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Original Article

Determining Artificial Ageing Methods and Evaluating Properties of Universal Composite Resin: A Review and Proposed Protocol.

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ABSTRACT

Objective: *Our aim is to propose a method for determining a more convenient artificial ageing (AA) protocol, which may be used to compare the optical / mechanical properties of universal to conventional composite.* **Methods:** *Artificial ageing (AA) protocol was adapted from (Alp et al., 2018) for thermocycling and (Jasin et al., 2018) for static immersion, which arbitrarily simulating 6 and 7 months of oral environment, respectively. In phase 1, Filtek Z350XT® will be used to be immersed in coffee solution (test group) with distilled (control group) water. Colour changes will be measured and calculated using the CIEDE2000 formula and data will be*

statistically analysed to determine a meeting time point of colour change occurrence. Measurement will be repeated thrice to validate the new static emersion method. In phase 2, this new static immersion AA protocol will be used to compare Omnichroma's ® colour stability with translucency to the Filtek Z350XT ®. In phase 3, SEM EDS will be used to measure quantitative and qualitatively the occurrence of subsurface properties changes in Omnichroma® and Filtek Z350XT ®. Finally, the discs' microhardness will be evaluated. **Significance:** *Presently, it is very difficult to precisely simulate the oral environment. In comparison to thermocycling, static immersion is a more convenient AA method. Establishing an evidence-based protocol may well potentially serve as a guide for researchers to assuredly select the more simplified AA protocol for future dental material development and clinical usage scenarios.*

Keywords: *Coffee, Colour Stability, Microhardness, Omnichroma, SEM-EDS, Static Immersion, Surface Roughness, Thermocycling, Translucency Parameter, Universal Composite*

INTRODUCTION

Artificial ageing via solution immersion and measurement of colour change using a spectrophotometer is a common method for evaluating colour stability of composite resins. Two methods are frequently used in the literature, namely static immersion and thermocycling staining challenge (as shown in table 1).

Table 1: Methods commonly used; Static Immersion and Thermocycling Stain Challenge

Static Immersion	Thermocycling
(Ertaş et al., 2006) (Ergücü et al., 2008) (Ibrahim et al., 2009) (Nasim et al., 2010) (Ardu et al., 2010) (Catelan et al., 2011) Kara et al., 2013) ((Hipólito et al., 2013) (Yew et al., 2013) (Kohli & Bhatia, 2015) (Tekçe et al., 2015) (Jasin et al., 2018) (Raja et al., 2019) (Roslan et al., 2019) (Suhaimi et al., 2019) (Ghani et al., 2021) (Al-Haj Ali et al., 2021) (Vyas et al., 2022)	(Souza et al., 2010) (Ren et al., 2012) (Morresi et al., 2014) (Alp et al., 2018) (Subaşı et al., 2018) (Papageorgiou-Kyrana et al., 2018) (Vasiliu et al., 2020) (Sulaiman et al., 2021)

Thermocycling artificial ageing method is considered to be arbitrarily simulating the oral environment (Gale & Darvell, 1999). Nevertheless, the vast majority of researchers still favour the static immersion method probably due to its relative convenience. Thermocycling is one method to analyze the effect of repeated thermal stress on the integrity of resin composite materials. However, authors rarely give a full account of the temperature and time settings. The varied number of cycles, temperatures, dwell time and intervals between baths hinder in the comparison of results across studies (Gale & Darvell, 1999). It has been reported that thermocycling for 10,000 cycles at 5°C to 55°C may approximately correspond to 1 year of clinical function (Morresi et al., 2014). A suggestion was made that an arbitrarily, 10,000 cycles of thermocycling is equivalent to a stretch of 1 year simulation in vivo (Gale & Darvell, 1999). Therefore, this formula was accepted by many researchers to conduct their studies. For instance, 5,000 cycles of thermocycling was used by Alp et al. to

