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Color stability of frequently used aesthetic restorative materials following in vitro exposure to chlorhexidine- and octenidine-based mouthrinses

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Abstract

Background Long-term color match is one of the most important characteristics of aesthetic restorative materials as discoloration constitutes a primary reason for otherwise unnecessary replacements. The aim of the present in vitro study was to evaluate the color stability of frequent dental materials (ceramic, composite, orthodontic adhesive) induced by common antiseptic mouthrinses taking into account black tea consumption and mechanical cleaning.

Methods Twenty-four disc-shaped specimens (8 × 2 mm) were made of the materials Ceram.x Spectra™ ST HV, Ceram.x Spectra™ flow, Ceramill® Zolid HT+ PS and Unitek™ Transbond™ LR. Each of the following solutions was tested on six pieces per material: Chlorhexamed forte (CHX), octenident® (OCTD), octenimed® (OCTM) and artificial saliva (control). Dental samples underwent a total of 30 discoloration cycles in which they were alternatively placed into artificial saliva, black tea and respective mouthrinse or only in artificial saliva. After every 10 cycles, discs were mechanically cleaned with toothbrush and toothpaste. After 30 cycles, dental specimens were submitted to professional polishing. Color shifts were measured at different time-points using the VITA Easyshade® V spectrophotometer and displayed as total color difference ΔE (mean ± standard error). A post-hoc Tukey test ($\alpha = 0.05$) was applied to the mean ΔE values after 30 cycles to determine discoloration discrepancies between various mouthrinses as well as the control. Moreover, photos of individual discs were taken at all measurement times to visualize potential color changes by eye.

Results All mouthrinses showed major color shifts in the clinically visible range compared to the control on all different dental materials tested. However, CHX caused significantly more discoloration than OCTM and OCTD. Established color changes could be almost completely removed by simple brushing and even further by professional polishing to clinically acceptable levels on all tested materials.

Conclusions Prolonged application of antiseptic mouthrinses may cause discoloration on different restorative materials. To maintain aesthetically satisfying conditions, patients should be educated about the importance of daily mechanical tooth brushing and regular professional polishing.

Keywords Discoloration, Dental materials, Octenidine, Chlorhexidine, Mouthrinse, Ceramic, Composite, Adhesive

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Background

In parallel to worldwide rising standards in society, the demand for dental restorative materials has markedly increased during the last years in accordance with emerging technologies to meet aesthetic expectations in the oral cavity. Nowadays, novel tooth-colored dental restorations can offer excellent aesthetic results and are technically designed for long-term retention. However, discoloration of these materials over time due to intrinsic or extrinsic factors poses a significant challenge for dentists and their patients. Intrinsic factors are directly linked to the composition and quality (e.g. ceramic, composite, orthodontic adhesive) as well as physical properties (e.g. surface roughness, long-term chemical stability) of the respective type of restorative material [1]. Otherwise, extrinsic factors favouring discoloration include e.g. dental plaque accumulation, consumption of chromogenic food and beverages (e.g. berries, curry, black tea, coffee), fluoride application and/or the frequent application of certain antiseptic mouthrinses [2–9].

Proper oral hygiene measures represent a crucial parameter for the prevention and control of common dental problems such as caries, periodontitis and gingivitis [10]. In that regard, antiseptic mouthrinses are widely used to support (daily) oral biofilm management, especially in otherwise inaccessible areas of the oral cavity or when mechanical plaque control by the use of a toothbrush is temporally or even permanently impaired (e.g. during fixed orthodontic treatments, after oral surgical interventions, in patients unable to brush their teeth due to illness or special needs) [11–13].

Chlorhexidine, a biguanide antiseptic, has established itself over the last decades as the gold standard in dentistry for the prevention and treatment of infections in the oral cavity, which has been verified by numerous clinical and laboratory studies [14]. The cationic molecule is characterized by a broad spectrum of antimicrobial activity and a long-lasting adherence to negatively charged oral surfaces, thus favouring a prolonged depot effect needed to prevent immediate washout of the active by continuous salivary flow [15]. Despite all its advantages, however, several reports have shown that the administration of chlorhexidine-containing mouthrinses is associated with frequent local adverse effects such as mucosal irritation, xerostomia, dysgeusia, promotion of calculus formation, discoloration of teeth and restorative materials as well as potentially harmful hypersensitivity reactions accompanied by severe anaphylaxis [16–18]. Beyond that, the meanwhile frequently described manifestations of reduced susceptibility to the active chlorhexidine, including even cross-resistance to antibiotics and antifungals, currently raises concerns about its use for preventive and therapeutic measures [19–23].

