BMC Oral Health

https://doi.org/10.1186/s12903-026-08477-2

Article in Press

The effects of three whitening toothpastes on the color stability, microhardness, and surface roughness of contemporary one-shade composite systems

Received: 24 November 2025

Accepted: 23 April 2026

Published online: 12 May 2026

Cite this article as: Sahin M., Ucuncu M.K., Celiksoz O. *et al.* The effects of three whitening toothpastes on the color stability, microhardness, and surface roughness of contemporary one-shade composite systems. *BMC Oral Health* (2026). https://doi.org/10.1186/s12903-026-08477-2

Merve Sahin, Musa Kazim Ucuncu, Ozge Celiksoz & Oktay Yazicioglu

We are providing an unedited version of this manuscript to give early access to its findings. Before final publication, the manuscript will undergo further editing. Please note there may be errors present which affect the content, and all legal disclaimers apply.

If this paper is publishing under a Transparent Peer Review model then Peer Review reports will publish with the final article.

ARTICLE IN PRESS

© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

ARTICLE IN PRESS

Merve Sahin[1]

Musa Kazim Ucuncu[2]

Ozge Celiksoz[3]

Oktay Yazicioglu[4]

[1]Istanbul Kent University, Faculty of Dentistry, Department of Restorative Dentistry, Istanbul, Türkiye

[2]Health Sciences University, Hamidiye Faculty of Dental Medicine, Department of Restorative Dentistry, Istanbul, Türkiye

[3]Eskisehir Osmangazi University, Faculty of Dentistry, Department of Restorative Dentistry, Eskisehir, Türkiye

[4]Istanbul University, Faculty of Dentistry, Department of Restorative Dentistry, Istanbul, Türkiye

Corresponding author:

Musa Kazım Üçüncü

E-mail: ucuncumusakazim@gmail.com GSM: +905071975712

Affiliations: Health Sciences University, Hamidiye Faculty of Dental Medicine, Department of Restorative Dentistry, Istanbul, Türkiye

Address: Selimiye Mah. Tıbbiye Cd. 34668 Üsküdar/Istanbul

ARTICLE IN PRESS

Disclosure Statement and Acknowledgements

The authors do not have any financial interest in the companies whose materials are included in this article

Ethics approval and consent to participate

Since our manuscript does not involve any human- or animal-derived material, obtaining ethics committee approval was not required.

ARTICLE IN PRESS

The effects of three whitening toothpastes on the color stability, microhardness, and surface roughness of contemporary one-shade composite systems

ABSTRACT

Background: The aim of this study was to evaluate the mechanical (surface roughness & microhardness) and optical (color stability) properties of two modern one-shade dental composites following exposure to three different whitening toothpastes.

Methods: A total of 192 disc-shaped specimens (n=8; per subgroup) were prepared from two one-shade composites: ONE (Charisma One, Kulzer, Germany) and VITTRA APS (Advanced Polymerization System) Unique (FGM, Germany). Specimens were assigned to four brushing media: Opalescence Whitening, Signal White Now Glossy Shine, Colgate Optic White Expert, and distilled water (control). Discs (2 mm × 10 mm) were fabricated using a Teflon mold, stored at 37°C for 24 h in tab water. Microhardness (Vickers; 200 g/10 s), surface roughness (contact profilometry), and color parameters (VITA Easyshade V; CIEDE2000) were recorded before and after a toothbrushing simulation of 10,000 cycles. Toothpastes were used as a 1:3 (v/v) slurry. Data were analyzed using two-way ANOVA with Tukey's HSD test ($p<0.05$).

Results: All surface roughness values increased after toothbrushing; the greatest change was observed in the VW group (0.056 ± 0.017), whereas the smallest change occurred in the OCO group (0.006 ± 0.040) ($p<0.05$). In all subgroups, microhardness values decreased following brushing, and the most pronounced reduction was found in the OW group (-10.12 ± 3.24). This decrease was statistically significant when compared with the OS, OC, OCO, and VW groups ($p<0.05$). With respect to color stability, only the VW group remained below the clinically acceptable threshold ($\Delta E_{00} = 1.8$). Nevertheless, no statistically significant differences were detected among the experimental groups. Nor, interestingly, did any group other than VW exhibit a change that fell within the clinically acceptable range.

Conclusion: The compositional architecture and formulation of dental composites, in conjunction with toothpaste constituents, may lead to divergent outcomes when subjected to various mechanical tests.

Keywords: CIEDE2000, microhardness, one-shade composite, surface roughness, toothpaste

ARTICLE IN PRESS

ARTICLE IN PRESS

INTRODUCTION

One of the most pivotal means of removing microbial dental plaque—the principal culprit behind dental caries and periodontal diseases, and a key player in their progression—is toothbrushing [1,2]. In this context, the synergistic interplay between toothbrushes and toothpastes emerges as indispensable for the preservation of both dental health and esthetics [3,4]. Toothbrushes and toothpastes, hailed as the most frequently chosen and arguably the most essential oral hygiene instruments, are routinely employed by individuals striving to maintain optimal oral health [5,6]. In this ever-evolving field, a diverse array of therapeutic and functional formulations—tailored to address various dental maladies and challenges—continues to be developed, encompassing both innovative toothpaste compositions and advanced toothbrush designs [3,7–10].

In this era where esthetic dentistry enjoys unprecedented popularity [11], advances in material chemistry have inevitably influenced modern composite materials as well. Replacing traditional polychromatic composites, one-shade composites, designed to offer both ease of use and simplified shade selection for clinicians, have emerged as a testament to this progress [12]. Beyond their therapeutic role in preserving oral health, the influence of oral hygiene instruments on the color stability of esthetic dental restorative materials, whether beneficial or detrimental, remains a subject of ongoing debate and investigation [13–15]. Notably, the recent development of restorative dentistry has introduced materials capable of mimicking the color of the surrounding dental tissues post-polymerization, thus achieving the so-called “chameleon effect” [12,16]. Toothpastes, on the other hand—complex semi-solid mixtures composed of a multitude of chemical constituents [3,17]—can exert various effects on the surface of esthetic composite resins, owing to their abrasive, whitening, and other active ingredients [18,19]. Yet, despite the proliferation of modernized dental composites, studies specifically examining the effects of toothbrushing with toothpaste on these new materials remain limited [12]. Accordingly, it remains unclear to what extent the “chameleon effect”–driven optical performance promised by one-shade composites is preserved after toothbrushing with a range of whitening toothpastes routinely used in daily oral hygiene, particularly given the marked compositional heterogeneity of these products. This uncertainty underscores the need for studies employing a unified experimental model in which color stability is assessed alongside surface roughness and microhardness.