correspond to 6 months of aging of CAD-CAM monolithic glass-ceramic (Alp et al., 2018) and 10,000 cycles of new-generation translucent zirconia in coffee solution was used by Subasi et al. to simulate a 1 year in vivo (Subaşı et al., 2018), 1000 cycles were used by Ren et al., (Ren et al., 2012) and 20000 cycles of thermocycling were adopted by Sulaiman et al. to further age the cements to a 2-year clinical function(Sulaiman et al., 2021a). Meanwhile, as previously mentioned, vast majority of researchers are in approval of using another method which is static immersion(Ghani et al., 2021; Jasin et al., 2018; Raja et al., 2019; Roslan et al., 2019). In a study by Ergucu et al., following the baseline measurements, the nanocomposite specimens were immersed in coffee for 7 days and after just one week, all materials exhibited significant colour changes compared to baseline(Ergücü et al., 2008). In the first part of our proposed experimental study, we will adapt the method used by Jasin et al., for their static immersion and by Alp et al., for their thermocycling protocol. This allows the determination of a meeting time point of colour change occurrence, within those two artificial ageing methods. To our knowledge, there have been no reports elucidating their comparison and the time of occurrence of their colour change and there is no standardization of methods used by researchers in terms of static immersion protocol. We are attempting to determine the equivalence of both methods in order to choose static immersion based on scientific evidence. This is because static immersion is more convenient, requires no machine, requires no close monitoring of the machine, is less expensive, less technically sensitive, and has less uncertainty than thermocycling, especially if the machine fails. We will then proceed to the second study phase, where only static immersion protocol will be used.

Table 1: Adaptation of protocol used in Phase 1 of this experimental study.

	Static Immersion	**Thermocycling**	**Static Immersion and Thermocycling**
	(Jasin et al., 2018)	(Alp et al., 2018)	Our proposed research (Phase 1)
Material used	Microhybrid composite resin	CAD CAM monolithic glass-ceramic	Filtek Z350XT (nanofilled composite)
Staining Solution	Coffee, cocoa,	Coffee	Coffee
Methods	Static immersion into staining solutions, colour measurement were made at $7^{th,}$ 14^{th}, 21^{st}, and 28^{th} Day All samples were immersed into their staining solutions of 5 ml for 20 minutes daily, for 28 days to simulate a clinical exposure	Themocycling, 5000 cycles. The baths were set at 5°C and 55°C; each bath transfer time was 10 seconds, and the dwell time was 30 seconds	Both methods of static immersion and thermocycling used by Jasin eta al., and Alp et al., to determine the equivalence of both methods and choose one on the basis of convenience
Results	Visual and spectrophotometer to measure colour changes (not specified)	A spectrophotometer was used to measure colour and the CIEDE 2000 formula was used to calculate colour changes (ΔE).	We will be measuring colour with a spectrophotometer and calculating colour and translucency changes with the CIEDE2000 formula.

Dental resin composites have undergone remarkable refinements in recent years, owing primarily to filler size reduction, which has improved the polishability and wear resistance. In particular, nanotechnology has been a significant propelling force behind many of the recent advancements in advanced resin composite properties (Ferracane, 2011) Nevertheless, despite the ongoing improvements in resin composites, there is still concern regarding the chemical and enzymatic degradation of these materials in the oral environment (Carvalho

et al., 2012). In addition to the deterioration caused by saliva, the consumption of foods and beverages also has the potential to soften and increase the surface roughness of resin composites. However, this potential is dependent on the type of resin composite that is used.

