INFLUENCES OF DIFFERENT SOLUTIONS ON THE COLOUR STABILITY AND MICROHARDNESS OF CONTEMPORARY LIGHT CURED COMPOSITES: AN *IN VITRO* STUDY

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Abstract

Despite the accumulation of dental plaque, adsorption of colourant pigment from foods and beverages was found to influence the quality of aesthetic restorations. The study aimed to evaluate the influence of different solutions on colour stability and microhardness of microhybrid and nanofilled composite resins. This project was a laboratory based experimental study. Three types of composite resins were used; microhybrid (Filtek™ Z250), nanohybrid (Filtek™ Z250 XT) and universal nanocomposite (Filtek™ 350 XT). A total of 135 disc-shaped specimens were prepared and were randomly divided into five subgroups (n = 9): artificial saliva, mouthwash, carbonated drink, energy drink and coffee. Samples were immersed in the solutions at time intervals of 24 hours, 7 days, 14 days, 21 days and 28 days. All specimens were assessed for the staining effect using a spectrometer (QE65000) and microhardness evaluation using the Vickers indenter (LECO LM 248AT, St. Joseph, Michigan, USA). Data on spectrometer colour spectrum and surface hardness were subjected to a one-way ANOVA and Tukey HSD test at a significance level of P < 0.05. There was a significant difference in discolouration between all three types of composite resins when immersed in all solutions regardless of the pH values ($P < 0.05$). Vickers hardness test showed statistically significant microhardness among all composite resins on Day 28. The staining medium caused significant discolouration and decreased the hardness of all the materials tested. Microhybrid composite resin reported the lowest colour variations and resistance to hardness test.

Keywords: Composite resins, Colour stability, Staining, Microhardness.

Introduction

Composite resin has become more predictable restorations that can last longer in function as their formulations and clinical procedures have advanced, making them an indispensable choice for both anterior and posterior regions. However, maintenance is still needed with time for discolouration, microleakage, and partial fracture of the restorations, despite the materials' satisfactory results (1). The dental industry has been exposed to a new brand of a nano-based composite resin composed of nanomer and nanocluster fillers through nanotechnology.

The most essential satisfactory factor for the composite resin is not only limited to a correct initial colour match but also maintaining its durability, the acceptance of appearance and a long-term ceaselessness to any exposure conditions (2). Intermittent or continuous exposure to the oral cavity promotes direct interaction between the chemical agents mainly pH constituents found in saliva, foods and beverages with the composite resin's surface (3). Additionally, diverse interactions of pH also contribute to material degradation in terms of surface hardness.

Table 1: Types of composite resins used in the study.

Trade Name	Types	Composition		Average particle siz (um)
		Matrix	Filler	
Filtek™ 250	Microhybrid composite resin	Bis-GMA, TEGDMA and Bis-EMA (6) resins	60% weight of fumed silica/zirconia, non-agglomerated/ non-aggregated nanometer surface-modified silica particles	0.6
Filtek™ Z250 XT	Nanohybrid composite resin	BIS-GMA, UDMA, Bis-EMA, PEGDMA and TEGDMA	63.3% weight of modified zirconia/silica, non-agglomerated/ non-aggregated 20 nanometer surface-modified silica particles	3
Filtek™ Z350 XT	Universal nanocomposite resin	Bis-GMA, TEGDMA and Procrylat resins	68% weight of combination of ytterbium trifluoride filler with a range of particle sizes from 0.1 to 5.0 microns, a non-agglomerated/ non-aggregated surface-modified 20 nm silica filler, a non-agglomerated/ non-aggregated surface modified 20 nm silica filler, and a surface-modified aggregated zirconia/silica cluster filler	0.6 to 10

* BIS-GMA: Bisphenol A diglycidyl ether dimethacrylate; BIS-EMA: Bisphenol A diglycidyl methacrylate ethoxylated; TEGDMA: triethylene glycol dimethacrylate; PEGDMA: Polyethylene dimethacrylate

Table 2: The compositions of components used to prepare artificial saliva.