ARTICLE IN PRESS

Beyond, the present study aims to comparatively evaluate the performance of two novel one-shade composite resins when subjected to brushing with different types of toothpastes, focusing on key physical properties such as color stability, surface roughness, and hardness. The null hypothesis of the study posits that brushing composite surfaces with toothpaste does not induce any significant changes in these physical characteristics.

MATERIAL METHODS

Determination of sample size and study design

ARTICLE IN PRESS

Prior to commencing the procedures, a power analysis was conducted using the statistical software G*Power 3.1.7 to determine the group sample sizes. The statistical power of the study was expressed as 1-ß (where ß represents the probability of a Type II error). Based on the data obtained from the study by Khamverdi et al., the effect size (d) was calculated as 1.8042901 [20]. With a significance level of $\alpha = 0.05$ and a statistical power of 90%, the minimum required sample size for each subgroup was determined to be seven. In this in vitro laboratory investigation, separate composite disk specimens were prepared for the assessment of microhardness, color stability, and surface roughness. To ensure methodological standardization, all specimen preparations were performed by a single operator (M.K.U.). Based on the power analysis, each subgroup comprised seven composite disks, yielding a total of 192 specimens for the study (n=8; per subgroup). Disks measuring 10 mm in diameter and 2 mm in thickness were fabricated using a custom Teflon mold. A Mylar strip was first placed on a glass plate, the mold was positioned on top, and—prior to light curing—a second Mylar strip and a glass slide were applied to the upper surface to promote uniform polymerization. After removal from the mold, the upper surfaces of the composite discs were finished and polished using a dedicated polishing system (Opti1Step™ Polisher, 8002 Cup Form; Balz-Zimmermann-Strasse 7, Kloten, Switzerland) in strict accordance with the manufacturer's instructions. Two composite materials were evaluated: a one-shade composite (VITTRA APS Unique, FGM, Brazil) and a nanohybrid composite (ONE Charisma Topaz, Kulzer, Germany). Light curing was performed with the designated curing unit in accordance with the manufacturer's instructions. The experimental workflow, which involved four different toothpaste formulations, is illustrated in the flow diagram (Figure 1). Detailed material compositions and application protocols are presented in Table 1.

Brushing procedure

The prepared composite discs were brushed using a mechanical simulator. Three toothpastes, each diluted to one-third (v/v), were applied with a pre-selected medium-bristled toothbrush (Colgate Extra Clean 1+1) within the simulator. The protocol comprised 10,000 circular brushing cycles (18 mm in diameter) at a speed of 40 mm/s and a force of 250 g, approximating one year of brushing [21].

Surface roughness measurement

To quantify surface roughness, a contact-type, multilingual profilometer (Surftest SJ-410, Mitutoyo, Tokyo, Japan) was employed. The instrument operated with a cut-off length of 0.2 mm and a horizontal evaluation length of 2 mm. A standard stylus probe mounted on the detector was used for all measurements. Following each run, the Ra values displayed on the device's touch-screen were transferred to a computer. For each specimen, three independent traces were recorded and their mean value was calculated. The instrument was calibrated prior to the measurement of each specimen [22].

Microhardness measurement

The surface microhardness of the composite discs was assessed using a Vickers hardness tester (Falcon 400, Innovatest Europe BV, Maastricht, The Netherlands). A load of approximately 200 g was applied for 10 seconds to generate indentations characteristic of the Vickers indenter. To ensure standardisation and obtain reliable values, measurements were taken at the disc centre and at near-central locations,

ARTICLE IN PRESS

maintaining a minimum spacing of 100 µm between indentations. The diagonal lengths of each indentation were measured with a 40× ocular lens, and the corresponding hardness values were recorded [23].

Assessment of color stability

Measurements were acquired using a spectrophotometer (VITA Easyshade V, VITA Zahnfabrik, Bad Säckingen, Germany). Prior to acquisition, specimen surfaces were dried and cleaned, and measurements were recorded at two time points (pre- and post-brushing). All measurements were performed by a single operator (M.S.). Under the D65 standard illuminant and against a neutral gray background, values were recorded in the CIELAB color space as L*, a*, and b*. Color stability was quantified using the CIEDE2000 color-difference formula, and ΔE_{00} values were computed [24].

$$\Delta E_{00} = \sqrt{\left(\frac{\Delta L'}{k_L S_L}\right)^2 + \left(\frac{\Delta C'}{(k_C S_C)}\right)^2 + \left(\frac{\Delta H'}{k_H S_H}\right)^2 + R_T\left(\frac{\Delta C'}{k_C S_C}\right)\left(\frac{\Delta H'}{k_H S_H}\right)}$$

ARTICLE IN PRESS

Differences in lightness, chroma, and hue are denoted by $\Delta L'$, $\Delta C'$, and $\Delta H'$, respectively. The parametric weighting functions S_L, S_C, and S_H—which normalize the overall color difference according to the position of the color pair in the L′, a′, and b′ coordinates—were computed accordingly. The parametric factors kL, kC, and kH were set to 1:1:1. The rotation term RT accounts for the interaction between hue and chroma differences and was expressed mathematically as:

$$R_T = -\sin(2\Delta\theta) \cdot R_C.$$

All statistical analyses were performed using GraphPad Prism (GraphPad Software, San Diego, USA). The normality of data distribution was evaluated with the Shapiro–Wilk and Q-Q plot test. Differences in the mean ΔE_{00} values among composite resin groups were assessed by two-way ANOVA, followed by Tukey's HSD post hoc test for multiple comparisons. Assessed by Levene's test was the homogeneity of variances, and established was homogeneity. Statistical significance was defined as $P < 0.05$ for all analyses.

RESULTS

The surface roughness outcomes were not significantly affected by composite type (F=1.86; p=0.177). In contrast, toothpaste type (F=7.21; p<0.05) and the composite × toothpaste interaction (F=3.45; p<0.05) were statistically significant. Based on the roughness values recorded before and after brushing with various toothpastes, the mean differences were calculated for both composite brands (Table 2-3). The greatest difference was observed in the VITTRA & Opalescence (VW) group (0.056 ± 0.017), whereas the smallest difference was detected in the ONE & Control (OCO) group (0.006 ± 0.040). When the ONE composite subgroups were evaluated individually, the lowest differences were also found in the ONE & Control (OCO) subgroup. Among the VITTRA composites, the VCO subgroup exhibited the lowest values. Statistically significant differences were identified between VW–OW, VW–VCO, OW–OS, OS–OCO, and VW–OCO ($p<0.05$). Between these specific pairs, therefore, the impact of the brushing regimen and toothpaste type appears more pronounced.