Clinicians prefer composite materials and restoration techniques that allow for the adoption of simplified clinical protocols in order to reduce chair time and technique sensitivity. Because colour selection can be difficult and reliant on environmental and operator factors, a trend to simplify shade selection has resulted in the creation of so-called universal composites (de Abreu et al., 2021). “Universal resin composite" was previously used to describe the composite's ability to restore teeth of any size and location (anterior and posterior). This term, however, is currently being used to describe the ease in tooth colours selection because the material has a higher “blending in effect” due to its unique filler particles. The universal composite carries less shades, whether single or group-shaded materials. As a result, creating practically unnoticeable restorations with fewer colours is made easier. (Perdigão et al., 2021)

Omnichroma (Tokuyama Dental America, Inc.) was the first genuine universal composite resin. It is a single-shaded material that is indicated to match all 16 Vita Classical shades (VITA North America, Linda, CA). It also includes an opaque shade known as Blocker to use as dentin shade in translucent areas such as class IV restorations. The composition of Omnichroma consists of a blend of an identical 200 nm spherical particles of silicon dioxide (SiO2) and zirconium dioxide (ZrO2), 75%–80% filler by weight (Perdigão et al., 2021). Resin composites containing the spherical filler with an average particle size of 260 nm have structural color, and thus, may show excellent color adaptation (Pereira Sanchez et al., 2019). However, it has been reported that the color stability of a few of the current universal composite resins is not ideal (Sulaiman et al., 2021). Although the color adaptation coefficient of Omnichroma has been shown to be improved compared to composite resins of previous generations (Pereira Sanchez et al., 2019) another study reported opposite results. Single-shaded materials such as Omnichroma may be unpredictable because they undergo a decrease in value and increase in chroma (Iyer et al., 2021). The information provided is of great importance to fulfill partially the lack of data regarding the use of this new generation of universal composites in anterior restorations. Further studies should be performed to evaluate these composites in other parameters, such as TP, optical scattering, and color stability (de Abreu et al., 2021). Thus, the results of present study showed that the effect of both color and translucency shift is dependent on materials and that only Omnichroma exhibited a large blending effect compared to the others resin composites analyzed (Durand et al., 2021). There are conflicting and limited reports on the colour and translucency stability of single-shaded universal composites. Recent universal composite resin discoloration data is scarce and is based solely on manufacturer-supplied data, so in vitro investigations are required for predictable clinical outcome. Abreu 2021 suggests more research into these composites in this parameter.

Color stability is defined as the capacity of any dental material to keep its original colour. According to previous research, there are two major groups of factors that contribute to the colour stability of composite resin. The factors are classified as either intrinsic or extrinsic. In terms of intrinsic factors, they influence the composition of the composite resin, such as the matrix or methacrylate of the composite itself, the type of filler content, and the photoinitiator of the material. Extrinsic factors such as the type of food colourant used, the surface finish, and the effect of bleaching can all have an impact on the colour stability of the composite material (Ashok & Jayalakshmi, 2017). Several common monomers found in the matrix of resin composite material have hydrophilic properties, often causing discoloration overtime due to water uptake. However, another study showed that microfiller presented the most significant discoloration; however, it is primarily composed of UDMA which is a less hydrophilic monomer compared to TEDGMA and BIS-GMA (Alp et al., 2018). When absorbed, water acts as a plasticizer which breaks the interface of the silane and filler particles. This degradation and swelling of the matrix induce microcracks at the surface allowing staining particles to penetrate internally. Although coffee is known to cause staining in resin composite materials, the degree of staining would be determined by the penetration of the staining particles and hydrophilic properties of the monomer (Souza et al., 2010). Extrinsic factors include plaque accumulation and surface stains caused by various types of food colourants, as well as the roughness of the surface finish. According to Ashok et al., coffee contains a higher

concentration of chromatogen than tea and cola. Adding sugar and milk powder to tea or coffee may also increase the colour change of dental materials. In addition to this, a rough surface finish may attract and accumulate plaque, as well as absorb more water and food colourant. Removing the outermost resin layer by polishing procedures is essential to achieving a stain resistant, more esthetically stable surface (Ergücü et al., 2008). Smooth surface finish, on the other hand, has been shown to improve colour stability (Ashok & Jayalakshmi, 2017). Coffee was used as staining element in this proposed study because it is usually used in in-vitro study ((Alp et al., 2018); (Ibrahim et al., 2009); (Jasin et al., 2018); (Suhaimi et al., 2019)). Coffee has been shown in previous studies to cause significant staining. The coffee's temperature may have acted as an ageing factor, hastening the degree of intrinsic staining. Temperature can also affect the discoloration of a composite material due to its effect on the photoinitiators (Souza et al., 2010).

