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ORIGINAL ARTICLE

Assessment of colour modifications in two different composite resins induced by the influence of chlorhexidine mouthwashes and gels, with and without anti-staining properties: An in vitro study

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Abstract

Objectives: Chlorhexidine (CHX)-based products are the most effective chemical agents used in plaque control and oral disinfection. One of their side effects is tooth and restoration staining. For this reason, CHX products with anti-discolouration systems (ADS) have been developed. The aim of this in vitro study was to compare different CHX-based products (gel and mouthwash) with or without ADS in composite colour modification.

Methods: Two hundred specimens were created, 100 of which were made of packable composite and 100 of flowable composite. After 24 h, colour coordinates (L^*, a^*, b^*, C^*, $h°$) were recorded using a spectrophotometer (T0). Then, all samples were subjected to a CHX/tea staining model and immersed in human saliva for 2 min. Composite specimens were divided in 10 groups ($N = 20$). Control groups (PC, FC) were soaked in distilled water and test groups (PG, PGads, FG, FGads, PM, PMads, FM and FMads) were immersed in CHX-based solutions or brushed with CHX gel. Then the cycle was repeated 6 times, and colour differences (ΔE_{ab} and ΔE_{00}) were finally calculated.

Results: Through flowable composites, FC and FG showed the highest colour differences, respectively $\Delta E_{ab} = 3.48 \pm 1.0$, $\Delta E_{00} = 2.24 \pm 0.6$ and $\Delta E_{ab} = 2.95 \pm 1.3$, $\Delta E_{00} = 1.53 \pm 0.6$. In the composite groups instead, PM and PMads showed the highest colour differences, respectively $\Delta E_{ab} = 2.78 \pm 1.3$, $\Delta E_{00} = 1.94 \pm 0.8$ and $\Delta E_{ab} = 2.71 \pm 1.4$, $\Delta E_{00} = 1.84 \pm 0.9$.

Conclusions: CHX-containing products are able to cause stains on restorative composite materials. Discolouration is more likely to occur in flowable composites than packable composites, and ADS-containing products cause fewer pigmentations than CHX products without ADS. Packable composites showed more staining after mouthwash treatment, whereas flowable composites underwent higher discolouration after treatment with gels.

KEYWORDS
chlorhexidine, colour, composites, flowable, in vitro study, stain

1 | INTRODUCTION

Chlorhexidine gluconate (CHX) is the most effective chemical agent for plaque control.[1,2] It is classified as a bis-biguanide antiseptic, and is active against Gram-positive and Gram-negative bacteria, including aerobes and anaerobes, and leads to the rupture of the bacterial cell wall.[3]

Following periodontal therapy, rinses or mouthwashes are often suggested as post-therapy care.[1,4] CHX at a concentration of 0.12% is frequently used due to its antibacterial, anti-inflammatory and anti-biofilm characteristics. It has been shown that rinsing with 0.12% CHX after non-surgical periodontal therapy reduces probing depth to a greater extent compared with non-surgical periodontal therapy alone.[5]

The most common reported side effects of long-term CHX use include taste alteration, irritation of the oral mucosa and both tooth and restoration (composites and cements) discolouration.[3,6,7] Side effects are reversible upon discontinuation of use but remain a major limitation on patient compliance.[8]

Based on in vitro and in vivo studies, a literature review concluded that the major etiological mechanism of extrinsic dental staining associated with cationic antiseptics was the precipitation of dietary chromogens on dental and oral surfaces.[9]

Three mechanisms are potentially associated with the CHX staining side effect: (1) the Maillard reaction; (2) the formation of pigmented metal sulphides and (3) reactions between tannin and polyphenols from drinks, food and CHX itself. The Maillard reaction occurs in the biofilm between proteins and sugars, producing glycosylamine that is unstable and is rearranged into ketosamines. This reaction is catalysed both by CHX and a series of polymerisation reactions, resulting in the brown-coloured pigments also known as 'melanoidins'.[10]