The microhardness outcomes were not significantly affected by the main effect of both composite and toothpaste type (F=1.315 & F=0.547 respectively; $p>0.05$). In contrary, the composite × toothpaste interaction were statistically significant (F=5.929; $p<0.05$). According to the hardness results, the highest numerical change after brushing was found in the OW group (-10.12 ± 3.24), while the smallest change was recorded in the VW group (-1.30 ± 5.70). Post hoc analysis revealed statistically significant differences between the OW group and the OS, OC, OCO, and VW groups ($p<0.05$) (Table 4-5). A reduction in microhardness values after brushing was observed in all subgroups.

Two-way ANOVA showed a significant composite × toothpaste interaction for color stability (F=6.74; $p<0.05$) and the main effects of toothpaste type (F=3.16; $p<0.05$), whereas the main effects of composite type were not significant (F=3.57; $p>0.05$) (Table 6). The group with the highest ΔE_{00} value was VCO (3.32 ± 0.60), whereas the VW group exhibited the most favorable color stability (1.76 ± 0.63). All ΔE_{00} values are presented in Table 7, and brushing the ONE composite with different toothpastes did not result in a statistically significant difference in color stability compared with the control group. For the VITTRA composite, however, the VCO group differed significantly from the other groups ($p<0.05$). When the composites were compared under identical brushing conditions, a statistically significant difference emerged only in the water-only control group (VCO) ($p<0.05$); no such inter-composite divergence was observed for any of the toothpaste groups (Table 7).

DISCUSSION

The null hypothesis was rejected in light of the obtained findings. With respect to surface roughness, toothpaste alone emerged as a strong influencing factor, and, in addition, the combination of toothpaste and composite type demonstrated a synergistic effect, which was statistically significant in terms of its impact on surface roughness (Table 6). Regarding surface microhardness, the interaction between the composite material and the toothpaste type demonstrated a strongly significant effect ($p<0.05$). However, when each factor was analyzed independently, neither the composite material nor the toothpaste type showed a statistically significant main effect (Table 6). As for color change, toothpaste on its own significantly affected color stability, and, moreover, the combined effect of toothpaste and composite type also showed a statistically significant influence on color stability (Table 6).

This study’s key strengths and originality lie in its focus on contemporary one-shade composites, the inclusion of whitening toothpastes with distinct formulations, and the incorporation of a water-only control for robust comparison. Moreover, by evaluating multiple endpoints—optical outcomes, microhardness, and surface roughness—within a single experimental model under a standardized toothbrushing simulation, the study provides an integrated and clinically meaningful appraisal of material performance. The clinical success of composite

ARTICLE IN PRESS

restorations, particularly in the anterior region, has been reported within a wide range of approximately 50–100%, and it is evident that the type of material and its mechanical properties are closely associated with failure and the onset of esthetic complications (such as color mismatch, loss of anatomical form, and discoloration) [25]. It should be borne in mind that laboratory investigations of the physical or mechanical properties of dental composites may not always correlate with their clinical performance. Nevertheless, conducting multiple tests under laboratory conditions in place of in vivo studies—which are both costly and time-consuming—represents one of the more rational approaches for generating predictions about clinical outcomes [26,27].

Various types of toothbrushing simulators have been employed in studies designed to evaluate the abrasivity of toothpastes [21,28,29]. According to the current standard in this field, BS EN ISO 11609:1998, test devices may be constructed as flat-bed systems or configured to more closely replicate human brushing kinematics, incorporating vertical reciprocating or rotary motions. In this context, novel systems are also being developed to approximate real-life brushing performance as closely as possible [28]. On hard tissues—particularly dentine—it has been shown that different simulator designs, such as linear back-and-forth versus rotary motion, can yield statistically distinct levels of wear [29,30]. Accordingly, and in line with previous literature, toothpastes in the present study were diluted at a 1:3 ratio, and brushing was performed under a 250 g load using a circular motion [21,31]. **Based** on the understanding of brushing-induced changes, it has been proposed that two distinct wear mechanisms may occur in anterior and posterior composite restorations: first, fatigue-related wear; and second, abrasion associated with lateral crack formation and filler particle loss, particularly in anterior composites [32]. Whether particle size in composite materials directly affects clinical performance remains a matter of investigation, and in vitro studies continue to report divergent findings [22,33]. Although non-contact, three-dimensional optical profilometers are highly informative for surface roughness assessment—offering detailed and visually rich topographical data [34]—contact profilometers are still frequently preferred when rapid and standardized measurements are required [22,26,35]. Hardness, defined as the resistance of a material to deformation under an applied force, can be measured through both invasive and non-invasive approaches. By applying very small or relatively large loads, surface microhardness can be quantified without causing substantial damage, using scales such as Brinell, Knoop or Vickers. Among these, the Vickers test is widely adopted: the diagonals of the indentation left by a diamond indenter are measured, and the corresponding hardness value is calculated by the device. In accordance with ISO standards, a load of 200 g was applied for a dwell time of 10 seconds in the present study, and surface microhardness values were recorded in Vickers units [23].

ARTICLE IN PRESS

CIEDE2000 is a widely preferred system for calculating color differences in dental materials [36], and it also enables the assessment of various optical abilities such as masking [37]. Beyond dental resins, CIEDE2000 has been increasingly applied in recent years across different disciplines and materials [38–42]. Although color differences can also be calculated using the CIELAB formula [33], the perceptibility and acceptability thresholds for these two systems differ according to the literature [43,44]. In addition, substantial discrepancies may arise in the calculated values depending on the chosen formula [45]. CIEDE2000 is essentially an improved version of CIELAB and incorporates specific weighting functions for lightness (S_L), chroma (S_C), and hue (S_H), together with parametric factors (k_L, k_C, k_H), as well as the rotation term R_T used to correct chroma and hue differences in the blue region of the color space [46]. Sharma further reported that the equations used in CIEDE2000 include multiple sources of discontinuity, particularly in the computation of h* [24]. Although this formulation does not define a unique color space of its own, it employs modified L*, a*, b* coordinates and has been shown to be effective for quantifying small color differences. In a key threshold-defining study aimed at standardizing interpretation among researchers, the 50:50% perceptibility threshold for CIEDE2000 was determined as 0.8, and the clinical acceptability threshold as 1.8 [44]. On this basis, the present study used the CIEDE2000 formulation to determine color stability values.

In the present study, which included two modern one-shade composite types, VITTRA was found to have a higher filler content than ONE. We hypothesize that the silanized glass phase in VITTRA may theoretically limit water sorption and degradation of the organic matrix, thereby providing an advantage in terms of color stability and microhardness [47,48]. Furthermore, VITTRA incorporates an advanced APS initiator system, which may allow for improved depth of cure and, indirectly, enhanced mechanical properties [49]. In contrast, the presence of both UDMA and TEGDMA in ONE is indicative of a more flexible matrix, and, combined with its lower filler loading, may result in greater water uptake and surface solubility compared with VITTRA [50,51]. This, in turn, could potentiate the whitening and abrasive effects of the toothpastes on the ONE composite.