More recently, octenidine (OCT) was introduced as another suitable biguanide antiseptic to be utilized in oral rinsing solutions [24–27]. Although sharing a similar chemical formula, OCT differs from chlorhexidine by the lack of an amide- and ester structure in its molecule, which results in better tissue tolerance and lower toxicity due to possible metabolites [28]. Indeed, OCT-containing mouthrinses have been proven in various clinical trials to effectively inhibit plaque formation, gingivitis and oral microbial growth, being either superior or comparable to chlorhexidine. Moreover, OCT was safe and well tolerated by all study participants with only minimal drawbacks (e.g. taste disturbances, mild buccal tissue irritations) [25]. In addition, cationic OCT molecules are equally able to attach to surfaces via electrostatic interactions, allowing an antimicrobial depot effect [29, 30]. Due to its rapid and unspecific mode of action based on purely physical interaction with microbial membranes [31–34], OCT is at the same time highly effective against different (multidrug) resistant bacteria and fungi [35–39], with no reported clinically relevant resistances towards the active to date [22, 40–43].

While the capability of chlorhexidine-based mouthrinses to cause discoloration on various restorative materials has already been broadly investigated under laboratory conditions [2, 5, 7, 44–46], there is a lack of data for commercial products containing OCT. Using in vitro models, possible color changes in a clinically relevant range can be easily investigated under standardised conditions either by visual inspection or by spectrophotometry, determining the color difference ΔE according to the Commission internationale de l'éclairage (CIE) L*a*b* system [47]. Recently, Sarembe et al. described a novel in vitro cyclic treatment protocol to evaluate the staining potential of common mouthrinses on human enamel, with additional consideration of alternating immersion in black tea and toothbrushing [48]. Thus, this new testing model mimics the reality of dental hygiene by consumers more realistically and further takes into account the expected adherence of staining products, such as antiseptic mouthrinses and chromogenic beverages, to the tooth surface as well as their interaction with each other. In that regard, we are unaware of any published experimental studies on the color stability of different dental materials against chlorhexidine- and OCT-based mouthrinses considering the effect of chromogenic nutritional ingredients and tooth brushing. Thus, the aim of the present in vitro study was to investigate possible color changes expressed as ΔE on frequently used aesthetic restorative materials (ceramic, composite, orthodontic adhesive) after cyclic exposure to commercial antiseptic mouthrinses utilizing black tea as chromogen and simulating mechanical cleaning by

Table 1 Tested commercial mouthrinses

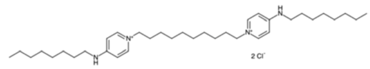
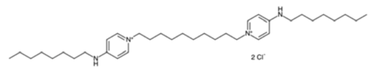
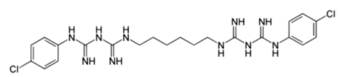
Brand name	Active	Manufacturer
octenimed®	Octenidine dihydrochloride 1 mg/ml 	Schülke & Mayr GmbH, Norderstedt, Germany
octenident®	Octenidine dihydrochloride 0.8 mg/ml 	Schülke & Mayr GmbH, Norderstedt, Germany
Chlorhexamed forte	Chlorhexidine digluconate 2 mg/ml 	GlaxoSmithKline Cosumer Healthcare GmbH & Co. KG, Munich, Germany

Table 2 Tested aesthetic restorative materials

Brand name	Composition	Manufacturer
Unitek™ Transbond™ LR light cure orthodontic adhesive for bonding of lingual retainers	Bisphenol A Diglycidyl Ether Dimethacrylate (Bis-GMA), Triethylene Glycol Dimethacrylate (TEGDMA), Silica	3 M Unitek, Neuss, Germany
Ceram.x Spectra™ ST HV high viscosity nanohybrid composite (A2 shade)	Bisphenol A Diglycidyl Ether Dimethacrylate (Bis-GMA), Ethoxylated Bisphenol A Dimethacrylate (Bis-EMA), Triethylene Glycol Dimethacrylate (TEGDMA), Silica, Zirconia	Dentsply Sirona Deutschland GmbH, Bensheim, Germany
Ceram.x Spectra™ ST flow flowable nanohybrid composite (A2 shade)	Bisphenol A Diglycidyl Ether Dimethacrylate (Bis-GMA), Triethylene Glycol Dimethacrylate (TEGDMA), Silica, Zirconia	Dentsply Sirona Deutschland GmbH, Bensheim, Germany
Ceramill® Zolid HT+PS highly translucent, zirconiumoxid ceramic (A2 shade)	Zirconium Dioxide (ZrO_2), Yttrium Oxide (Y_2O_3), Aluminum Oxide (Al_2O_3)	Amann Girrbach, Koblach, Austria

tooth brushing. On top, our laboratory approach even included in a final step comprehensive polishing of all tested dental materials to further determine the reversibility of observed discoloration simulating oral hygiene performed by professionals.

Methods

Treatment groups

As indicated in Table 1, three different commercial mouthrinses were investigated for their discoloration potential on dental materials: two octenidine (OCT)-containing products, octenimed® (OCTM) and octenident® (OCTD), as well as the chlorhexidine-based solution Chlorhexamed forte (CHX). As a control in the in vitro test procedure, the rinsing solutions were replaced by laboratory made artificial saliva composed of 1.20 g potassium chloride, 0.85 g sodium chloride, 2.50 g sodium monohydrogen phosphate, 0.15 g calcium chloride dihydrate, 0.05 g magnesium chloride hexahydrate, 5 g carboxymethylcellulose and 30 g sorbitol in 960.25 g aqua purificata. This carboxymethylcellulose-based artificial saliva composition [49] is also used in various artificial saliva products such as E-Saliva (Kunze Indopharm BV, Den Haag, Netherlands).