Extrinsic staining factors include coloration by absorption of dyes from exogenous sources, such as smoking, coffee, tea, red wine assumption. Also, mouthwashes have been reported to stain composite resin restorations in varying degrees.[11] Because of this, daily rinsing with chlorhexidine is not promoted.[2]

In order to counter tooth staining, CHX with an anti-discolouration system (ADS) was developed. The ADS system is made of sodium meta-bisulphite and ascorbic acid, two molecules that seem to be capable of interfering with the main processes that lead to the formation of pigmentation.[12]

Based on a recent literature review, there is moderate evidence from non-brushing studies that the addition of an ADS to CHX-mouthwash does not appear to affect its properties with respect to gingival inflammation and plaque scores and reduces tooth surface discolouration. Also, in brushing studies, there is moderate quality evidence that ADS does not affect the anti-plaque and anti-gingivitis efficacy of CHX. Most comparisons and the meta-analysis including these suggest the absence of a significant effect of ADS on tooth staining when mouthwash is used in addition to toothbrushing.[10]

To date, there is no scientific data available regarding whether a CHX-based liquid product such as mouthwash or a gel product is more effective in staining composite restorations, or which type of composite resin is more sensitive to the phenomenon of CHX-induced pigmentation.

Therefore, the aim of this in vitro study was to evaluate the staining ability of CHX-based mouthwashes and gels on two different composite resins, packable and flowable, compared to the staining caused by the same products with the adjunct of an ADS.

In particular, the null hypotheses tested were that: (1) there is no difference in discolouration between packable and flowable composites after contact with CHX-based products; (2) there is no difference in discolouration on composite resins between CHX-containing gels and mouthwashes; and (3) there is no difference in discolouration on composite resins between CHX-containing products and CHX + ADS-containing products.

2 | MATERIALS AND METHODS

Two hundred resin composite blocks (8 mm × 6 mm × 5 mm) were created using a parallelepiped silicon mould in order to obtain identical samples. Half of them were made of a packable composite (Premise™, Kerr Italia Srl) and the other 100 samples were made of a flowable composite (Premise™ Flowable, Kerr Italia Srl).

All specimens were realised through three different increments of composite, each of which was light-cured with a blue-led medium intensity lamp for 20 s (1400 mW/cm^2) (Mectron Starlight Pro, Mectron SpA). Before the last curing phase, a Mylar sheet was placed on top of the composite in order to obtain a perfectly smooth surface. Samples were removed from the mould and then polished using a composite diamond-coated polishing kit (TWIST™ DIA for Composite, Kuraray Noritake Dental Inc.).[13] After that, specimens were stored in distilled water at 37° for 24 h.[14] Samples were then gently air-dried[15] and colour coordinates (L^*, a^*, b^*, C^*, $h°$) were recorded at T_0 using an intraoral spectrophotometer (VITA Easyshade V, VITA Zahnfabrik, Germany) by placing the tip perpendicular to the sample surfaces with a grey background and natural daylight.[16]

After initial colour measurements, all blocks were subjected to a previously published staining chlorhexidine/tea model,[8] and then immersed in stimulated human saliva (collected from the same experimenter, without food ingestion at least 2 h before saliva collection) for 2 min.

Subsequently, composites blocks were randomly divided into 10 groups ($N = 20$).[17] Test groups were immersed in CHX-based solutions (Curasept® SPA, Saronno, Italy), or brushed with CHX-based gels (Curasept® SPA), whereas control groups were soaked in distilled water:

1. PG (composite: 2′ CHX gel 0.50%),
2. PG-ADS (composite: 2′ CHX gel 0.50% with ADS),
3. FG (flow: 2′ CHX gel 0.50%),
4. FG-ADS (flow: 2′ CHX gel 0.50%),
5. PM (composite: 2′ CHX mouthwash 0.20%),
6. PM-ADS (composite: 2′ CHX mouthwash 0.20% with ADS),

TABLE 1 Description of the tested composite resins.