When evaluating aesthetics, one of the most important optical properties to consider is translucency. The translucency of resin-based composites is caused by the interaction of the refractive indices of the filler particles and the resin matrix. The greater the discrepancies, the greater the translucency. It is known that the content and type of inorganic filler particles, as well as the type and fraction of organic matrix, can all affect the material's translucency. According to (Salgado et al., 2018), the translucency of the material affects the colour stability of dental composite resin. The colour stability of the high-translucent shade was the lowest across all resin-based composite brands.

At present, the market provides a diverse selection of aesthetically pleasing conventional resin-based composites. Despite that, colour mismatch and composite discoloration continue to be two of the most common reasons for restoration replacement, so colour matching and long-term colour stability are two of the most significant challenges these newer formulations attempt to address. In addition to that, to achieve natural-looking restorations based on optical properties and tooth anatomy, conventional composite systems necessitate mastery of multiple layers of dentin, body, and enamel composites. As a consequence of this fact, the majority of the restorations that were attempted were unsuccessful due to the difficulty of technically mastering the multilayering technique.

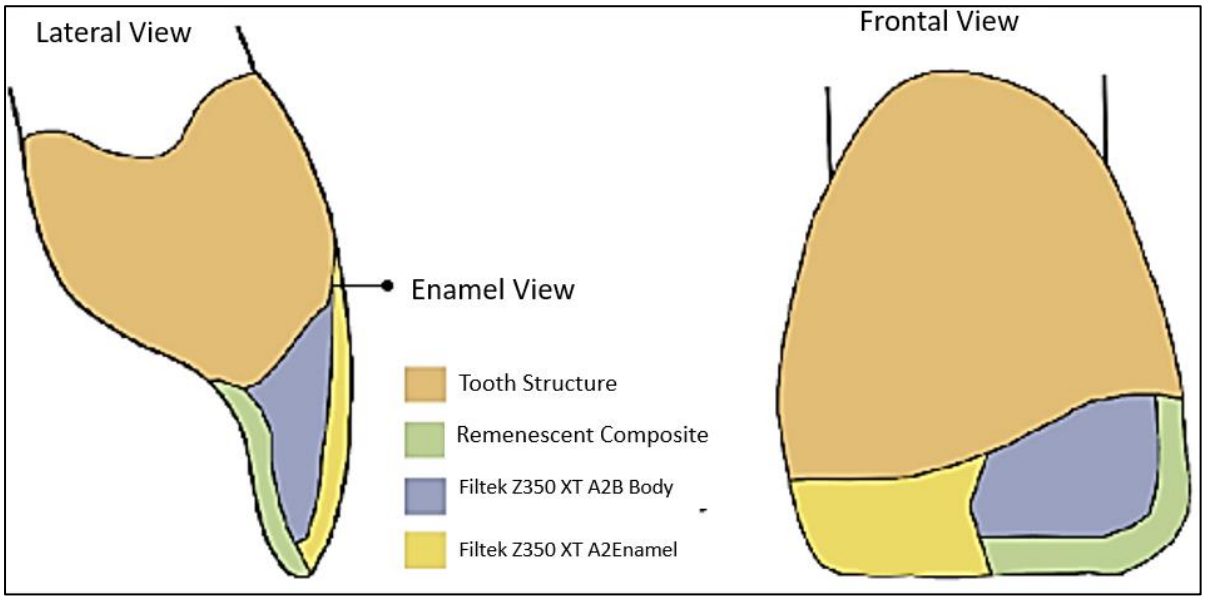

Figure 1: Color maps show layers of composite resin planned to be used in the restoration. The frontal view shows a cutaway portion and the surface layer (da Silva, 2017)

