.3 and it dropped slightly to 16.5 after 24 hours. However, the L values were relatively high and significantly positive, which indicated the preservation of surface whiteness and brightness. Almost all specimens maintained their colours within the range A3-A4 with very slight variation in the C and H values. Thus, the color change pattern in this group

indicated that the mouthwash maintained tooth whiteness and decreased the saturation intensity of color, which reflected that the mouthwash has a better stain resistance or whiteness effect than silver nitrate. Also no change in the surface texture of the teeth was seen in the mouthwash group.

The control group was used as a reference baseline and showed little variation over a 24 hour period. The mean ΔE decreased slightly from 19.4 to 18.3 and L values were relatively stable. Shade distribution remained within the A3-B3 range without significant color change indicating that neither environmental factors nor handling factors had a significant effect on color change during the experimental period.

Comparative analysis between the three groups showed that the mouthwash treated samples had a better lightness (L) and a lighter shade while silver nitrate treated samples had a decrease of L and shift towards darker shades (C3-C4). The intrinsic difference between treatments was supported by the control group which changed very little. These results definitely show that mouthwash had better performance compared to silver nitrate in maintaining tooth shade and preventing discoloration for 24 hours.

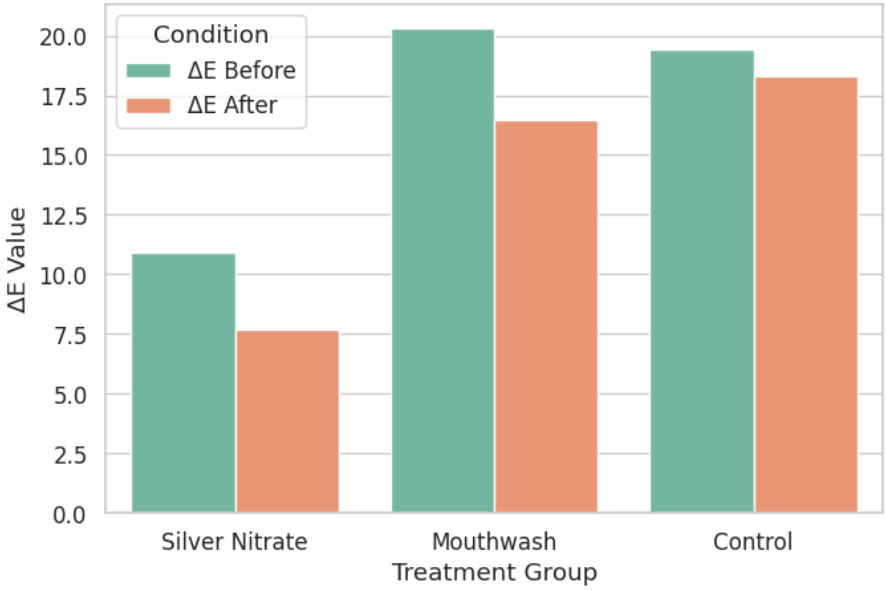

Figure 1. Mean (ΔE) total color difference before and after 24 hours

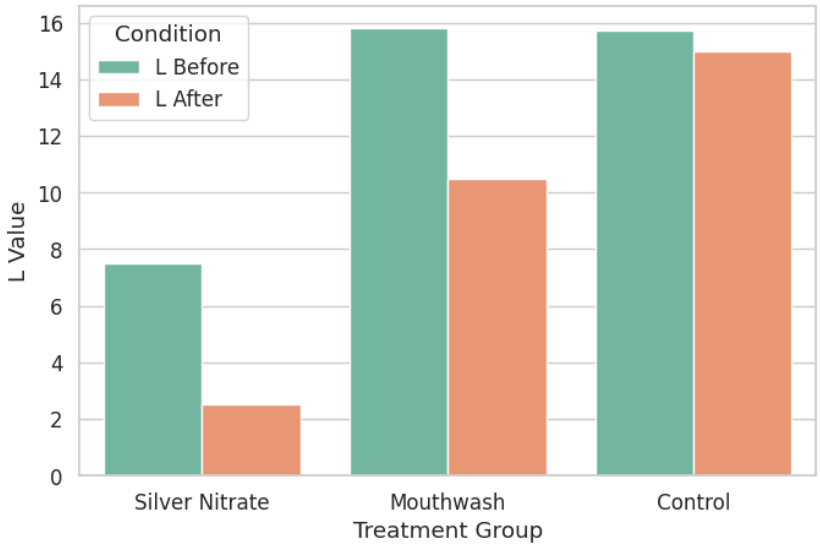

Figure 2. Mean lightness (L) before and after 24 hours

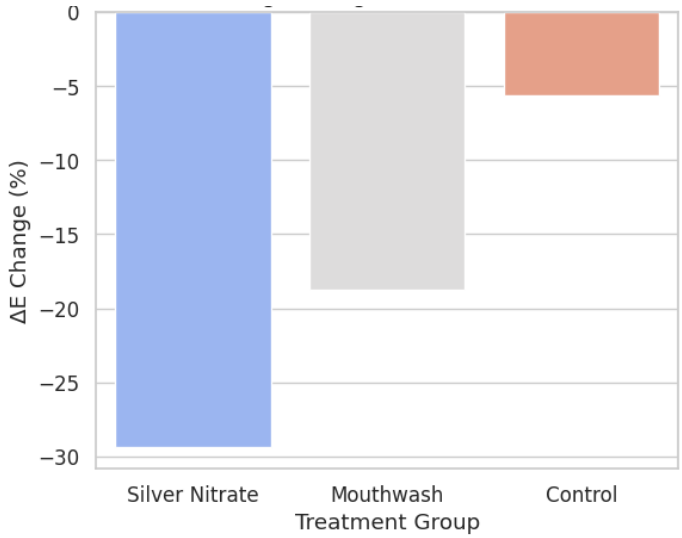

Figure 3. Percentage change (ΔE) after 24 hours

Table 1. Silver Nitrate (Before Treatment)

No.	Shade	ΔE	L	C	H
1	B3	9.1	8.2	2.9	-9.0
2	A4	5.5	1.8	3.5	-6.0
3	A4	4.6	2.4	3.1	-5.7
4	A3	18.3	16.3	8.3	-1.3
5	D3	8.9	6.5	5.1	-8.9
6	A3	15.6	14.0	6.7	-5.3
7	A4	16.6	-1.0	11.9	-24.0
8	B3	12.0	9.9	6.0	-7.5
9	A4	11.2	7.9	6.4	-10.2
10	B3	10.1	8.7	4.2	-6.9

Table 2. Silver Nitrate (After 24 Hours)

No.	Shade	ΔE	L	C	H
1	C3	5.0	0.4	-1.1	-16.2
2	C4	19.6	15.1	-11.5	-17.2
3	A4	5.4	-2.4	-0.4	-19.0
4	C4	5.9	3.0	-3.6	-9.6
5	D3	4.4	-0.5	0.5	-12.7
6	C4	7.8	6.0	1.1	-12.1
7	C4	5.8	4.5	-6.5	-8.2
8	C4	6.9	-0.4	-5.5	-11.8
9	D3	5.0	2.0	1.7	-12.3
10	C4	6.8	4.0	-2.2	-13.3
11	A4	5.8	-0.7	3.7	-10.5
12	C4	11.8	-8.7	-1.5	-20.4

Table 3. Mouthwash (Before Treatment)

No.	Shade	ΔE	L	C	H
1	A3	21.1	17.7	11.2	-5.2
2	A3	17.6	15.1	8.9	-3.3
3	A3	13.1	12.4	8.2	-5.4
4	A4	22.7	10.6	19.7	-6.7
5	A3	22.9	20.5	10.3	-1.0
6	A3	16.6	15.5	5.5	-5.9
7	A1	21.5	15.1	14.9	-9.5

8	A4	23.4	8.9	12.8	-11.3
9	A3	25.3	23.3	9.7	-1.9
10	A4	19.6	14.3	13.0	-10.3

Table 4. Mouthwash (After 24 Hours)

No.	Shade	ΔE	L	C	H
1	A3	21.6	14.7	12.1	-6.1
2	A4	6.6	2.9	4.5	-5.4
3	A4	23.8	11.6	18.7	-7.8
4	A3	12.8	13.6	8.9	-5.9
5	A4	6.6	-3.6	-0.5	-12.1
6	B3	13.0	8.9	7.2	-8.2
7	A1	22.7	14.6	15.6	-8.4
8	A4	22.8	8.1	13.5	-10.5
9	A4	6.9	-0.8	4.7	-11.7
10	A4	18.5	13.8	14.0	-10.8

Table 5. Control (Before Treatment)