Composite	Particles	Resin type	Resin matrix	Filler	Producer
Premise™	Nanohybrid	Methacrylate	Bis-GMA, bisphenol-A, ethoxylate dimethacrylate and TEGDMA	Weight 84%, volume 69% Non-agglomerate silice nanoparticles (0.02 μm), pre-polymerised filler (30–50 μm), and barium silicate glass (0.4 μm)	Kerr Italia Srl
Premise™ Flowable	Nanohybrid flowable	Methacrylate	Bis-GMA, bisphenol-A, ethoxylate dimethacrylate and TEGDMA	Weight 72.5%, volume 56% Non-agglomerate silice nanoparticles (0.02 μm), pre-polymerised filler (30–50 μm), and barium silicate glass (0.4 μm)	Kerr Italia Srl

TABLE 2 Description of the CHX-based products tested.

Product	Composition	Producer
Mouthwash CHX 0.20% with ADS	Aqua, Xilitol, Propylene glycol, PEG-40 Hydrogenated Castor Oil, VP/VA Copolymer, Sodium citrate, Poloxamer 407, Ascorbic acid, Sodium metabisolfite, Sodium DNA, Aroma, Chlorhexidine digluconate, Sodium benzoate, Citric acid, C.I. 42,090	Curasept S.p.A.
Mouthwash CHX 0.20% without ADS	Aqua, Xilitol, Propylene glycol, PEG-40 Hydrogenated Castor Oil, VP/VA Copolymer, Sodium citrate, Poloxamer 407, Aroma, Chlorhexidine digluconate, Sodium benzoate, Citric acid, C.I. 42,090	Curasept S.p.A.
Gel CHX 0.50% with ADS	Aqua, Propylene glycol, Hydroxypropylcellulose, VP/VA Copolymer, PEG- 40 Hydrogenated Castor Oil, Chlorhexidine digluconate, Sodium DNA, Sodium acetate, Sodium citrate, Sodium metabisulfite, Ascorbic acid, Aroma, Acetic acid	Curasept S.p.A.
Gel CHX 0.50% without ADS	Aqua, Propylene glycol, Hydroxypropylcellulose, VP/VA Copolymer, PEG- 40 Hydrogenated Castor Oil, Chlorhexidine digluconate, Sodium acetate, Aroma, Acetic acid	Curasept S.p.A.

7. FM (flow: 2′ CHX mouthwash 0.20%),
8. FM-ADS (flow: 2′ CHX mouthwash 0.20%) with ADS),
9. PC (composite: 2′ dH_2O),
10. FC (flow: 2′ dH_2O).

Materials used in the study are described in Tables 1 and 2.

After this, every specimen was immersed in a black tea solution (prepared with 5 Lipton tea bags in 1 L of hot water for 5 min) for 1 h.[3] Specimens were then rinsed with distilled water and the cycle saliva/CHX/tea was repeated for six times.[8]

At the end of the last cycle, specimens were rinsed with distilled water, gently air-dried and colour coordinates measured as described previously through the spectrophotometer (T_1).

Colour differences (ΔE) were calculated using CIELAB traditional formula (ΔE_{ab})[17] and CIEDE2000 modified formula (ΔE_{00})[13] as described below:

$$\Delta E^*_{ab} = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

$$\Delta E^*_{00} = \sqrt{\left(\frac{\Delta L'}{K_L S_L}\right)^2 + \left(\frac{\Delta C'}{K_C S_C}\right)^2 + \left(\frac{\Delta H'}{K_H S_H}\right)^2 + R_T\left(\frac{\Delta C'}{K_C S_C}\right)\left(\frac{\Delta H'}{K_H S_H}\right)}$$

Manuscript was prepared following CRIS Guidelines (Checklist for Reporting In-vitro Studies).[18]

2.1 | Statistical analysis

The statistician was blinded to the groups when performing the analysis. Statistical analysis was performed using STATA program version 17 (StataCorp LP). Means, standard deviations, counts and percentages were used to summarise the data. Data from colour coordinates (CIE L^*, a^*, b^*, C^* and $h°$) were statistically analysed using one-way analysis of variance (one-way ANOVA) and Tukey's multiple comparison test with Bonferroni correction. A one-way ANOVA was performed to compare the effects of colour differences ΔE_{00} and ΔE_{ab} value among the materials. Paired *t*-tests were used to compare continuous measures between groups. A *p*-value of ≤0.05 was considered statistically significant.