In this proposed study, we chose the conventional nanocomposite Filtek Z350 XT as the benchmarking composite resin to be evaluated in the first phase and later compared with Omnichroma (Universal composite resin). According to the dental supplier, this is due to the Filtek Z 350 XT being widely available in the Malaysian market and widely used by general practitioners. It is also because many studies have shown that Filtek Z 350XT has good colour stability when compared to other composites tested. Furthermore, it shares same type of filler particles with Omnichroma, namely nanofiller particles, which contribute to its good polishability properties, making it comparable to the universal composite. In this study, the Filtek Z350XT with the enamel shade A2B was chosen, as shown in the Figure above because this enamel layer is intended to be placed exposed to the oral environment.

Color stability of esthetic dental materials should be acceptable for a successful restoration. Degree of color change is perceived visually or measured with photometric instruments. The degree of accuracy with which the color measurements were made varied by the instrument and the type of material surface. In order to measure the colour changes, it has been shown that dental spectrophotometers provide the highest overall accuracy and precision among different shade selection methods as compared to visual evaluation (Tabatabaian F, 2021). Previously, the use of CIELab formula was vastly used by researchers to measure the color changes in dental materials. However, the preferred formula for determining colour differences for clinical interpretation perceived by the human eye, CIEDE2000 is better at reflecting colour differences than the CIELab (Gómez-Polo et al., 2016). According to the CIEDE2000 total color difference formula,18 color change may be calculated using the L* C* h* (lightness, chroma, and hue) color parameters. Although many studies have previously used CIELab color difference formula to evaluate color stability, the CIEDE2000 color difference is a recommended formula that corrects for small color differences and improves visual-color difference perception (Fairchild, 2019). The translucency may also be evaluated before and after immersion by using a CIE color difference of the material over black and white backgrounds. Furthermore, to better predict the acceptability of clinical use, the data of color alteration recorded on a spectrophotometer, will be converted to the National Bureau of Standards (NBS) system. According to this system, ΔE values can be described by the subsequent equation: NBS unit = ΔE × 0.92 (Vyas et al., 2022). This will be measured and converted at the end of the artificial aging procedures.

ΔE NBS Criteria	
0–0.5 Trace:	Remarkably slight alteration
0.5–1.5 Slight:	Slight alteration
1.5–3 Noticeable:	Observable alteration
3–6 Appreciable:	Apparent alteration
6–12 Much:	Remarkably apparent alteration
12 or more Very much:	Alteration to other color
NBS unit = ΔE × 0.92	

National Bureau of Standards (NBS) system of expressing color differences.

Adopted from (Vyas et al., 2022)

Scanning electron microscopy (SEM) with energy-dispersive X-ray spectroscopy (EDS) mapping analysis will be performed to further investigate the specimen's subsurface and the effect of the staining solution on both conventional and universal composite resin because changes will affect. Because the subsurface properties of the composite resin tested have rarely been reported, SEM-EDS analysis will be used to investigate how the material's microproperties and elemental changes after being immersed in coffee staining solution. SEM images of the morphology and cross-sectional surface of a polished composite specimen will be evaluated qualitatively and quantitatively analysed using a Scanning electron microscopy (SEM) with energy-dispersive X-ray

spectroscopy (EDS) mapping analysis. This study's SEM-EDS analysis protocol was adapted from a journal article by (Han et al., 2021). EDS is a standard method for identifying and quantifying elemental compositions in a very small sample of material (even a few cubic micrometres). The atoms on the surface are excited by the electron beam in a properly equipped SEM, emitting specific wavelengths of X-rays that are characteristic of the atomic structure of the elements. These X-ray emissions can be analysed using an energy dispersive detector (a solid-state device that discriminates between X-ray energies). The composition of the atoms on the specimen surface is determined by assigning appropriate elements. This technique is known as energy dispersive X-ray spectroscopy (EDS), and it is useful for determining the composition of a specimen's surface. (Ebnesajjad, 2014). EDS can be used for qualitative (the type of elements) as well as quantitative (the percentage of the concentration of each element of the sample) analysis. In most SEMs, dedicated software enables auto-identification of the peaks and calculation of the atomic percentage of each element that is detected. One more advantage of the EDS technique is that it is a non-destructive characterization technique, which requires little or no sample preparation.