Dental materials

In total four restorative materials frequently used in dentistry were tested (for details see Table 2): two different nanohybrid composites (high viscosity and flowable) in A2 shade from the Ceram.x Spectra™ ST family, one zirconia ceramic (Ceramill® Zolid HT+PS in A2 shade) and one light cure adhesive for orthodontic bonding of lingual retainers, brackets and occlusions (Unitek™ Transpond™ LR).

Preparation of test specimens

To produce samples from each composite as well as the orthodontic adhesive, a 2 mm thick disc made of polytetrafluoroethylene (PTFE, Technoplast Kunststoffe, Lahnstein, Germany) with a hole with a diameter of 8 mm was placed on a microscope glass slide [50]. The materials Ceram.x Spectra™ ST flow and Unitek™ Transpond™ LR were introduced into the mold, a glass slide was put on top and the excess was extruded by manual finger

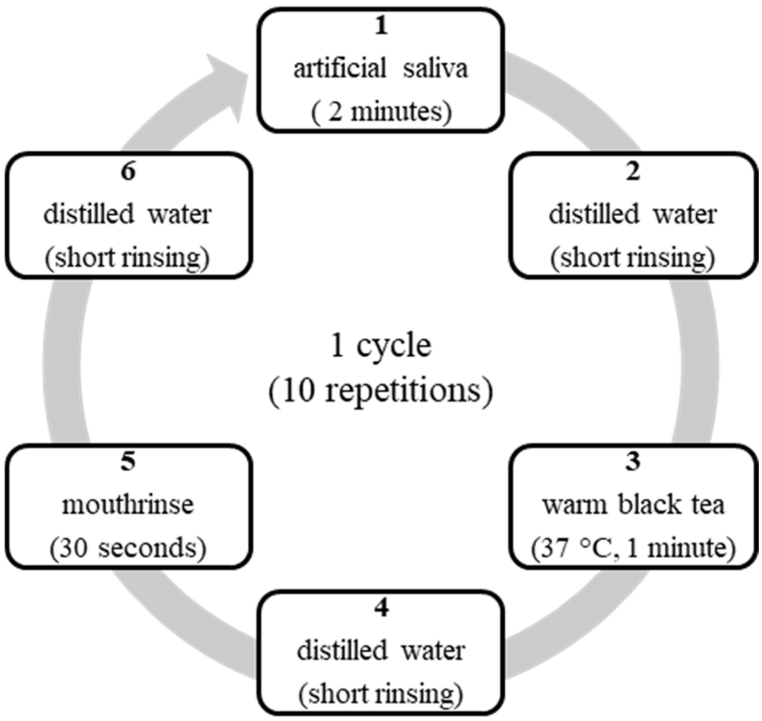


Fig. 1 Six-step cyclic treatment of dental samples including the immersion in respective mouthrinses in vitro

pressure. Otherwise, after placed into the mold, the high viscosity composite Ceram.x Spectra™ ST HV was compacted with a dental ball tamper. To achieve a smooth and homogenous surface, the materials were further compressed with an additional glass slide. Afterwards, the composite and adhesive specimens were polymerized according manufacturers´ instruction on both sides for 20 and 10 s, respectively, with a light density of 1.47 mW/cm^2 using an UV lamp (Elipar™ Deep-Cure S, 3 M, Neuss, Germany). Cured excess material was removed from the edges using a medium rotating diamond roller (Intensiv, Montagnola, Switzerland) under water cooling. The disc-shaped specimens made of composite were finished with a flat composite polisher (Kerr, Bioggio, Switzerland) under water cooling, whereas the adhesive discs were not polished.

By contrast, ceramic discs were milled from a block of Ceramill® Zolid HT+PS using a milling machine (Ceramill Matik, Amman Girrbach, Koblach, Austria) Subsequently, the specimens were sintered at 1450 °C for two hours including a 5 h cooling phase according to the manufacturer´s instructions. After sandblasting with 110 µm aluminum oxide (SHERA Werkstoff-Technologie GmbH & Co KG, Lemförde, Germany) at 2.5 bar, a layer of Paste Glaze transparent (Ceramotion, Dentaurum, Ispringen, Deutschland) was applied to each disc twice with a brush and after a drying time of 6 min fired for 1 min at 750 °C. Finally, all samples were finished with a diamond polisher for ceramics (Kerr, Bioggio, Switzerland) under water cooling.

In total 24 identical disc-shaped specimens of each dental material with a diameter of 8 mm and a thickness of 2 mm were produced. Each of the three mouthrinses as well as artificial saliva (control) was tested on 6 individual discs per restorative material ($n=6$).