In many studies, increased roughness and reduced gloss have been reported after toothbrushing due to abrasive effects [52]. A reduction in gloss may directly influence the L* parameter in the CIEDE2000 formula, and, because of the formula's dependence on L*, ΔE_{00} values exceeding the clinically acceptable threshold may have been detected. As particle size increases, the hardness of the material tends to be higher than that of materials with smaller particle sizes; accordingly, surface roughness values often follow a similar trend [32]. In the present study, microhardness values decreased in all subgroups, with the most pronounced reduction observed in the ONE + Opalescence group (-10.12 ± 3.24). The lower filler content and the presence of TEGDMA in ONE may have contributed to this outcome [51,53]. In addition, the silica-based abrasive system and surfactants in Opalescence whitening toothpaste may have promoted surface wear and matrix softening, thereby exacerbating the microhardness reduction in the ONE + Opalescence group. It is noteworthy that the roughness values remained below the clinically acceptable threshold of 0.2 µm Ra [54], although a certain increase in surface roughness was observed in all subgroups after brushing. The greatest change occurred in the VW group, while the smallest change was recorded in the OCO group. Considering the mean values, ONE groups generally exhibited higher surface roughness than VITTRA, which may primarily be attributed to the higher filler loading in VITTRA. Although a high filler content is often regarded as advantageous, it does not invariably guarantee lower roughness; following toothpaste use, the type of abrasive and the presence of surface-active agent can also adversely affect the glass filler phase [55]. Composite surface roughness may be further influenced by abrasives such as silica/hydrated silica, calcium aluminum borosilicate, and calcium and tetrasodium pyrophosphate, as well as by matrix-weakening chemical components including SLS, pH-adjusting bases/acids, and

ARTICLE IN PRESS

hydrogen peroxide [3]. The use of only water in the control group, in the absence of such mechanical and chemical agents, explains the minimal increase in roughness observed in this group.

It has been reported that, following brushing with toothpaste, color stability values on composite surfaces generally tend to increase compared with control groups [56]. In the present study, all three toothpastes used had whitening properties but differed structurally from one another. Existing literature suggests that, after the use of whitening toothpastes, color stability of composite surfaces may be improved relative to controls. However, the behavior observed in hardness tests may differ [56]. In terms of color stability, the only composite–toothpaste combination that yielded a value below the clinically acceptable threshold was Opalescence & VITTRA (ΔE_{00}=1.76 ± 0.63). It is evident that all other values exceeded the corresponding cut-off. When toothpastes were compared with respect to color stability, no statistically significant differences were detected between the composites, with the exception of the control (water) group. In the control groups brushed with water alone, the absence of any chemical or colloidal medium between the toothbrush and the composite surface—and, consequently, the lack of a toothpaste-derived interfacial film or residue—may render brushing-induced microstructural surface alterations more pronounced. This, in turn, could increase light scattering and thereby promote greater optical deviation, yielding poorer color-stability outcomes. By contrast, the lack of a significant difference between the composites in the toothpaste groups may plausibly be attributed to a comparable residue- and/or polishing-like effect, which is likely to drive a convergence of ΔE_{00} responses across both composite types. In a related study using three similar toothpastes on ceramic blocks, color differences were found to be far above clinically acceptable limits, and surface roughness was shown to increase regardless of the toothpaste used [57]. In our study, although color stability was influenced synergistically by both composite type and toothpaste type, composite type alone was not identified as a determining factor. Given its higher filler content and APS initiator system, VITTRA may be regarded as having color stability that is comparable to or better than that of ONE. Although the Opalescence & VITTRA group demonstrated color stability within clinically acceptable limits, whitening toothpastes may not invariably secure the anticipated level of color stability across all one-shade composite systems. In summary, certain mechanical properties of single-shade composites—such as surface hardness and roughness—can vary as a function of their organic and inorganic composition [58].

If the present laboratory-based study had been conducted under in vivo conditions, it is likely that different results would have been obtained. This discrepancy would primarily arise from inherent limitations of in vitro methodologies, such as the absence of biokinetic phenomena and the inability to fully replicate the unique characteristics of oral fluids [59]. This in vitro brushing simulation may not fully reproduce the complex conditions of the oral environment (e.g., salivary dynamics, biofilm formation, pH fluctuations, patient-specific brushing habits, and variability in dietary and toothpaste use); therefore, the observed changes in the mechanical properties of the tested composites and their interactions with different toothpastes should be interpreted with caution, as they may not directly reflect in vivo behavior or long-term clinical performance. Although a correlation between surface roughness and gloss has been reported in the literature [60], gloss measurements could not be performed in this study due to

ARTICLE IN PRESS

ARTICLE IN PRESS

the unavailability of a glossmeter. Furthermore, insofar as the VITA Easyshade is regarded—based on current scientific evidence—as a reliable instrument for color assessment and is extensively employed in the literature, ambient illumination and background conditions during the pre- and post-measurement sessions were rigorously standardized. Ensuring a stable, low-variability lighting environment, preferably under neutral illumination; using a neutral background (e.g., grey tones); minimizing the presence of surrounding colors (such as colored gloves, drapes, lipstick, etc.) to reduce reflections toward the sensor or the tooth; keeping the specimen surface clean and dry with a standardized drying procedure; positioning the probe tip in full contact with the surface—ideally perpendicular to the specimen—while applying a standardized pressure; and predefining and documenting the measurement protocol in a clear and consistent manner are all essential prerequisites for obtaining standardized and reproducible color measurements.

CONCLUSION

In this study, the effects of three whitening toothpastes with distinct formulations on the mechanical and optical properties of two novel one-shade dental composite resins were critically evaluated. When examined in terms of the most favourable pairing, brushing VITTRA composites with Opalescence toothpaste yielded the best colour stability and the smallest reduction in surface hardness. Although the VITTRA & Opalescence combination was associated with the greatest increase in surface roughness, the lowest roughness increase was likewise observed within a VITTRA subgroup. In one-shade composites, surface roughness tends to increase and microhardness values tend to decrease following toothbrushing simulation. Following toothbrushing with whitening toothpastes, clinically acceptable color stability cannot be consistently guaranteed across all one-shade composite systems. However, the magnitude and nature of these alterations may vary depending both on the intrinsic characteristics of the dental material itself and on the specific compositional features of the toothpaste used during brushing. Additionally, it is important to exercise clinical caution when recommending whitening toothpastes to patients. Therefore, when one-shade composites are used in areas with high esthetic demands, clinicians should exercise particular caution when recommending whitening toothpastes and should monitor tooth shade and surface texture at periodic recalls, thereby helping to preserve the esthetic and mechanical performance of the restorative materials.