3 | RESULTS

Colour differences at T_0 and T_1 were calculated using ΔE_{ab} and ΔE_{00} different formulas and are presented in Table 3.

PM samples showed the highest colour change ($\Delta E_{ab} = 2.78 \pm 1.3$, $\Delta E_{00} = 1.94 \pm 0.8$), followed by PM-ADS specimens ($\Delta E_{ab} = 2.71 \pm 1.4$, $\Delta E_{00} = 1.84 \pm 0.9$). Packable composite brushed with gel showed the least colour modification, especially PG ($\Delta E_{ab} = 1.84 \pm 0.7$, $\Delta E_{00} = 1.34 \pm 0.5$) and PG-ADS blocks ($\Delta E_{ab} = 1.21 \pm 0.5$, $\Delta E_{00} = 0.83 \pm 0.4$).

TABLE 3 ΔE_{ab} and ΔE_{00} mean values and standard deviations.

	ΔE_{ab}	ΔE_{00}
Premise		
PG	1.84 ± 0.7	1.34 ± 0.5
PG ads	1.21 ± 0.5	0.83 ± 0.4
PM	2.78 ± 1.3°	1.94 ± 0.8°
PM ads	2.71 ± 1.4°	1.84 ± 0.9°
PC	1.94 ± 0.7	1.28 ± 0.3§
Premise flowable		
FG	2.95 ± 1.3*°	1.53 ± 0.6°
FG ads	2.79 ± 1.3°	1.47 ± 0.7°
FM	2.63 ± 1.1°	1.46 ± 0.5
FM ads	2.60 ± 1.1°	1.37 ± 0.5°
FC	3.48 ± 1.0*°^	2.24 ± 0.6*°^&$£〃

Note: ΔE_{ab}: Statistically significant differences ($p < 0.05$) were observed between *PG versus others, °PG ads versus others and ^PC versus others. ΔE_{00}: Statistically significant differences ($p < 0.05$) were observed between *PG versus others, °PG ads versus others, ^PC versus others, §PM versus others, $FG versus others, &FG ads versus others, 〃FM versus others and 〃FM ads versus others.

Flowable-made specimens instead showed the highest colour change when treated with CHX-based gels, especially FG ($\Delta E_{ab} = 2.95 \pm 1.3$, $\Delta E_{00} = 1.53 \pm 0.6$) and FG-ADS ($\Delta E_{ab} = 2.79 \pm 1.3$, $\Delta E_{00} = 1.47 \pm 0.7$). Minor colour variations instead were reported with flowable blocks soaked in mouthwashes (FM: $\Delta E_{ab} = 2.60 \pm 1.1$ and $\Delta E_{00} = 1.36 \pm 0.5$, FM-ADS: $\Delta E_{ab} = 2.63 \pm 1.1$ and $\Delta E_{00} = 1.37 \pm 0.5$).

Concerning the CIELAB traditional formula (ΔE_{ab}), PM-ADS and FG-ADS samples showed a higher colour variation than, respectively, PG-ADS and PG. These differences were statistically significant ($p = 0.607$ and $p < 0.001$).

CIEDE2000 modified formula (ΔE_{00}) analysis instead reported statistically significant differences between PM-ADS and PG-ADS ($p < 0.001$), and between FG-ADS and PG-ADS ($p = 0.001$).

For both CIELAB and CIEDE2000 formulas, the packable composite control group showed similar values compared to samples treated with gels. Whereas the flowable composite control group behaved surprisingly differently, exhibiting dramatic colour changes far superior to all other samples analysed in the study.