Proposed Methodology

1. This is an Experimental In Vitro study that will be carried out in the Faculty of Dentistry UiTM, Sungai Buloh Campus. In accordance with its objectives, this study is divided into three phases;
    a. Prior to all measurements, samples will be prepared in the laboratory using customised stainless-steel moulds.
    b. In the first phase, staining immersion methods such as thermocycling ageing and static immersion will be used testing only a conventional composite resin. However, only static immersion method will be used in the second phase.
    c. Laboratory data of color and translucency stability of universal and conventional composite resin will be statistically analyzed.
    d. Scanning electron microscopy (SEM) with energy-dispersive X-ray spectroscopy (EDS) mapping analysis of the specimens will be evaluated quantitatively and qualitatively to further investigate the morphology of subsurface properties of the specimens after immersion in coffee staining solution.
    e. Finally, because the process is quite destructive to the specimen, a microhardness test will be performed using Vicker's test machine at the very end of the experiment.
2. Composite Selection

Table 2: Resin composite materials with corresponding composition and selected shades (Pereira Sanchez et al., 2019)

	Material	Composition	Manufacturer	Shade
Test Group	Omnichroma® (Tokuyama, Tokyo, Japan)	UDMA, TEGDMA, uniform sized supra-nano spherical filler (260 nm spherical SiO2-ZrO2), composite filler (SiO2-ZrO2)	Tokuyama Dental, Tokyo, Japan	Universal
Control Group	Filtek Z350 XT® (3M, ESPE)	Bis-GMA, UDMA, TEGDMA, Bis-EMA, PEGDMA, BHT, non-agglomerated/non-aggregated 20 nm silica filler and 4 to 11 nm zirconia filler, aggregated zirconia/silica cluster	3 M ESPE, St Paul, MN	A2B

Phase 1:

a. In the first phase of the experiment, Filtek Z350XT was chosen as the benchmark of the conventional composite resin.
b. Sample size determination
c. Sample Preparation

The mould will be overfilled with the composite material and covered with cling film (100µm in thickness) followed by a Perspex plate on either side using finger pressure for 1 minute to remove excess composite and voids.

The plates will then be clamped with 4 finger screws before they were subjected to curing using 4 Halogen lights (110 V 150 W, light spectrum 400-500 nm) Solidilite V® (Shofu Inc. Kyoto, Japan) for 3 minutes on each side for both groups.

The discs will be subjected to flash and irregularities removal using 1500 grit abrasive paper on a flat table, manually. Following this, they will be polished using Shofu Supersnap Rainbow kit® and Shofu One Gloss® (Shofu Inc. Kyoto, Japan), for 30 seconds. (Chen et al., 2020) The prepared discs had a dimension of 10.0±0.1-mm-diameter and 1.0±0.1-mm-depth measured using an electronic digital calliper.

d. Surface Roughness Baseline Measurements

In the first phase of the study, surface roughness measurement will only be taken at baseline, for standardization purposes. The surface roughness of specimens was measured using a profilometer accurate to 0.01 µm. Three measurements of surface roughness will be performed for each specimen, and mean Ra was used for the statistical analysis. The measurements will be performed in the central area of each specimen at intervals of 2.0 mm, and the average reading was designated as the intact Ra value for that specimen. Resolution was 0.01 µm, interval (cut-off length) was 0.8 mm, transverse length of 2.4 mm, and 0.5 mm/second stylus speed under load of 0.75 mN). The profilometer was calibrated using a standard precision reference specimen after each measurement. For each specimen, the arithmetic means of the results of measurements taken in three different directions will be determined.