Component	Concentration (mg/L)	Product No.
Sodium chloride, NaCl	400	S9625
Potassium chloride KCl	400	P3911
Calcium chloride dehydrate, $CaCl_2.2H_2O$	795	C7902
Sodium phosphate dibasic dehydrate, $NaH_2PO_4.H_2O$	1.6	71643
Sodium sulphide hydrate, $Na_2S.9H_2O$	5	13466
Urea	1000	U5378
Potassium thiocyanate, KSCN	300	207799

*Components used in artificial saliva preparation is manufactured by Sigma-Aldrich, MERCK KGAA, Darmstst, Germany

Despite colour stability, composite resin requires outstanding mechanical, as well as physical properties (4) and their usage, has now been extended. The development of new nano-based composite resin generally has superior physical and mechanical properties compared to conventional composites (5). Due to the improvement, composite resins are now being selected as one of the materials to restore a load-bearing surface for posterior teeth with a minimal intervention approach (6). Ideally, it is expected that composite resin exhibits a more stable and water-permeable restorative material. However, a drawback related to the water uptake by polymer networks hindering the success rate of clinical achievements (3).

The lifetime colour stability of the composite resin is as important as its mechanical properties. Discolouration of the composite resin depends on several factors such as the types of composite resin, staining agents, immersion contact time and surface roughness (7). The staining effect of composite resin can be assessed either visually or using the spectrometer or colorimeter. As recommended, instrumental assessment was reported to give a more reliable findings as they eliminate the subjective interpretation inherent by visual colour comparison (8).

Since composite resin is used to restore the missing tooth structure, the strength of its surface to withstand the mastication forces is important. The microhardness test is useful to evaluate the influence of aging, surface reaction and water absorption (9) and it can be measured either using the Vickers or Knoop indenters to determine the surface resistance and localised surface deformation (10). This aspect is important to ensure long-term clinical sustainability efficiency (11).

Thus, the aims of this *in vitro* study were to examine and compare the colour changes and microhardness between the three different types of composite resins; microhybrid composite resin, nanohybrid composite resin and universal nanocomposite resin after exposure to different staining solutions (artificial saliva, mouthwash,

carbonated drink, energy drink and coffee). The null hypotheses for this study were:
1. Acidic pH could influence the colour stability of composite resin.
2. Immersion solutions caused significant microhardness alteration in between the tested composite resins.

Materials and Methods

Sample preparation

Three commercially available composite resins of A2 shade with different types of fillers were used in this study (Table 1). 45 disk-shaped samples sized of ±8 mm (diameter) and ±1.5 mm (thickness) were fabricated using a customised silicone mold for each type of composite resin, rendering a total of 135 samples according to previous published study (12). All specimens were polished with medium, fine and superfine aluminium oxide-impregnated polishing discs (Sof-Lex XT, 3M ESPE, St. Paul. MN, USA) using a slow speed headpiece for 30 seconds to achieve a parallel and flat surface (13).

Immersion protocol

Five immersion solutions were used in the study: artificial saliva, mouthwash, carbonated drink, energy drink and instant coffee. The artificial saliva was prepared according to the composition used in a previous published study (3), as shown in Table 2. All specimens were equally divided and immersed into 5 ml solution according to the five solution groups in airtight containers. To achieve a constant and standardised room temperature (37^{o}C), all samples were stored in the KS 4000 i control shaker incubator (IKA, Staufen, German). The pH value of each solution was measured and recorded before immersion on a weekly basis using a digital 827 pH Lab Meter (Metrohm, Herisau, Switzerland). After seven days of immersion, samples were rinsed with distilled water and blotted dry with absorbance paper. Immersion solutions were freshly replaced every seventh day. Observations were made at time intervals of 24 hours – baseline (D1), Day 7 (D7), Day 14 (D14), Day 21 (D21) and Day 28 (D28). The immersion protocols were adapted similarly to a previous study (14).