Experimental approach

The protocol for the in vitro test procedure is depicted in Figs. 1 and 2. As a starting point, all test samples were placed in a petri dish, immersed in 20 ml of artificial saliva and stored at 37 °C for 24 h to allow that the different dental materials are hydrated as uniform as possible, resembling the initial situation in the oral cavity. Afterwards, the specimens were shortly rinsed with distilled water, blotted dry with an air pusher and subjected to the baseline color determination (T0).

Subsequently, all specimens underwent a 6-step cyclic treatment (Fig. 1), in which they were alternately immersed during one cycle in artificial saliva (2 min), exposed to warm black tea solution (37 °C, 1 min) and soaked in the respective mouthrinse or in artificial saliva (30 s). After each step, dental discs were shortly rinsed with distilled water. As shown in Fig. 2, each sample was subjected to a total number of 30 cycles (10 cycles per day

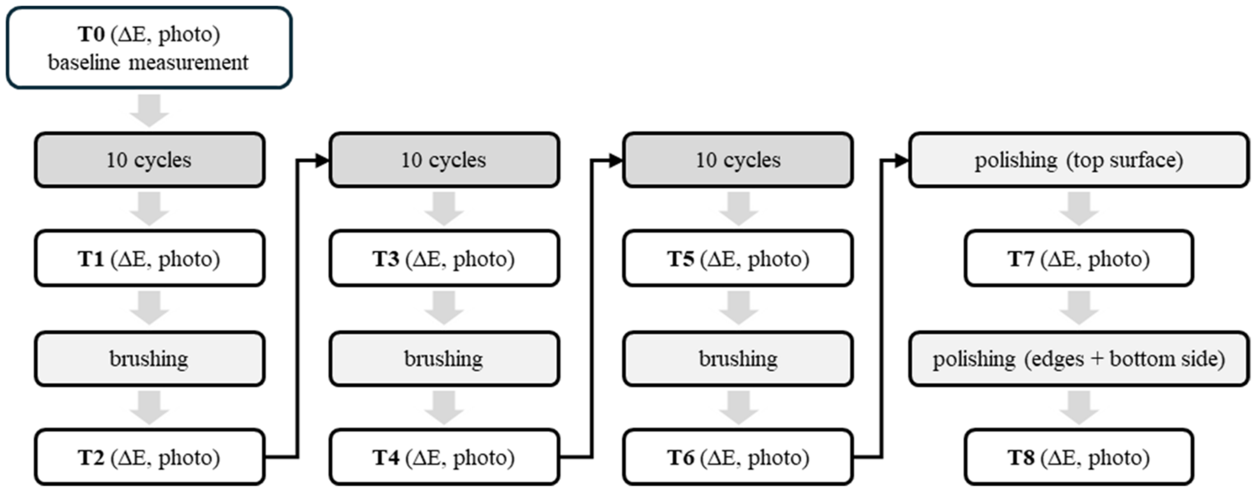


Fig. 2 Flowchart representing the entire in vitro test procedure. As a starting point, all dental discs were pre-treated with artificial saliva for 24 h at 37 °C to allow uniform hydration of the different dental materials followed by the first baseline color measurement (time-point T0)

on 3 consecutive days), with color measurements being carried out after every 10 cycles (T1, T3, T5). Next, the discs of each dental material were additionally brushed for 4 s, rinsed with distilled water and measured again optically (T2, T4, T6). At the end, all specimens were initially polished on the top surface using a suitable polishing device followed by an optical measurement (T7). After a last complete polish including also lateral edges and the bottom side of each dental disc, a final analysis on staining was conducted (T8).

Preparation of black tea

One bag (2 g) of English Breakfast Tea (Twinings) was brewed in 100 ml of freshly boiled distilled water (100 °C) for 4 min. The black tea solution was prepared freshly every day and stored at 37 °C until use in the cyclic test procedure.

Brushing of test specimens

Brushing of dental discs was conducted by applying the "Daily Clean" program of an electric toothbrush (Oral-B iO Series 9, Procter & Gamble Company, Cincinnati, United States) using a common brush head (Ultimate Clean, Oral-B iO) as well as toothpaste (medium abrasive Colgate® Total® Original, Colgate Palmolive Company, NYC, US). An amount of 10 g of toothpaste was mixed with 4 g of distilled water to achieve a consistency that remained evenly on the bristles but could be easily removed from the samples by rinsing with distilled water. The specimens were held with tweezers and the brush was moved 4 times for 1 s each from right to left with the toothbrush's pressure sensor "green".

Final polishing of dental discs after cyclic treatment

Samples made of composite and ceramic were initially polished on the top surface (T7) using a composite and ceramic polisher, respectively. Otherwise, discs prepared from the orthodontic adhesive were cleaned utilizing a polishing brush (Stoddard ApS, Aarhus, Denmark) and paste (RDA 120, Prophy Paste CCS, ProphyCare®, Directa, Upplands Vasby, Sweden) by moving 5 times from the left to the right over the sample, thereby applying constant manual pressure. At last, also the lateral edges and the bottom side of all specimens were equally polished with the help of a polishing brush and paste as described before and subjected to a final color measurement (T8).