e. Staining solution preparation

Using test tubes, all the composite discs will be immersed in 20 ml distilled water at (37±2) °C and will be kept in a dark oven for 24 hours to allow for complete equilibration in water (ISO 2015)

The black instant coffee (Nescafe® Classic, Malaysia) solutions will be made by combining 8 grams of each powder with 100 ml of boiling distilled water, according to the manufacturer's standardisation instructions. The mixture will be manually stirred until homogenous and set aside to cool down to room temperature (37±2) °C.

In the static Immersion group, to simulate clinical exposure, samples will be immersed in 5 ml coffee staining solutions (test group) and in distilled water (control group) for 20 minutes every day for 28 days using static immersion staining methods (Jasin et al., 2018).

In thermocycling group, after baseline measurements, discs will be aged in a coffee solution (test group) and distilled water (control group) using 5000 thermocycles (Zecttron Automatic Thermocyclic Dipping Machine ATDM®). The baths were set to 5°C and 55°C, with a 10 second transfer time and a 30 second dwell

time (Alp et al., 2018). Distilled water and staining solutions will be changed daily, and the containers will be covered to prevent the solutions from evaporating.

f. Colour Measurement.

Prior to colour measurement all the sample disc from static immersion group and thermocycle group will then be cleaned using an ultrasonic cleaner (Jeio Tech® Co. Ltd., South Korea) for 10 minutes. Samples will be rinsed with distilled water and blotted dry with tissue paper for colour measurement.

Spectrophotometer (Konica Minolta) CM-5® calibration will be set for the colour measurement. All samples will be placed on the measuring head of spectrophotometer (one at a time) and covered with a black cover. The 3 variables C*H*L* measurements will be performed 3 times. The mean values will be automatically calculated and recorded by the spectrophotometer.

The spectrophotometer will be calibrated for reflectance mode with a 6 mm aperture plate according to manufacturer's instructions prior to measurements. Specimens were removed from each aging solution and ultrasonically cleaned for 10 minutes in deionized water. Measurements were performed three times and averaged for each specimen with CIE standard illuminant D65 over a white background. A small amount of saturated sucrose solution was placed between the specimen and the backing to increase optical continuity during measurement, providing a more clinically relevant measurement. The CIE L (lightness), C (chroma) and H (hue) values were determined over a white background at baseline and after exposure using the CIE D65 illuminant and the CIE 2° standard colorimetric observer. The inverse of colour stability, that is, the colour changes due to exposure, for each specimen was calculated according to the CIEDE2000 formula (ΔE00).

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

where ΔL, ΔC, and ΔH are the changes in CIE L', C', and H', respectively, due to the exposure and the remaining terms within this formula are previously well described.

Colour changes measurement was repeated for;

a. Static Immersion group for every 7th, 14th, 21st, 28th days. (Jasin et al., 2018)

b. Thermocycling group: every 1000 cycle until completed 5000 cycles. (Alp et al., 2018)

		T1(Baseline)	T2(Day 7)	T3(Day 14)	T4(Day 21)	T5(Day 28)	
Static Immersion	Coffee						
	Distilled water						
		Tt1(baseline)	Tt2(1000 cycles)	Tt3(2000 cycles)	Tt4(3000 cycles)	Tt5(4000 cycles)	Tt6(5000 cycles)
Thermocycling staining challenge	Coffee						
	Distilled water						

Statistical data analysis

From the data obtained correlation analysis will be statistically used to measure the correlation between colour changes of the materials in both staining methods; thermocycling and static immersion.To reconfirm the equivalence analysis of the colour changes in the two methods, the procedure will be repeated again twice. Finally, a convenient method of static immersion will be used in the second phase of the study.