ARTICLE IN PRESS

DECLARATIONS

Ethics approval and consent to participate

Since our manuscript does not involve any human- or animal-derived material, obtaining ethics committee approval was not required.

Consent for publication

Not applicable

ARTICLE IN PRESS

Availability of data and materials

The data associated with this article may be made available upon reasonable request and with appropriate justification.

Competing interests

Not applicable

Funding

Not applicable

Authors' contributions

M.S., O.C., M.K.Ü. and, O.Y. wrote the main manuscript text. The laboratory phase of the study was conducted by M.S., O.C. and, M.K.U. M.K.U. and, O.Y. contributed to the statistical analysis and interpretation of the results. M.S. and, O.C. prepared figures and tables. All authors reviewed the manuscript.

Acknowledgements

ARTICLE IN PRESS

REFERENCES

1. Nyvad B, Takahashi N. Integrated hypothesis of dental caries and periodontal diseases. *J Oral Microbiol*. 2020;12(1):1710953.
2. Iba B, Adamu VE. Tooth brushing: An effective oral hygiene measure. *Orapuh Journal*. 2021;2(2):1-7.
3. Ucuncu MK, Guven K, Yazicioglu O. Investigation of the constituents of commercially available toothpastes. *Int J Dent Hyg*. 2024;22(4):913-932.
4. Alleh AO, Iyevhobu KO. Effect of Toothpaste on Oral Microbial Flora. *International Journal of Research Publications*. 2022;100(1).
5. Vijayakumar P, Mahesh J, Prasad SV, Indrapriyadharshni G, Karthikeyan R, Revanth MP. Reason for Preference of Toothpaste among Buyers–A Cross-

ARTICLE IN PRESS

sectional Survey. *Journal of Primary Care Dentistry and Oral Health*. 2024;5(2):56-61.

6. Pérez-Portilla T, Ortíz-Benitez DL, Lucas-Rincón SE, et al. The importance of toothbrushing and oral hygiene in maintaining oral health. Published online 2023.

7. Lyne A, Ashley P, Saget S, Porto Costa M, Underwood B, Duane B. Combining evidence-based healthcare with environmental sustainability: using the toothbrush as a model. *Br Dent J*. 2020;229(5):303-309.

8. Kakodkar G, Lavania A, De Ataide IDN. An in vitro SEM study on the effect of bleaching gel enriched with NovaMin on whitening of teeth and dentinal tubule occlusion. *J Clin Diagn Res*. 2013;7(12):3032.

9. Matos J, Almeida H, Ascenso RMT, Novo C, Freire M. Development of a sustainable toothbrush with the aid of additive manufacturing technologies. In: *Challenges for Technology Innovation: An Agenda for the Future*. CRC Press; 2017:61-64.

10. Arantes DC, Limeira FIR, Yamauti M, Moreira AN, Abreu LG, Magalhães CS. Comparison of clinical efficacy of pro-argin and NovaMin toothpastes in relieving dentin hypersensitivity: A systematic review and meta-analysis. *Oral Health Prev Dent*. 2019;17(5):403-412.

11. Alnuwaiser MK, Alqudairy MS, Alshehri FA, et al. Recent advancement in anterior teeth esthetics: A narrative review. *Bulletin of Pioneering Researches of Medical and Clinical Science*. 2024;4(2-2024):13-21.

12. Yeşilova KN, Turkun S. Tek Renkli Monokromatik Kompozit Rezinlerle İlgili Bir Durum Değerlendirmesi. *Selcuk Dental Journal*. 2025;12(2):323-330.

13. Keleş ZH, Aydın S, Çağdaş E, Şişmanoğlu S. Effect of Charcoal-containing Mouthwashes on Color Stability of Nanofill and Microhybrid Composites. *Essentials of Dentistry*. 2024;3(3):81-86.

14. Pinto P, Carvalho A, Silva FS, Gomes JR, Carvalho O, Madeira S. Comparative toothbrush abrasion resistance and surface analysis of different dental restorative materials. *Tribol Int*. 2022;175:107799.

15. Köroğlu A, Şahin O, Küçükekenci AS, Dede DÖ, Yıldırım H, Yilmaz B. Influences of toothbrushing and different toothpastes on the surface roughness and color stability of interim prosthodontic materials. *Materials*. 2022;15(17):5831.

16. Eliezer R, Devendra C, Ravi N, Tangutoori T, Yesh S. Omnichroma: one composite to rule them all. *Int J Med Sci*. 2020;7(06):6-8.

17. Üçüncü MK, Yazicioglu O, Güven K. Marketlerden satın alınabilecek çocuk diş macunlarını oluşturan bileşenlerin analizi. *Akdeniz Tıp Dergisi*. 2024;10(3):470-484.

18. Yilmaz MN, Gul P, Unal M, Turgut G. Effects of whitening toothpastes on the esthetic properties and surface roughness of a composite resin. *J Oral Sci*. 2021;63(4):320-325.

ARTICLE IN PRESS

ARTICLE IN PRESS

19. Manis RB, Da Silva TM, Franco TT, Dantas DCB, Franco LT, Huhtala MFRL. Influence of whitening toothpaste on color, roughness, and microhardness of composite resins. *European J Gen Dent*. 2017;6(02):92-98.

20. Khamverdi Z, Kasraie S, Rezaei-Soufi L, Jebeli S. Comparison of the effects of two whitening toothpastes on microhardness of the enamel and a microhybride composite resin: an in vitro study. *J Dent (Tehran)*. 2010;7(3):139.

21. Biçer Z, Yaman BC, Çeliksöz Ö, Tepe H. Evaluation of the color stability of resin composites subjected to artificial aging procedures: immersion in different solutions, brushing, and thermal cycling. *Odontology*. 2025;113(3):1003-1020.

22. Üçüncü MY, Üçüncü MK. Comparison of the mechanical properties of various microhybrid dental composites. *Eurasian Dental Research*. 2023;1(3):58-64.

23. Azmy E, Al-Kholy MRZ, Fattouh M, Kenawi LMM, Helal MA. Impact of nanoparticles additions on the strength of dental composite resin. *Int J Biomater*. 2022;2022(1):1165431.