Colour assessment

One randomly selected sample from each solution of each type of composite resin was selected and underwent three indentations of colour assessment using the visible and near infrared range of spectrum between 400 nm and 900 nm. The reflectance spectra were obtained using QE65000 Spectrometer (Ocean Optic, Dunedine, Fl, USA). Three different areas were evaluated at different wavelengths and converted into a single mean value. In addition to the standardisation of the colour spectral changes representation, the dual-wavelength ratio between 460 nm and 880 nm reflectance values was calculated (i.e. R460nm/ R880nm). In spectral analysis for remote sensing application, dual-wavelength ratio has shown a capability to decrease the effect of external influences and the spectral distortion due to variations in irradiance, angle, orientation, and shading (15).

Microhardness testing

One randomised selected sample from each type of composite resin and immersion solutions was selected and underwent microhardness testing. A diamond pyramid shaped indenter (LECO LM 248AT, St. Joseph, Michigan, USA) was automatically placed approximately at a distance of 1 mm from each measurement point using a 50 g test load at 15 seconds dwelling time. The average value of five indentations were expressed into Vickers Hardness Number (VHN) in kg/mm^2.

Statistical analysis

All data were recorded and transformed into Microsoft Excel Sheet, tabulated and analysed using a One-way ANOVA (Analysis of Variance) with post-hoc Tukey HSD (Honestly Significant Difference) Test Calculator application (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp) at a designated significance level of $P < 0.05$.

Results

Immersion solutions' pH test

The test groups and their respective pH values are summarised in Table 3. As presented, carbonated drink as found to be the most acidic solution (pH 2.45), followed by energy drink (pH 3.62), mouthwash (pH 5.33), artificial saliva (pH 6.66) and instant coffee (pH 6.76).

Colour reflectance spectroscopy assessment

The outcome of spectroscopy colour reflectance assay (Table 4), the normalisation of the spectra value (Table 5) and the colour changes exhibited by the three types of composite resin are visualised according to the immersion solutions as shown in Figure 1. All composite resins experienced slight gradual colour changes and in consistent with the ANOVA one-way analysis which reported that all immersion groups had significantly changed the colour of all composite resins ($P < 0.05$). The artificial saliva was found to cause a gradual discolouration to all types of composite resin tested. However, the nanotechnology composite resins (nanohybrid composite and universal nanocomposite resins) were found to be more resistant to discolouration when in contact with mouthwash, carbonated drink and energy drink. Although both nanohybrid composite and universal nanocomposite resins were found to maintain their colours, but when they were in contact with instant coffee, both materials obtained a lower reflectance value compared to microhybrid composite resin.

Table 3: Type, pH value and manufacturer of the immersion solutions.

Storage Group	Mean pH	Brand	Manufacturer
AS	6.66	-	Sigma-Aldrich, MERCK KGAA, Darmstst, Germany
MW	5.33	Oral7®	Oral 7 International Pty. Ltd., Balcatta WA, Australia
CD	2.5	Coca-Cola®	Coca-Cola Refreshments Malaysia Sdn. Bhd., Petaling Jaya Selangor, Malaysia
ED	3.6	100PLUS	Fraser & Neave Holdings Bhd., Kuala Lumpur, Malaysia
IC	6.76	NESCAFÉ®	Nestlé (Malaysia) Berhad, Selangor, Malaysia

* AS: artificial saliva; MW: mouthwash; CD: carbonated drink; ED: energy drink; IC: instant coffee

Table 4: Spectroscopy means and standard deviations (SD) of colour reflectance between groups of tested composite resins.