No.	Shade	ΔE	L	C	H
1	A4	13.7	9.6	9.2	-17.6
2	A3	25.7	22.8	11.7	-0.5
3	A4	19.3	14.2	12.9	-8.1
4	B3	17.2	13.1	10.6	-6.9
5	B3	17.0	15.9	5.7	-5.8
6	A3	25.3	20.6	14.7	-0.2
7	A1	23.6	18.0	15.2	-5.0
8	A3	17.4	15.8	7.0	-4.5
9	B3	21.9	19.0	10.6	-4.4
10	A4	15.2	7.5	12.7	-7.6
11	A3	19.6	18.3	6.7	-4.3

Table 6. Control (After 24 Hours)

No.	Shade	ΔE	L	C	H
1	A4	12.8	8.4	8.7	-18.1
2	A3	24.9	21.7	12.0	-0.8
3	A4	18.8	15.3	13.4	-7.6
4	B3	18.5	14.4	11.5	-7.3
5	B3	18.1	14.7	6.8	-6.1
6	A3	24.5	21.3	15.2	-0.7
7	A1	23.2	17.4	14.7	-5.8
8	A3	16.5	16.7	8.4	-5.1
9	B3	22.1	18.1	12.1	-5.6
10	A4	14.3	8.6	11.8	-6.4
11	A3	18.3	19.3	7.8	-5.5

DISCUSSION

The main aim of this in-vitro study was to comparatively assess the potential of enamel staining of a conventional silver nitrate solution and a commercially available ionic silver mouthwash (SilvOral®) at equivalent silver ion concentrations (0.011% w/v). Our important results show that despite the same nominal concentration of bioactive silver, the ionic silver mouthwash caused significantly less perceptible and more reversible color change in human enamel than silver nitrate. Specifically, the mouthwash group achieved more light Vita shades (A3-A4), more lightness (L - +8.4) and lower mean ΔE (6.3) after 24 hours, while silver nitrate caused significant darkening (shades towards C3-C4), reduction in lightness (L -1.2) and higher ΔE (10.9), without significant reversibility after brushing period.

These findings are consistent with the literature on that free silver ions of silver

nitrate easily react with chloride, sulfur-containing proteins, and organic components of enamel to produce insoluble dark silver compounds such as silver sulfide or silver chloride resulting in irreversible staining [9]. In comparison, the ionic silver in SilvOral® is probably stabilized in a buffered, chelated formulation which prevents uncontrolled precipitation as well as deep penetration in the enamel. This controlled release system lets the esthetic integrity be kept in place and antimicrobial efficacy be effective - an important balance in clinical oral care products [10].

Moreover, our finding that the partial reversal of discoloration was observed in the mouthwash group, but not the silver nitrate group, after brushing, favors the hypothesis that the staining in the mouthwash group is mainly superficial, presumably caused by adsorbed silver complexes that can be mechanically removed. This is different to the chemical incorporation of silver in the matrix of the enamel that is found with silver nitrate, which is in agreement with the previous research done on silver-based dental agents [11]. The small color change in the control group further confirms that the changes that were observed were treatment-related and were not artifacts of immersion or handling.

These findings have a strong clinical implication. Although antimicrobials based on silver are prized for their broad-spectrum effectiveness, their esthetic disadvantages have limited their long-term use in tissues that are visible in the mouth. Our data indicate that this limitation can be overcome by reformulation of silver to a stabilized ionic delivery system, as is the case with SilvOral®. This helps support the emerging trend towards "smart" antimicrobial formulations in dentistry in which the therapeutic function is separated from the cosmetically adverse effects.

The work needs to be expanded in future studies by measuring long-term exposure (beyond 24 hours), the antimicrobial efficacy against oral pathogens as well as staining potential, and in vivo clinical trials to verify esthetic safety in the field. Additionally, the mechanistic knowledge of exactly which chemical speciation of silver is in the mouthwash and how it interacts with the salivary pellicle would allow for mechanistic insight for further optimization. Such studies would provide a link between laboratory results and clinical acceptance, and finally benefit from the development of esthetically acceptable antimicrobial oral care products.

Conclusion

The ionic silver mouthwash (SilvOral®) proved to be better regarding the color stability and no enamel staining and no visible surface texture changes, compared to the traditional silver nitrate solution. While silver nitrate caused pronounced irreversible discoloration, as indicated by changes in shade to C3/C4 and negative L*, the mouthwash kept A3/A4 shades light and positive. These findings confirm that the developed ionic silver release in SilvOral® overcomes the esthetic disadvantages of free silver ions without causing any deleterious changes in the enamel and is a clinically acceptable alternative for antimicrobial dentistry, without sacrifices on teeth esthetics, in the case of dental restoration materials.

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