Colorimetric evaluation

Dental samples were carefully dried with an air pusher before each optical analysis (T0–T8). To ensure equal conditions for each disc, all measurements were performed by the same operator in a closed, windowless room, with a constant artificial light source using the VITA Easyshade® V color spectrophotometer (VITA Zahnfabrik, Bad Säckingen, Germany) against white paper background (Bio Top 3® extra 160 g/m^2, Mondi) according to the manufacturer's instructions. The spectrophotometer was calibrated before every measurement by placing the probe tip against the calibration block. Values were recorded by using the Commission internationale de l'éclairage (CIE) L*a*b* standard system for color matching, which resembles a three-dimensional color space: the L* value corresponds with the lightness, the a* value represents the red-green component and the b* value the blue-yellow component. Hence, the CIE ΔE values describe the total color difference between the initial situation (before starting the cyclic procedure, T0) and the treated specimens (T1-T8) in all three color dimensions and was calculated using the following formula:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2(\Delta b^*)^2}$$

Statistical analysis

Our sample size estimation based on the results of a staining protocol with chlorhexidine, OCT and black tea on teeth [48] resulted in a sample size of six specimen per group.

Color shifts were expressed as ΔE values (mean ± standard error; $n=6$ per dental material) for all treatment groups and time-points (T1-T8). In addition, mean ΔE values at T5 (after 30 cycles) were further statistically analysed by pairwise comparison applying Tukey-HSD post-hoc test to figure out disparities in the discoloration potential between various mouth rinses as well as the control. The level of significance (α) was set to 0.05. The statistical evaluation of the collected and converted data was carried out using the software R (R Core Team, version 4.2.1).

Photographic images

To visualize potential color changes on dental materials, pictures were taken from individual discs at all indicated measurement time-points (T0-T8) by the same operator in a closed, windowless room, using a reflex camera (CANON EOS 40D, Tokyo, Japan) with constant positioning and settings against white paper background (Bio Top 3® extra 160 g/m^2, Mondi).

Results

Regardless of the type of restorative material used in the present in vitro approach, all mouthrinses provoked a major color shift in the clinically visible range over time, which was more pronounced for CHX than OCTM and OCTD, respectively (Fig. 3). Due to the cyclic treatment including brushing steps, a zig-zag progression of mean ΔE values was obtained for all test groups except the control (Fig. 3A). In detail, increased mean ΔE values measured on T1, T3 and T5 induced by alternately immersing dental discs in black tea and antiseptic mouthrinses, could be markedly – but not completely—reduced by brushing with toothpaste after every 10 cycles (T2, T4, T6). Indeed, the results further showed that mean ΔE values built up slightly and steadily for all mouthrinses and dental materials before (T1, T3, T5) and after brushing (T2, T4, T6), respectively. However, final professional polishing, including not only the top surface but also lateral edges and the bottom side of each dental disc, led to an almost complete reduction of respective discoloration at measurement point T8, demonstrating their reversibility. Exemplary photographic images shown in Fig. 3B for one dental disc per material for the initial state (T0) and after 30 cycles (T5) including brushing (T6) and final polishing (T8) further visually confirm the results obtained by spectrophotometry.

To ascertain statistically relevant differences in the discoloration potential between various mouthrinses as well as the control, respectively, calculated mean ΔE values after 30 cycles (T5), without taking into account final oral cleaning steps, were further analysed pairwise in more detail (Table 3). Hence, at measurement time T5, all antiseptic mouthrinses were associated with statistically significant color shifts compared to the control group ($p < 0.0001$). Thereby, CHX was associated with the highest mean ΔE difference for all dental specimens tested. The pairwise comparison further revealed that CHX caused significantly more discoloration than both OCT-containing mouth rinses on all restorative materials ($p < 0.05$). Notably, that effect was statistically strongest between OCTD and CHX for all dental specimens as well as among OCTM and CHX for the composite-based samples ($p \leq 0.0001$). With the exception of the ceramic material Ceramill® Zolid HT+PS, no statistically significant differences in discoloration were observed on T5 between OCTD and OCTM.

Discussion

A crucial property of aesthetic restorative materials is their long-term color stability, thereby avoiding time- and cost-intensive replacements as well as dissatisfaction of patients. In that context, the aim of the current in vitro study was to compare the discoloration potential of commercially available chlorhexidine- and OCT-based mouthrinses on frequent dental materials (one zirconium-ceramic, two nanohybrid-composites, one orthodontic adhesive) when black tea was used as chromogenic nutrient and to investigate the reversibility by mechanical cleaning and professional polishing. In this presented standardized in vitro model, experimental conditions were adopted to mimic the real-life situation in the human oral cavity as accurately as possible. To the best of our knowledge, most of the laboratory studies published so far evaluated possible color shifts of various dental materials only after soaking or rinsing with certain mouthrinses, neither taking into account the additional influence of chromogenic nutritional factors nor the effect of regular mechanical and/or professional oral cleaning. Discoloration of dental test specimens was assessed both visually as well as by using spectroscopy recording objective ΔE values in order to eliminate