Phase 2:

a. Using only the Static Immersion method, the above procedure will be repeated for both conventional composite resin Filtek Z350XT® and Omnichroma® Universal Composite resin.
b. Sample size determination

Prior to static immersion in coffee solution, baseline measurements of colour and microhardness will be taken, via a spectrophotometer and Vicker's test machine, respectively.

c. Sample measurement of colour, will be obtained; Data for static immersion will be collected on the days determined in phase 1.
d. Colour changes (ΔE) and translucency parameter (TP_{00}) changes will be calculated by using CIEDE2000 formula via Microsoft® Office Excel 2016. CIEDE2000 (1:1:1) colour difference formula will also be used to calculate the translucency parameter (TP_{00}):
e. Data Collection and Statistical Analysis

Overall colour changes, translucency parameter changes for Omnichroma® (Tokuyama Dental, Tokyo, Japan) Universal Composites and Filtek® Z350XT (3M, ESPE) will be calculated using Microsoft® Office Excel 2016 and compared at different experimental stages using Two Way Repeated Measure ANOVA between group analysis on IBM® SPSS 28 Software. For each optical and mechanical property changes, Omnichroma® (Tokuyama Dental, Tokyo, Japan) Universal Composites and Filtek® Z350XT (3M, ESPE) at different experimental stages will be compared using Two Way Repeated Measure ANOVA (within group analysis of experimental effect) followed by pairwise comparison and confidence interval adjustment at a significance level of 0.05. Lastly, to determine the relationship between the amount of color alteration recorded on a spectrophotometer to the clinical environment, data will be converted to the National Bureau of Standards (NBS) system. According to this system, ΔE values can be described by the subsequent equation: NBS unit = $\Delta E \times 0.92$.

Phase 3:

Scanning electron microscopy (SEM) with energy-dispersive X-ray spectroscopy (EDS) mapping analysis and microhardness testing will be carried out on samples after statically immersed in coffee staining solution.

After immersion is complete, 3 samples per group will be randomly selected to undergo SEM EDS analysis. Selected composite resin samples will be dried at room temperature and cleaned with acetone, and will be subjected to polishing procedure after embedding in acrylic resin block. The surface morphology of the composite resin will be observed and analysed using SEM-EDS (Mira3®, Tescan, Czechia).(Han et al., 2021)

Because of the destructive nature of the procedure towards the specimen disc, the measurement of microhardness for both composite types will be taken at baseline and after completion of staining challenge. The microhardness of each composite type will be measured on the test specimen surface by a Vicker's microhardness tester (Shanghai Taiming Optical Instrument Co., Ltd., Shanghai, China) with a 50-gf load (0.490 N) for 15 s. The Vickers microhardness was calculated from the expression HV = 0.1891 F/d 2, where HV is the Vickers hardness, F is the test load (N), and d is the mean value of the indentation's diagonal lengths (μm). (Han et al., 2021)

Limitations of Study

1. This is an in vitro, study. It does not simulate the actual clinical scenario of oral environment.
2. This study compares only one single shaded universal composite and one conventional nanohybrid composite, focusing on colour stability and translucency parameters for optical properties and surface roughness and microhardness stability for physical properties.
3. Colour matching is not being investigated.
4. Coffee staining solution was selected in comparison to distilled water to put focus on the elucidation of staining methods and suitability of CIE DE2000 in terms of acceptability through the NBS.

Significance of Study

This research has been proposed and passed DRP on 23 May 2022. Ethics has been approved and exempted on 16 August 2022. The research may give information about:

1. This research may serve as a guide for future researchers to choose the simplified procedure in terms of choice of artificial ageing staining methods.
2. The results obtained from this study may facilitates to the long-term longevity of resin-based composites in terms of dental material development as well as clinical usage scenarios.
3. The result obtained with regards to the color stability of the single shaded universal composite may answer the previous contradicting reports.
4. Surface and subsurface optical and mechanical properties of Universal Composite in relation to a conventional composite after being immersed statically within coffee staining solution.

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