Immersion Group	Composites	D1	D7	D14	D21	D28
AS	MHCR	1.90 (0.09)	1.91 (0.10)	1.88 (0.09)	1.87 (0.10)	1.85 (0.10)
	NHCR	1.93 (0.01)	1.90 (0.02)	1.88 (0.02)	1.78 (0.03)	1.74 (0.04)
	UNCR	1.80 (0.06)	1.59 (0.15)	1.55 (0.16)	1.51 (0.18)	1.49 (0.25)
MW	MHCR	1.77 (0.03)	1.73 (0.03)	1.71 (0.03)	1.70 (0.02)	1.64 (0.10)
	NHCR	2.00 (0.05)	1.96 (0.03)	1.96 (0.00)	1.94 (0.01)	1.87 (0.02)
	UNCR	1.92 (0.17)	1.95 (0.05)	1.96 (0.06)	1.97 (0.05)	2.00 (0.05)
CD	MHCR	1.86 (0.04)	1.83 (0.06)	1.82 (0.04)	1.82 (0.04)	1.74 (0.04)
	NHCR	1.84 (0.03)	1.75 (0.04)	1.70 (0.07)	1.67 (0.06)	1.64 (0.06)
	UNCR	2.00 (0.04)	2.02 (0.01)	2.11 (0.02)	2.11 (0.00)	2.13 (0.08)
ED	MHCR	1.84 (0.04)	1.85 (0.04)	1.85 (0.07)	1.79 (0.08)	1.73 (0.10)
	NHCR	1.99 (0.03)	1.95 (0.01)	1.95 (0.00)	1.93 (0.04)	1.91 (0.03)
	UNCR	2.00 (0.01)	2.01 (0.01)	2.01 (0.01)	2.04 (0.01)	2.22 (0.03)
IC	MHCR	1.77 (0.03)	1.73 (0.06)	1.65 (0.03)	1.59 (0.01)	1.53 (0.03)
	NHCR	1.63 (0.09)	1.60 (0.12)	1.13 (0.26)	1.04 (0.34)	0.68 (0.37)
	UNCR	1.80 (0.06)	1.65 (0.06)	1.62 (0.16)	1.53 (0.25)	1.51 (0.22)

* AS: artificial saliva; MW: mouthwash; CD: carbonated drink; ED: energy drink; IC: instant coffee; MHCR: microhybrid composite resin; NHCR: nanohybrid composite resin; UNCR: universal nanocomposite resin

Table 5: Normalisation spectra value (R460/R880nm) between groups of tested composite resins.

Immersion Group	Composites	D1	D7	D14	D21	D28
AS	MHCR	1.97	1.98	1.95	1.94	1.92
	NHCR	1.94	1.92	1.89	1.75	1.71
	UNCR	1.76	1.48	1.44	1.38	1.32
MW	MHCR	1.79	1.71	1.88	1.88	1.78
	NHCR	2.04	1.99	1.72	1.96	1.52
	UNCR	2.04	1.99	2.03	2.02	1.60
CD	MHCR	1.89	1.69	1.85	1.90	1.67
	NHCR	1.83	1.97	1.65	1.95	0.95
	UNCR	2.03	2.00	2.10	2.03	1.50
ED	MHCR	1.87	1.69	1.85	1.83	1.60
	NHCR	1.98	1.94	1.63	1.96	0.80
	UNCR	2.01	2.01	2.12	2.05	1.36
IC	MHCR	1.79	1.57	1.77	1.80	1.50
	NHCR	1.56	1.86	1.60	1.88	0.42
	UNCR	1.76	2.04	2.08	2.25	1.36

* AS: artificial saliva; MW: mouthwash; CD: carbonated drink; ED: energy drink; IC: instant coffee; MHCR: microhybrid composite resin; NHCR: nanohybrid composite resin; UNCR: universal nanocomposite resin

Table 6: Comparison means and standard deviations (SD) of colour changes between groups of tested composite resins on D1 and D28

Composites	Immersion Period	Staining Solutions				
		AS	MW	CD	ED	IC
MHCR	D1	1.90 (0.09)	1.77 (0.03)	1.86 (0.04)	1.84 (0.04)	1.77 (0.03)
	D28	1.85 (0.10)	1.64 (0.10)	1.74 (0.04)	1.73 (0.10)	1.53 (0.03)
NHCR	D1	1.93 (0.01)	2.00 (0.05)	1.84 (0.03)	1.99 (0.03)	1.63 (0.09)
	D28	1.74 (0.04)	1.87 (0.02)	1.64 (0.06)	1.91 (0.03)	0.68 (0.37)
UNCR	D1	1.80 (0.06)	1.92 (0.17)	2.00 (0.04)	2.00 (0.01)	1.80 (0.06)
	D28	1.49 (0.25)	2.00 (0.05)	2.13 (0.08)	2.22 (0.03)	1.51 (0.22)