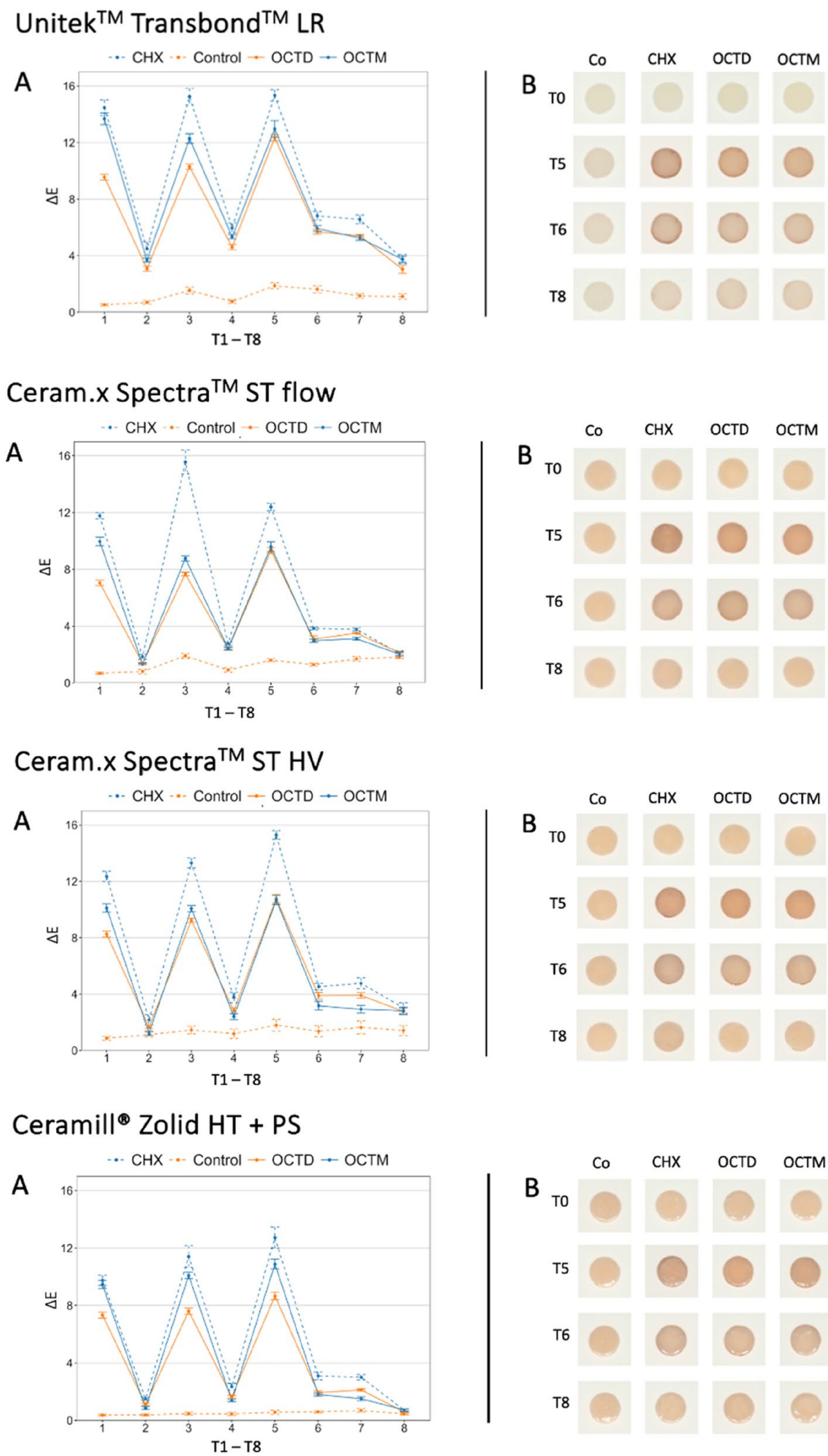


Fig. 3 Progression of color changes for different dental materials following treatment with various mouthrinses. **A** Mean ΔE values (± standard error; $n = 6$) for all treatment groups and time-points (T1-T8). **B** Representative examples of photographic images of one disc per dental material treated with respective solutions at selected time-points T0, T5, T6 and T8. CHX, Chlorhexamed forte; OCTD, octenident®; OCTM, octenimed®; Co (control, artificial saliva)

Table 3 Pairwise comparison of mean ΔE values of different mouthrinses and the control at time-point T5. Mean ΔE differences, 95% confidence intervals (CI) and p-values adjusted for the number of comparisons for various dental materials are shown. CHX, Chlorhexamed forte; OCTD, octenident®; OCTM, octenimed®; control (artificial saliva)