4 | DISCUSSION

This study evaluated the effects of four CHX-based products on the colour stability of two composite resins, one packable and one flowable composite. The tested products were CHX-based mouthwashes and gels, with and without the ADS system, and a control solution made of stimulated human saliva. Overall, the results show that both test and control solution produced variable colour changes on the surfaces of all specimens.

The ability of CHX-based products to create pigmentation on the surfaces of composite restorations was also recently demonstrated by Ebrahimzade et al.[19] who immersed composite blocks in 0.2% CHX twice a day, for 1 min, for 2 weeks. At the end of this period, through a spectrophotometer analysis, the samples showed an increase in *a*, *b* and *L* values.

Also, Hasani et al.[20] in 2019 soaked different composite specimens in a 0.2% CHX solution, 1 min/day. After 1 months, all the samples presented significant colour changes, although this could be due also to the long-time treatment, not acceptable form a clinical point of view.

Flowable composite showed a greater colour change than packable composite. This is likely due to the fact that resin composites containing less filler particles are more prone to colour variations, as they might have higher water absorption which allows penetration of pigmenting agents, resulting in discolouration. This aspect was highlighted in a very recent systematic review, which aimed to investigate whether mouthwashes could affect the colour of direct composite resin restorations and concluded that mouthwashes are responsible of a modification in colour.[11]

Moreover, regarding the colour variation produced by CHX mouthwash compared to that produced by CHX gel, the results were found to be conflicting. Packable composite samples showed greater colour change when treated with mouthwash, while flowable composite samples showed greater colour change when treated with gel. In this regard, a systematic review conducted in 2015, in which the efficacy of a CHX gel compared to a CHX mouthwash was evaluated on plaque, bleeding, gingival inflammation and tooth colour change scores, highlighted that the CHX mouthwash produces a greater colour change than the gel.[21] Since flowable composites are usually used as liners for cavities and are rarely in contact with the oral environment, the application of CHX gel instead of mouthwash would seem preferable to minimise the pigmentation of composite restorations.

Similarly to what happens on teeth and oral mucosa, products containing the ADS system (G ADS, M ADS) are able to produce less colour variation on composite restorations when compared to the respective products not containing the ADS system (G, M).

This aspect was also confirmed by a recent systematic review in which most of the studies analysed showed that the ADS system determined a reduction of chlorhexidine-induced pigmentation.[10] This systematic review aimed to investigate whether the addition of an anti-pigmentation system (ADS) to chlorhexidine-based mouthwashes was effective in preventing tooth surface pigmentation, as well as to evaluate whether chlorhexidine combined with ADS maintained its effectiveness in reducing plaque and gingivitis.

Also a recently published paper aimed to assess colour changes in teeth and composite resins under the influence of CHX, with and without ADS. A total of 40 nanoceramic and nanohybrid composite specimens of size 10 mm diameter and 0.5 mm thickness were prepared, cured for 20 s and polished with a composite polishing kit. Two mouthrinses comprising CHX and CHX with ADS were used. Baseline colour values of composite resins were recorded using an ultraviolet spectrophotometer. After baseline spectrophotometric measurements, all the samples were subjected to the mouthrinses, and the post-immersion colour values of the samples were then recorded using the same spectrophotometer.

Reflectance values showed a statistically significant difference between CHX and CHX with ADS among nanoceramic and nanohybrid composite samples.[22]

In daily clinical practice, there are several factors that can influence the colour stability of restorative-prosthetic materials. The present research, since it was performed in vitro, only partially simulates the conditions found intraorally, and for this reason, further in vivo clinical studies will be necessary to be able to obtain a more clinically-oriented correlation with the clinical aspect.

In particular, contrary to what might have been expected, the control samples, subjected to a saliva/distilled water/tea washing cycle, showed greater colour changes than some test groups subjected to the washing cycle in saliva/chlorhexidine/tea.