24. Sharma G, Wu W, Dalal EN. The CIEDE2000 color-difference formula: Implementation notes, supplementary test data, and mathematical observations. *Color Res Appl*. 2005;30(1):21-30. doi:10.1002/col.20070

25. Demarco FF, Collares K, Coelho-de-Souza FH, et al. Anterior composite restorations: A systematic review on long-term survival and reasons for failure. *Dental materials*. 2015;31(10):1214-1224.

26. Ucuncu MK, Celiksoz O, Sen E, Yucel YY, Dinc B. Investigation of the degree of monomer conversion in dental composites through various methods: an in vitro study. *Applied Sciences*. 2024;14(11):4406.

27. Ferracane JL. Resin-based composite performance: are there some things we can’t predict? *Dental materials*. 2013;29(1):51-58.

28. Meseli S, Alkan E, Korkut B, Kanar O, Tagtekin D. Abrasiveness and bleaching level of toothpastes on composite resins: a quantitative analysis using a novel brushing simulator. *Applied Sciences*. 2025;15(5):2314.

29. Parry J, Smith AJ, Sufi F, Rees GD. Effect of simulator design on in vitro profilometric assessment of toothpaste abrasivity. *Wear*. 2012;278:34-40.

30. White DJ, Schneiderman E, Colón E, St John S. A profilometry-based dentifrice abrasion Method for V8 brushing machines. Part I: Introduction to RDA-PE. *J Clin Dent*. 2015;26(1):1-6.

31. Nassar HM, Lippert F, Eckert GJ, Hara AT. Impact of toothbrushing frequency and toothpaste fluoride/abrasivity levels on incipient artificial caries lesion abrasion. *J Dent*. 2018;76:89-92.

32. Arsecularatne JA, Chung NR, Hoffman M. An in vitro study of the wear behaviour of dental composites. *Biosurf Biotribol*. 2016;2(3):102-113.

ARTICLE IN PRESS

ARTICLE IN PRESS

33. Korkut B, Hacıalı C. Color stability of flowable composites in different viscosities. *Clinical and Experimental Health Sciences*. 2020;10(4):454-461.

34. Lainović T, Blažić L, Kukuruzović D, Vilotić M, Ivanišević A, Kakaš D. Effect of diamond paste finishing on surface topography and roughness of dental nanohybrid composites–AFM analysis. *Procedia Eng*. 2014;69:945-951.

35. Karatas O, Gul P, Gündoğdu M, Iskenderoglu DT. An evaluation of surface roughness after staining of different composite resins using atomic force microscopy and a profilometer. *Microsc Res Tech*. 2020;83(10):1251-1259.

36. Çağlayan E, Sasany R, Bolat BN, et al. Optical properties of repaired additively manufactured resin composites and zirconia and subtractively manufactured ceramics: a comparative study of composite resins. *BMC Oral Health*. 2025;25(1):144. doi:10.1186/s12903-025-05538-w

37. Miotti LL, Santos IS, Nicoloso GF, Pozzobon RT, Susin AH, Durand LB. The use of resin composite layering technique to mask discolored background: a CIELAB/CIEDE2000 analysis. *Oper Dent*. 2017;42(2):165-174.

38. Higueras M, Collado-Montero FJ, Medina VJ. Colorimetric evaluation of 3D printing polymers exposed to accelerated aging for Cultural Heritage applications. *Color Res Appl*. 2023;48(3):283-295.

39. Miletic V, Marjanovic J, Veljovic DN, Stasic JN, Petrovic V. Color stability of bulk-fill and universal composite restorations with dissimilar dentin replacement materials. *Journal of Esthetic and Restorative Dentistry*. 2019;31(5):520-528.

40. Boronkay G, Jámbor-Benczúr E, Máthé Á. Colour stability of the flowers of some rose varieties measured in CIEDE2000. *Horticultural Science*. 2009;36(2):61.

41. Polychronakis N, Lagouvardos P, Polyzois G, Sykaras N, Zoidis P. Color changes of polyetheretherketone (PEEK) and polyoxymethelene (POM) denture resins on single and combined staining/cleansing action by CIELab and CIEDE2000 formulas. *J Prosthodont Res*. 2020;64(2):159-166.

42. Xu BT, Zhang B, Kang Y, Wang YN, Li Q. Applicability of CIELAB/CIEDE2000 formula in visual color assessments of metal ceramic restorations. *J Dent*. 2012;40:e3-e9.

43. Paravina RD, Pérez MM, Ghinea R. Acceptability and perceptibility thresholds in dentistry: A comprehensive review of clinical and research applications. *Journal of Esthetic and Restorative Dentistry*. 2019;31(2):103-112.

44. Paravina RD, Ghinea R, Herrera LJ, et al. Color difference thresholds in dentistry. *Journal of Esthetic and Restorative Dentistry*. 2015;27:S1-S9.

45. De Oliveira DCRS, Ayres APA, Rocha MG, et al. Effect of different in vitro aging methods on color stability of a dental resin-based composite using CIELAB and CIEDE 2000 color-difference formulas. *Journal of Esthetic and Restorative Dentistry*. 2015;27(5):322-330.

ARTICLE IN PRESS

ARTICLE IN PRESS

46. Pant DR, Farup I. Riemannian formulation of the CIEDE2000 color difference formula. In: *Color and Imaging Conference*. Vol 18. Society of Imaging Science and Technology; 2010:103-108.

47. Aydınoğlu A, Yoruç ABH. Effects of silane-modified fillers on properties of dental composite resin. *Materials Science and Engineering: C*. 2017;79:382-389.

48. Rodríguez HA, Kriven WM, Casanova H. Development of mechanical properties in dental resin composite: Effect of filler size and filler aggregation state. *Materials Science and Engineering: C*. 2019;101:274-282.

49. Tapety C, Carneiro YKP, Chagas YM, Souza LC, Souza N de O, Valadas LAR. Degree of conversion and mechanical properties of a commercial composite with an advanced polymerization system. *Acta Odontológica Latinoamericana*. 2023;36(2):112-119.

50. Sideridou I, Tserki V, Papanastasiou G. Study of water sorption, solubility and modulus of elasticity of light-cured dimethacrylate-based dental resins. *Biomaterials*. 2003;24(4):655-665.

51. Barszczewska-Rybarek IM. Structure–property relationships in dimethacrylate networks based on Bis-GMA, UDMA and TEGDMA. *Dental Materials*. 2009;25(9):1082-1089.

52. Amaya-Pajares SP, Koi K, Watanabe H, da Costa JB, Ferracane JL. Development and maintenance of surface gloss of dental composites after polishing and brushing: Review of the literature. *Journal of Esthetic and Restorative Dentistry*. 2022;34(1):15-41.

53. Hu X, Marquis PM, Shortall AC. Influence of filler loading on the two-body wear of a dental composite. *J Oral Rehabil*. 2003;30(7):729-737.