	Unitek™ Transbond™ LR		Ceram.x Spectra™ ST flow		Ceram.x Spectra™ ST HV		Ceramill® Zolid HT+PS	
	mean ΔE diff [95% CI]	*p-value*	*mean ΔE diff [95% CI]*	*p-value*	*mean ΔE diff [95% CI]*	*p-value*	*mean ΔE diff [95% CI]*	*p-value*
CHX-control	13.46 [11.94,14.98]	< 0.0001	10.79 [9.82,11.75]	< 0.0001	13.49 [12.13,14.85]	< 0.0001	12.16 [10.43,13.89]	< 0.0001
OCTD– control	10.46 [8.93,11.98]	< 0.0001	7.72 [6.76,8.68]	< 0.0001	8.98 [7.62,10.33]	< 0.0001	8.09 [6.37,9.82}	< 0.0001
OCTM–control	11.09 [9.57,12.62]	< 0.0001	7.99 [7.03,8.95]	< 0.0001	8.88 [7.52,10.24]	< 0.0001	10.31 [8.59,12.04]	< 0.0001
OCTD–CHX	−3.00 [−4.53,−1.48]	0.0001	−3.06 [−4.02,−2.10]	< 0.0001	−4.51 [−5.87,−3.16]	< 0.0001	−4.07 [−5.79,−2.34]	< 0.0001
OCTM–CHX	−2.37 [−3.89,−0.84]	< 0.01	−2.79 [−3.75,−1.83]	< 0.0001	−4.61 [−5.97,−3.25]	< 0.0001	−1.85 [−3.57,−0.12]	< 0.05
OCTM–OCTD	0.64 [−0.89,2.16]	0.6527	0.27 [−0.69,1.23]	0.8591	−0.1 [−1.45,1.26]	0.9971	2.22 [0.49,3.95]	< 0.01

subjective interpretation inherent in a color comparison by naked eye only. Visual thresholds are of utmost importance as a quality tool and guide for the assessment and selection of dental materials and the evaluation of their clinical performance [51]. Thus, the color change was determined after every ten cycles as well as after each brushing step to accurately assess discoloration kinetics, rather than only once at the end of the procedure. Although CIE developed a new formula (CIEDE2000) in 2004, which according to some studies [52, 53] better reflects human perception and acceptance of tooth colors, we decided to use the version CIEL*a*b* to calculate ΔE because it is still regularly used in similar studies [5, 44, 48, 54–56] to investigate color changes in the oral cavity and to better compare results especially to Sarembe et al. [48], as identical mouthrinses were recently used on human molar crowns.

In that regard, the results of the current in vitro study revealed that cyclic immersion in black tea solution and different antiseptic mouthrinses, respectively, caused considerably increasing discoloration of all four tested restorative materials in the clinically visible range ($\Delta E \geq 3.3$ on T1, T3, T5). In fact, the most pronounced color shift was constantly monitored for CHX rather than OCTM and OCTD, regardless of the physical and chemical properties of the respective dental material utilized. In detail, discs made of the adhesive Unitek™ Transpond™ LR generally showed the highest changes in color upon cyclic treatment with the different antiseptic mouthrinses (maximum $\Delta E = 15.3$ for CHX at T5). Accordingly, specimens prepared out of zirconia ceramic Ceramill® Zolid HT+PS proved in the present in vitro approach more resistant to discoloration (maximum $\Delta E = 12.7$ for CHX at T5).

Surface roughness and surface composition are factors that must be taken into account in the discoloration behavior. Ramoglu et al. [57] found a surface roughness of 0.039 µm ± 0.008 µm for Unitek™ Transbond™ LR, which increased significantly to 0.121 µm ± 0.066 µm after an aging protocol with UV light and water spray. Reinhard et al. [58] reported a median surface roughness for Ceram.x Spectra™ ST after rubber cup polishing of 10.55 nm (Min 8.81 nm, Max 12.04 nm) and 3.86 nm (Min 3.43 nm, Max 4.21 nm) after erythritol air flow polishing. High filler content resin flow composites exhibited surface roughness and wear comparable to their paste counterparts [59]. The surface roughness of Ceramill® Zolid HT+PS after polishing was found to be 0.114 µm ± 0.023 µm [60]. The surface roughnesses in the literature for the materials examined are in a comparable range. It seems that the type of material has a decisive influence on the discoloration behavior and is more important than the initial surface roughness.

The observed high in vitro staining potential of CHX in combination with black tea, although at varying extend, is in good agreement with already published studies on human teeth [48, 54, 61–64]. There are various theories on why and how chlorhexidine-containing mouthrinses may cause discoloration in the oral cavity. However, it is most likely that the per se colorless cationic active precipitates anionic staining dietary chromogens present in food and beverages onto dental surfaces [65, 66]. The investigations by Addy et al. [67] led to the assumption that the dietary associated staining of teeth and acrylic material may be a precipitation reaction between adsorbed cationic antiseptics and chromogenic compounds producing a very stable complex. Carpenter et al. [68] reported a significantly increased staining behavior when chlorhexidine and tea were used together on teeth and suggested that this effect was due to an enhanced binding of tea polyphenols to the hydroxylapatite by chlorhexidine and recommended thorough rinsing after using the mouthwash/ before consuming chromogenic food. Since OCT also belongs to the group of positively charged antiseptics, a similar staining mechanism may be theoretically assumed for OCTM and OCTD. However, as the discoloration potential of CHX was statistically significantly higher after 30 cycles (T5) in direct comparison to both OCT-based mouthrinses, respectively, there might be different degrees of adhesion of antiseptic ingredients to the surface of restorative materials and/or organic chromogens. Notably, no significant differences in discoloration were observed on T5 between OCTD and OCTM, except for Ceramill® Zolid HT+PS. It can be speculated that the contrasting effect observed for the OCT-containing mouthrinses on the zirconium ceramic might be due to unequal additional ingredients (e.g. salts, organic acids) in the respective final commercial formulations, which may chemically and physically interact differently with the ceramic surface texture.