These results contrast with those of other studies, which instead found a lower colour change of the control samples compared to the test samples.[9,23]

The study by Addy et al. presented a single-blind randomised design with the aim of determining the in vivo staining potential of a 0.02% chlorhexidine formulation and Listerine phenolic mouthwash compared to a negative control. Fifteen subjects underwent scaling and polishing to make their teeth free of plaque, calculus and pigmentation, and tongue brushing was performed. Oral hygiene was suspended, and 8 times a day, the subjects rinsed first with the assigned formulation and then with 10 mL of black tea. On the fourth day, tongue and teeth pigmentation was assessed. The results showed that the rinse with the 0.02% chlorhexidine formulation produced significantly more pigmentation on the teeth and tongue than that induced by Listerine or the negative control.[9]

Ten years later, the same study group published an in vitro study that aimed to determine whether two 0.2% and 0.12% chlorhexidine-based mouthwashes containing ADS were capable or not of binding the chromogens in the diet. These mouthwashes were compared to a negative control rinse (water). Six acrylic samples were assigned to each group and immersed in saliva for 2 min, removed, washed in water and placed in the respective solution for another 2 min, then removed and washed again in water, and finally immersed for 60 min in tea. After removal from the tea solution, the samples were rinsed in water and allowed to dry and subsequently their optical density was detected using a spectrophotometer. The cycle was repeated until any tested solution produced an average optical density greater than 2.0. All chlorhexidine-based mouthwashes exceeded the optical density of 2.0 at 11 cycles, and significantly less pigmentation was observed for the negative water control.[23]

One of the major differences between the present study and the cited ones concerns the number of samples. It is likely that, having tested 200 composite resin samples, the results obtained from the present research could be considered more plausible than those obtained by analysing a much smaller number of specimens.

Differently from the above cited studies findings, Carpenter et al.[24] found greater colour change in saliva-pretreated specimens when exposed to tea alone than when exposed to chlorhexidine and tea together. This in vitro study aimed to analyse the role of saliva in the pigmentation mechanism induced by chlorhexidine on hydroxyapatite samples. Using different combinations of tea, chlorhexidine and saliva, the substances that bound to hydroxyapatite were analysed by electrophoresis. The results showed that the salivary-acquired biofilm reduced the binding of chlorhexidine and tea when used in combination but conversely, increased the binding of tea alone or chlorhexidine alone to hydroxyapatite.[24]

Therefore, to date, there is no uniform consensus in the scientific literature regarding this phenomenon.

Based on the findings of the present manuscript, the three initial null hypotheses were rejected.

5 | CONCLUSION

This in vitro study showed how CHX-containing products are able to cause stains on restorative composite materials. In detail, discolouration is more likely to occur in flowable composites than packable composites. ADS-containing products cause fewer pigmentations than CHX products without ADS. It was not clear which material undergoes more likely discolouration processes because packable composites showed more staining after mouthwash treatment, whereas flowable composites underwent higher discolouration after treatment with gels.

6 | CLINICAL RELEVANCE

6.1 | Scientific rationale for study

Chlorhexidine-based products are known to cause teeth pigmentation, and an anti-discolouration system has been introduced in order to minimises this side effect. While this phenomenon has been extensively studied on tooth surfaces, there aren't yet studies evaluating the ability of chlorhexidine-based products to pigment composite resins used for restorative dentistry. The aim of this in vitro study is to compare chlorhexidine-based gel and mouthwashes, with or without anti-discolouration system, in composite colour modification.

6.2 | Principal findings

Anti-discolouration system—containing products cause fewer pigmentations than chlorhexidine alone products, and discolouration is more likely to occur in flowable composites than packable composites. In case of mouthwash treatments, packable composites tend to show more staining, whereas flowable composites undergo higher discolouration after gel application.

6.3 | Practical implications

To minimise the pigmentation of composite restorations, it is advisable to suggest chlorhexidine-based products containing the

anti-discolouration system. Furthermore, since flowable composites are usually used as liners for cavities and are rarely in contact with the oral environment, the application of gel instead of mouthwash would seem preferable to minimise the pigmentation of composite reconstructions.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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