* AS: artificial saliva; MW: mouthwash; CD: carbonated drink; ED: energy drink; IC: instant coffee; MHCR: microhybrid composite resin; NHCR: nanohybrid composite resin; UNCR: universal nanocomposite resin

Table 7: Means and standard deviations (SD) of microhardness test for the three types of composite resins tested on D28

Composites	VHN (kg/mm²)				
	AS	MW	CD	ED	IC
MHCR	93.56 (1.77)	92.80 (4.94	91.60 (4.70)	93.86 (3.77)	85.78 (2.08)
NHCR	96.28 (1.87)	78.18 (6.15)	87.32 (1.08)	87.88 (1.99)	60.28 (6.01)
UNCR	76.72 (5.16)	79.54 (1.90)	63.20 (8.89)	77.18 (6.49)	81.68 (3.22)

* AS: artificial saliva; MW: mouthwash; CD: carbonated drink; ED: energy drink; IC: instant coffee; MHCR: microhybrid composite resin; NHCR: nanohybrid composite resin; UNCR: universal nanocomposite resin

The comparison of reflectance values after immersion between D1 and D28 are shown in Table 6 and graphically represented in Figure 2. After D28, microhybrid composite resin was reported to be the most stable material in maintaining its colour compared to nanohybrid composite and universal nanocomposite resins after immersion with the artificial saliva. There was a slight difference in colour changes between the three composite resins when exposed to instant coffee. Meanwhile, the universal nanocomposite resin obtained higher reflectance of the spectra value after immersion in carbonated drink and energy drink compared to the other two materials. Above all, the nanohybrid composite resin was reported to obtain the lowest reflectance value (0.68) and significantly changed its colour after immersion in instant coffee compared to microhybrid composite resin (1.53) and universal nanocomposite resin (1.51).

A statistically significant difference was determined in the reflectance values of all groups of immersion. There were statistically significant effects when exposed to artificial saliva ($P < 0.00032$), mouthwash ($P < 0.00001$), carbonated drink ($P < 0.00001$), energy drink ($P < 0.000226$) and instant coffee ($P = 0.028513$) on colour stability at $P < 0.05$. A statistically significant difference ($P < 0.05$) was also determined between the materials in respect of the reflectance value in artificial saliva with the universal nanocomposite resin was significantly lower ($P < 0.05$) than the microhybrid composite resin and the nanohybrid composite resin.

Meanwhile, immersion with mouthwash and energy drink, reported that the reflectance values of the microhybrid composite resin were significantly lower ($P < 0.05$) than nanohybrid composite and universal nanocomposite resins. However, in the carbonated drink and instant coffee groups, nanohybrid composite resin was significantly lower ($P < 0.05$) than the universal nanocomposite and the microhybrid composite resins.

Vickers microhardness assessment

As summarised in Table 7, the Vickers Hardness Number (VHN) of the three composite resins were ranged from 60.28 to 96.28 kg/mm^2. Microhardness test result was statistically different when applying ANOVA ($P < 0.000053$). By using Tukey's HSD test, it was reported that there was no statistically significant difference between composite resins used when immersed in the artificial saliva group. However, there was a statistically significant difference between the three composite resins when immersed in other solutions whereby the mean score for miccrohybrid composite resin was significantly different than the other two composite resins tested.

Discussion

This study was conducted to focus on the problems encountered by the surface of modern composite resins. conducted after 28 days presents a perceivable spectral colour variation to all three types of composite resin tested.