54. Bollenl CML, Lambrechts P, Quirynen M. Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: a review of the literature. *Dental materials*. 1997;13(4):258-269.

55. Khedr A, Barakat IF, Abdul Rahim R. Influence of Abrasive and Non-Abrasive Toothpaste on The Surface Properties, Stain Removal and Stainability of The Teeth Enamel (An in Vitro Study). *Al-Azhar Journal of Dental Science*. 2024;27(2):279-288.

56. da Silva TM, Dantas DCB, Franco TT, Franco LT, Huhtala MFRL. Surface degradation of composite resins under staining and brushing challenges. *J Dent Sci*. 2019;14(1):87-92.

57. Geduk ŞE, Sağlam G. The effect of whitening toothpastes on the surface properties and color stability of different ceramic materials. *BMC Oral Health*. 2024;24(1):1305.

58. SARIALIOGLU GüNGöR A, EROL B, DÖNMEZ N. Üç Farklı Tek Renkli Rezin Kompozitlerin Mekanik Özelliklerinin Değerlendirilmesi. *Selcuk Dental Journal*. 2023;10(4).

ARTICLE IN PRESS

59. Saeidnia S, Manayi A, Abdollahi M. From in vitro experiments to in vivo and clinical studies; pros and cons. *Curr Drug Discov Technol*. 2015;12(4):218-224.

60. Heintze Sd, Forjanic M, Ohmiti K, Rousson V. Surface deterioration of dental materials after simulated toothbrushing in relation to brushing time and load. *Dental Materials*. 2010;26(4):306-319.

TABLES

Table 1. The constituent information of all materials presented.

Name of brand	Model	Composition
Charisma	ONE Topaz (one-shade)	Barium Aluminium Boro Fluor Silicate Glass, TCD-Urethaneacrylate, Silica, UDMA, TEGDMA, Polymer, Titanium Dioxid, Fluorescent Pigments, Metallic Oxide Pigments, Organic Pigments, Aminobenzoicacidester, BHT, Camphorquinone. Charisma Topaz contains approximately 59% filler by volume, its filler particle size is 5 nm – 5 µm.
FGM	VITTRA APS Unique (one-shade)	Active ingredients: mixture of methacrylate monomers, photoinitiator composition (APS), co-initiators, stabilizers and silane. Inactive ingredients: boron–aluminum–silicate glass. Filler rate: 72-80/52-60 (wt/vol)
Opalescence	Whitening	Glycerin, Aqua, Silica, Sorbitol, Xylitol, Aroma, Poloxamer 407, Sodium Lauryl Sulfate, Carbomer, Sodium Benzoate, Sodium Fluoride, Sodium Hydroxide, Sucralose, Xanthan Gum, CI 42090 (Brilliant Blue FCF) , CI 19140 (tartrazine)
Signal	White Now Glossy Shine	Hydrogenated Starch Hydrolysate, Aqua, Hydrated Silica, Sodium Lauryl Sulfate, PEG-32, Aroma, Cellulose Gum, Sodium Fluoride, Sodium Saccharin, PVM/MA Copolymer, Calcium Aluminum Borosilicate, Trisodium Phosphate, Glycerin, Sodium Lauryl Sulfate, Lecithin, Tin Oxide, Caprylyl Glycol, Limonene, CI 74160, CI 77891.
Colgate	Optic White Expert	Glycerin, Propylene Glycol, Calcium Pyrophosphate, PEG/PPG-116/66 Copolymer, PVP, PEG-12, Tetrasodium Pyrophosphate, Sodium Lauryl Sulfate, Silica, Aroma, Sodium Monofluorophosphate, Sodium Saccharin, Phosphoric Acid, Hydrogen Peroxide, BHT, Limonene.
Control		Water

***Abbreviations:** [**UDMA**: Urethane Dimethacrylate, **TEGDMA**: Triethylene Glycol Dimethacrylate, **BHT**: Butylated Hydroxytoluene, **APS**: Advanced Polymerization System, **CI**: Color Index, **PEG-32**: Polyethylene Glycol 32, **PVM/MA:** Poly(vinyl methyl ether/maleic anhydride), **PEG/PPG-116/66:** Poly(ethylene glycol)/Poly(propylene glycol) 116/66, **PVP:** Polyvinylpyrrolidone, **nm:** nanometer, **µm:** micrometer, **wt/vol:** weight/volume*

ARTICLE IN PRESS

ARTICLE IN PRESS

Table 2. Surface roughness values before and after brushing

	Composite	Toothpaste	Mean (Ra)	Std. Deviation
Before brushing	ONE (O)	Opalescence (OW)	0.118	0.015
		Signal (OS)	0.135	0.020
		Colgate (OC)	0.119	0.024
		Control (OCO)	0.119	0.040
	VITTRA (V)	Opalescence (VW)	0.063	0.011
		Signal (VS)	0.067	0.017
		Colgate (VC)	0.064	0.011
		Control (VCO)	0.063	0.012
After brushing	ONE (O)	Opalescence (OW)	0.126	0.015
		Signal (OS)	0.189	0.048
		Colgate (OC)	0.155	0.048
		Control (OCO)	0.126	0.043
	VITTRA (V)	Opalescence (VW)	0.119	0.021
		Signal (VS)	0.116	0.021
		Colgate (VC)	0.106	0.026
		Control (VCO)	0.074	0.024

ARTICLE IN PRESS

Table 3. Pairwise comparisons in terms of surface roughness

Composite	Toothpaste	Mean differences + std.
ONE (O)	Opalescence (OW)	0.007 ± 0.008 ^{Ac}
	Signal (OS)	0.054 ± 0.040 ^{B}
	Colgate (OC)	0.050 ± 0.034 ^{AB}
	Control (OCO)	0.006 ± 0.040 ^{Ac}
VITTRA (V)	Opalescence (VW)	0.056 ± 0.017 ^{Bd}
	Signal (VS)	0.049 ± 0.021 ^{AB}
	Colgate (VC)	0.041 ± 0.027 ^{AB}

ARTICLE IN PRESS

	Control (VCO)	0.010 ± 0.022 ^{A}

Two-way ANOVA with Tukey HSD *$p<0.05$*

Statistically significant differences arising from comparisons among different toothpastes within each composite were denoted using different uppercase superscript letters. Statistically significant differences arising from comparisons between the two composites were denoted using different lowercase superscript letters.