Indeed, mouthrinses containing antiseptic agents are widely prescribed in the clinical setting mainly because of their ability to reach otherwise inaccessible areas of the oral cavity of patients where mechanical plaque control is temporally (e.g. after oral surgery) or in some cases even permanently not possible [69]. As OCTM and OCTD showed in our experiments significantly less discoloration on dental specimens compared to CHX before brushing (T5), with similar observations also reported by Sarembe et al. when using human enamel in vitro [48], OCT-containing mouthrinses offer additional advantages besides their potent antimicrobial efficacy.

Furthermore, data which were achieved in preliminary experiments without involving a nutritional factor during the setup of the present in vitro study, demonstrated significantly less staining after immersion of restorative specimens in CHX (data not shown), confirming earlier findings reported in the literature [2, 3, 5, 7, 44–46]. For that reason, patients should be advised to avoid discoloring food components (such as black tea, red wine, coffee, curry) in particular when using cationic oral rinsing solutions.

Moreover, we considered that the dental materials utilized in the present experimental approach have a quite individual retention capacity according to the manufacturer's information and therefore may absorb different amounts of liquids, such as hydrophilic chromogens and antiseptic mouthrinses, due their individual specific surface roughness and structure. By initially hydrating the prepared dental discs for 24 h in artificial saliva, we aimed to create almost similar conditions for all materials, at least as a starting point.

Importantly, simulated mechanical cleaning after every 10 cycles (T2, T4, T6) with common toothpaste and an electrical toothbrush mirroring routine oral hygiene measures as per general recommendations resulted in a substantial decrease of ΔE values, returning gradually arising discoloration to almost clinically acceptable levels [51]. This observation emphasizes the importance of simple tooth brushing being quite effective in controlling a large part of already accumulated discoloration on restorative materials. However, depending on the type of dental material used, the reversibility of staining by mechanical cleaning was varying. The most satisfying reduction in ΔE values upon brushing with toothpaste was achieved on the surface texture of the zirconium ceramic specimens, whereas the cleaning effect was least pronounced for the orthodontic adhesive.

In order to gain additional information about the maximum reversibility of observed discoloration due to professional oral hygiene measures performed by dentists, the present in vitro set-up involved a comprehensive polishing of all specimens as a final step. This additional procedure allowed for a further reduction of accumulated discoloration to clinically acceptable ΔE values ($\Delta E \leq 3.3$ at T8), for Ceramill® Zolid HT+PS and Ceram.x Spectra™ ST flow, at the same level like the control. Overall, these findings clearly point out the importance of regular polishing of dental restorations during professional prophylaxis treatments besides proper daily oral care at home in order to maintain also aesthetically pleasing results.

There may be some possible limitations in the presented in vitro study that could be addressed for future research. To ensure long-term aesthetic of restorative materials, color stability is not the only criteria to be considered. Another important factor is the overall change in surface roughness of individual dental materials in the oral cavity over time. In addition, under clinical conditions, the effect of the mouthrinses CHX, OCTM and OCTD on restorative materials may be different, being dependent on many other factors that could not be replicated in the present in vitro set-up. Thus, further studies will be needed to validate the impact of antiseptic mouthrinses on the long-term color stability of different dental materials in clinical practice.

Conclusion

This applied in vitro setup reproduced general oral hygiene using commercial antiseptic mouthrinses in combination with mechanical tooth brushing by considering the adherence of organic chromogens to the surface of frequently used restorative materials. It was demonstrated that all tested antiseptic mouthrinses showed a color shift in the clinically visible range on all dental materials, whereby the staining effect was significantly higher for CHX than for OCTM and OCTD, respectively. However, discoloration was almost completely removed by simple brushing with a toothbrush and toothpaste followed by professional polishing.

Abbreviations

CIE	Commission internationale de l'éclairage
CHX	Chlorhexamed forte
OCT	Octenidine
OCTD	octenident®
OCTM	octenimed®

Authors' contributions
Conceptualization M.L., S.B., M.A., A.M.; methodology S.B., M.L., M.A.; formal analysis D.B., B.L.; writing – original draft preparation S.B., M.L., M.A.; writing – review and editing D.B., M.C., X.R.-F., A.M., B.L.; visualization S.B., M.L., D.B., B.L.; supervision M.C., X.R.-F., A.M., B.L.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Ethical approval is not applicable as this was an in vitro study in which humans and animals, including materials/data, were not used for experimental purposes.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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