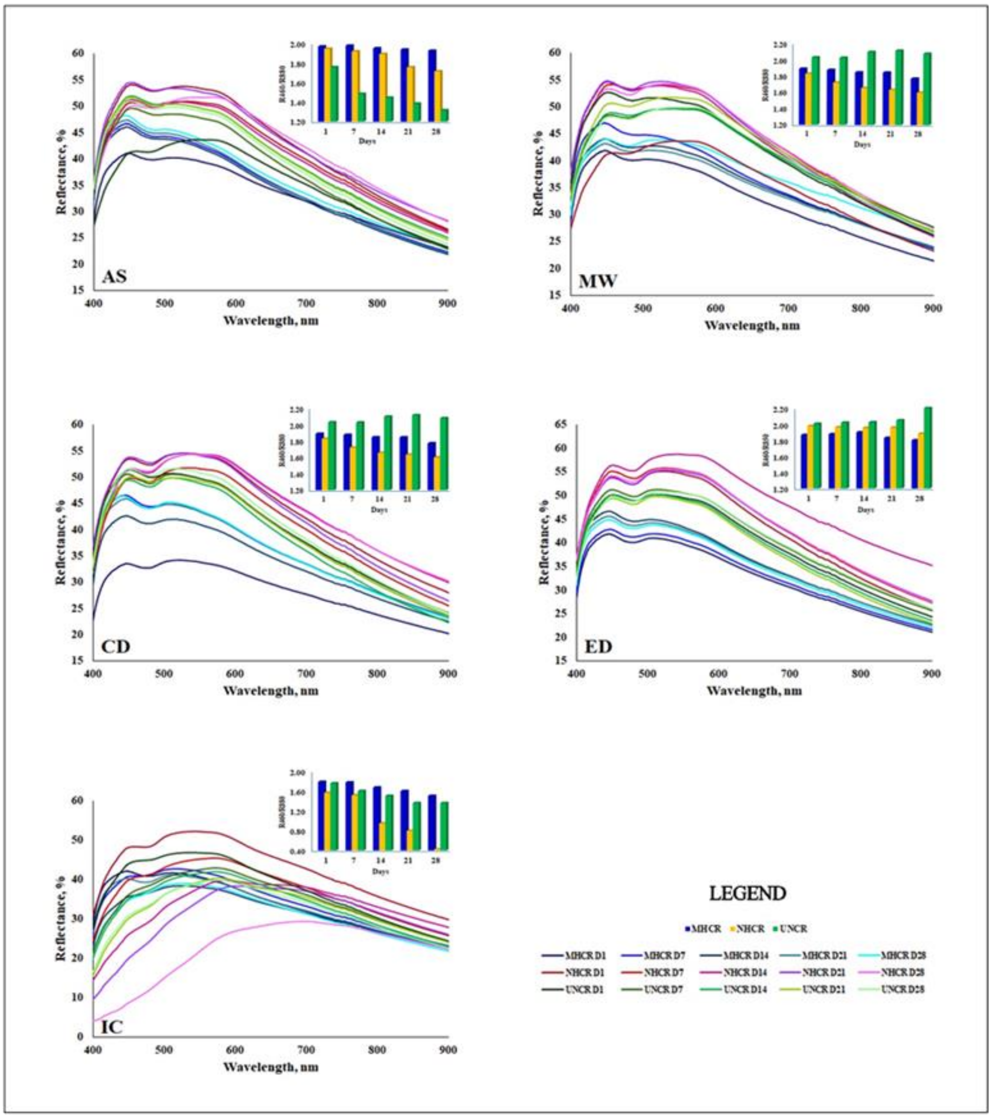

Figure 1: Mean reflectance value of the three composite resins throughout 28 days of experiment based on immersion groups (AS: artificial saliva; MW: mouthwash; CD: carbonated drink; ED: energy drink; IC: instant coffee).