Table 4. Microhardness values before and after brushing

	Composite	Toothpaste	Mean (VHN)	Std. Deviation
Before brushing	ONE (O)	Opalescence (OW)	61.48	2.30
		Signal (OS)	60.36	1.76
		Colgate (OC)	58.59	2.87
		Control (OCO)	58.52	2.03
	VITTRA (V)	Opalescence (VW)	56.48	2.71
		Signal (VS)	57.97	2.12
		Colgate (VC)	57.80	2.13
		Control (VCO)	56.31	2.68
After brushing	ONE (O)	Opalescence (OW)	51.35	4.86
		Signal (OS)	56.12	5.03
		Colgate (OC)	55.04	2.76
		Control (OCO)	54.94	2.79
	VITTRA (V)	Opalescence (VW)	55.17	4.94
		Signal (VS)	54.61	4.17
		Colgate (VC)	52.31	3.36
		Control (VCO)	49.94	1.51

ARTICLE IN PRESS

Table 5. Pairwise comparisons in terms of Vickers Microhardness

Composite	**Toothpaste**	**Mean differences ± std (VHN)**
ONE (O)	Opalescence (OW)	-10.12 ± 3.24 ^{Ac}
	Signal (OS)	-4.24 ± 6.33 ^{B}
	Colgate (OC)	-3.55 ± 3.40 ^{B}
	Control (OCO)	-3.58 ± 2.36 ^{B}
VITTRA (V)	Opalescence (VW)	-1.30 ± 5.70 ^{Ad}
	Signal (VS)	-3.35 ± 4.83 ^{A}

ARTICLE IN PRESS

	Colgate (VC)	-5.48 ± 4.27 ^{A}
	Control (VCO)	-6.37 ± 2.97 ^{A}

Two-way ANOVA with Tukey HSD *$p<0.05$*
Statistically significant differences arising from comparisons among different toothpastes within each composite were denoted using different uppercase superscript letters. Statistically significant differences arising from comparisons between the two composites were denoted using different lowercase superscript letters.

Table 6: Two-way ANOVA analysis of the effects of composite resin material and toothpaste type on colorr stability, surface roughness and microhardness

		Sum sq	**df**	**F**	**Effect Sizes (η²)**	***p***
color stability	C(composite type)	2.423	1	3.57	0.040	*0.064*
	C(toothpaste type)	6.430	3	3.16	0.106	***0.03***
	C(composite type) * C(toothpaste type)	13.726	5	6.74	0.227	***0.005***
	Residual	38.03	56		0.627	
surface roughness	C(composite type)	0.001541	1	1.867	0.032	*0.177*
	C(toothpaste type)	0.017861	3	7.218	0.278	***0.0003***
	C(composite type) * C(toothpaste type)	0.008543	3	3.452	0.156	***0.022***
	Residual	0.046186	56			
microhardness	C(composite type)	24.788	1	1.315	0.023	*0.25*
	C(toothpaste type)	30.909	3	0.547	0.028	*0.652*
	C(composite type) * C(toothpaste type)	335.263	3	5.929	0.241	***0.001***
	Residual	1055.503	56			

ARTICLE IN PRESS

Table 7: Comparison of the color stability of two different dental composites subjected to brushing with different toothpastes

ΔE_{00}	**ONE**	**VITTRA**
OPALESCENCE	2.36 ± 0.85^{Aa}	1.76 ± 0.63^{Aa}
SIGNAL	2.56 ± 1.08^{Aa}	2.19 ± 0.42^{Aa}
COLGATE	2.41 ± 0.53^{Aa}	2.44 ± 0.42^{Aa}
CONTROL	2.75 ± 0.37^{Aa}	3.32 ± 0.60^{Bb}

Two-way ANOVA with multiple comparisons (Tukey HSD) *$p<0.05$*
Different uppercase letters indicate statistically significant differences between groups in the same row. Different lowercase letters indicate statistically significant differences between groups in the same column.

ARTICLE IN PRESS

FIGURE

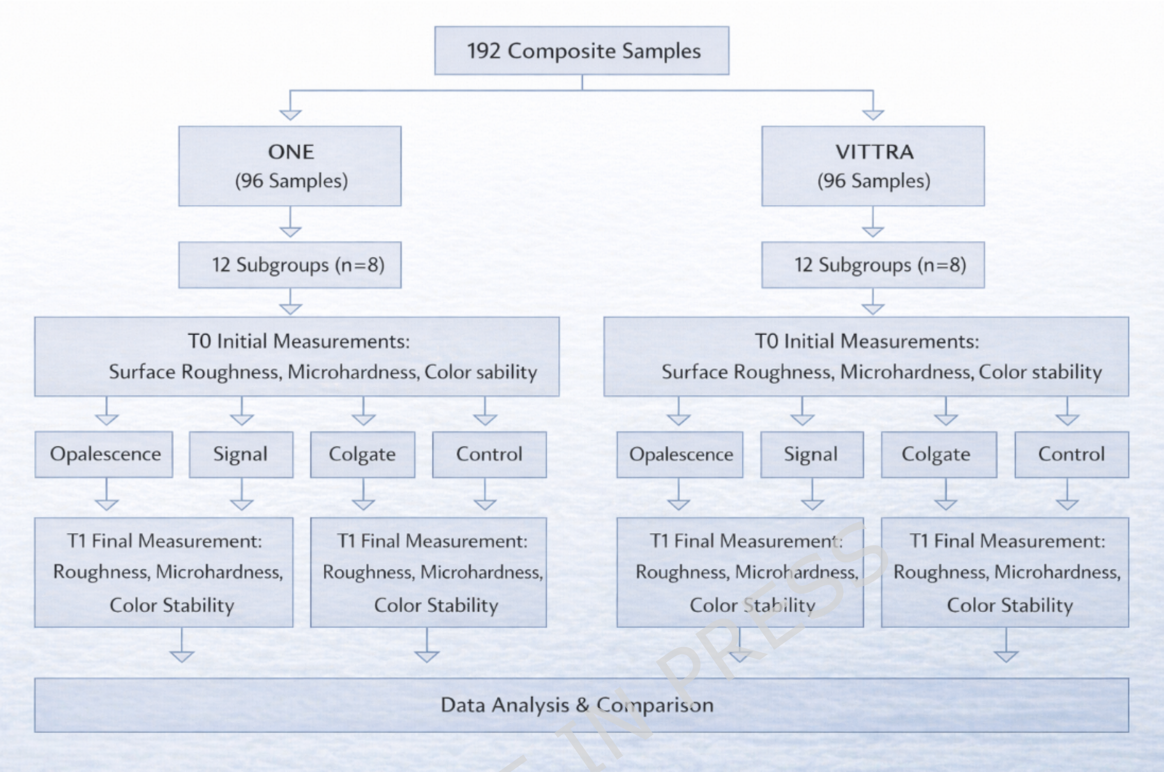

Figure 1. Flow diagram illustrating the procedures to be performed

ARTICLE IN PRESS