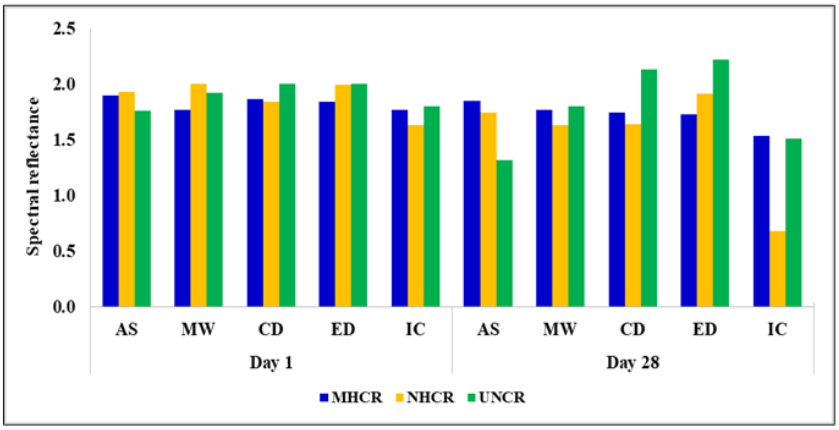

Figure 2: Comparison of mean reflectance values of CRs on D1 and D28 after immersion in different solutions (AS: artificial saliva; MW: mouthwash; CD: carbonated drink; ED: energy drink; IC: instant coffee).

Although instant coffee was not too acidic compared to other solutions, it was reported to significantly affected the colour stability of the three composite resins tested, with microhybrid composite resin to be more resistant than both nanohybrid composite resin and universal nanocomposite resin. These findings could be best described as the low pH medium is not the main affected reason of composite resin to discoloured. It is consistent with the previous study indicated that the changes of colour spectral in composite resin respectively caused by their different composition, filler and matrix contents (14). Regardless of the pH value, coffee was reported to cause the highest staining effect to all composite resins due to the polyphenol and tannin contents, and these findings were in accordance with the previous studies conducted by Malekipour et al. (17) and Pratomo et al. (18). Therefore, the first null hypothesis was rejected.

The colour instability of nanohybrid composite resin is mainly related to their filler composition and structure. The hydrophilic ethoxy group in triethylene glycol dimethacrylate (TEGDMA) increase the water absorption, thus allowing the penetration of water and colour pigments into the matrix or filler matrix interface, hence increasing the susceptibility to oral environmental stains (19). In addition, composite resin with less amount of filler contributes to a greater colour variations and will finally detach from the matrix, resulting in a concavity on the surface that allow the stain penetration (20). In accordance with Tavangar et al. (21) the current study demonstrates that both nano-based composite resins (Filtek^{TM} Z250 XT and Filtek^{TM} Z350 XT) present anamorphous crystalline phase that is affected by a simplified ageing protocol. This factor may clinically affect the relevant mechanical/ physical properties such as fracture toughness, polymer conversion and strength. Additionally, composite resin underwent acidic storage was also reported to cause a higher degree of micromorphological damage. The organic acids contained in food or beverages imposed a detrimental effect of the polymeric resins and composite resin, thus accelerating the degradation process that reduces the lifespan of composite resin (19). Despite the acidic media, phosphoric acid found in the mouthwash may alter the matrix composition by catalysing the ester groups in the dimethacrylate monomers of the composite resin. The hydrolysis of the ester groups forms a carboxylic and acid monomers, which accelerate the polymer network and decrease the internal pH of the matrix (22). The results of the current study is in corroboration with the previous studies which indicated that the hardness and colour stability of nanohybrid composite and universal nanocomposite resins were found to be lower than those of microhybrid composite resin (23). Above all, stain intensity and softening of the resin matrix vary respectively based on the type of the immersion solution, its pH, exposure frequency and permanence contact with the staining agent (24). Therefore, the second hypothesis which anticipated that immersion solution caused significant microhardness alteration in between composite resins tested was accepted.

Conclusion

Observations and results from the present study concluded that all immersion solutions used have affected the colour stability and microhardness of all tested composite resins. Properties alteration of all tested composite resins are material-dependent with a specified immersion medium and highly affected by the composite resin's composition, polymeric matrix and filler loading matrix. The micro-cracks or interfacial gaps between the filler and matrix allow stain penetration and discolouration. The microhybrid composite resin present higher stain-resistant and hardness in comparison with the nanohybrid composite and universal nanocomposite resins, regardless of the immersion pH value and time.

Competing Interests

The authors declare that they have no competing interests.

Ethical Clearance

No ethical clearance is required for the present study.

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