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COMPARISON BETWEEN THE EFFECTIVENESS OF TWO SEALANTS IN PREVENTION OF ENAMEL WHITE SPOT LESIONS - IN VITRO STUDY

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ABSTRACT:

Objectives: *This study was done to compare between the effectiveness of using highly filled and lightly filled sealants in preventing demineralization and resisting acid and abrasion through assessing enamel color change differences and sealant retention after induced challenges.* ***Methods:*** *100 human premolars were collected and allocated into 2 groups. The buccal surfaces of the 1st group were sealed with a 38% filled sealant (Opal®Seal™) and the 2nd group with an 18 % filled one (PRO SEAL®). Both groups were exposed to acidic and abrasive challenges. Enamel color changes were recorded spectrophotometrically at baseline (T0), after sealant application (T1), after first exposure to acidic challenge (T2), and after second exposure to acidic challenge and simulated toothbrushing (T3). Standardized photographs were taken for each tooth under ultraviolet light at baseline and at (T3). Teeth images were analyzed using computer software to estimate sealant coverage and fluorescence loss.* ***Results:*** *The best protection was seen in the Opal seal group after the 1st acidic challenge (p=0.000) when compared with PRO SEAL. Greater sealant material loss (68.78% ± 15.54) and fluorescence loss (82.46% ± 11.04) were seen in the PRO SEAL group (p=0.000). Both sealants changed the original enamel color similarly and exceeded the clinically detectable threshold (ΔE=3.7).* ***Conclusion:*** *Based on the results of this study; increasing the filler content strengthens the sealant, therefore, offering more protection against white spot lesions. Both sealants degrade with increasing the challenges. Both sealants change the initial enamel color similarly.* ***Keywords:*** *White spot lesions, orthodontic sealants, filled sealants, color change, demineralization, spectrophotometer.*

INTRODUCTION

Enamel decalcifications are the most common unfavorable side effects of fixed orthodontic appliances (Enaia, Bock & Ruf, 2011; Proffit, White & Sarver, 2003; Srivastava, Tikku, Khanna & Sachan, 2013). Improper oral hygiene measures and the nature of patients' diet in the presence of orthodontic attachments stimulate local plaque accumulation. The low pH of plaque deters the remineralization process (Øgaard, Larsson, Henriksson, Birkhed & Bishara, 2001). However, this can be reversed if the pH is raised for sufficient time (Arends & Christoffersen,

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1986; Garcia-Godoy & Hicks, 2008). The variety in compliance to good oral hygiene standards among patients leads to a wide range of white spot lesions prevalence (2% - 97%) (Boersma, Van der Veen, Lagerweij, Bokhout & Prahl-Andersen, 2005; Chapman, Roberts, Eckert, Kula & Gonza´lez-Cabezas, 2010). Therefore, the best treatment of white spot lesions is prevention (Meng et al., 2009). Studies showed that an 18% filled resin sealant (PRO SEAL®) provided the most protection against demineralization (Frazier et al., 1996; Ghiz et al., 2009). Although it has the advantage of being colorless, its wear resistance is limited as reported by other studies (Korbmacher-Steiner, Schilling, Huck, Kahl-Nieke & Amling, 2013; Leizer, Weinstein, Borislow & Braitmand, 2010; Meller & Schott, 2018). Recently, a higher filled sealant (38% filled, Opal®Seal™) was introduced with the claim that it has superior properties and withstands abrasion (Opal® Seal™ - Ultradent Products, 2019). However, there is no enough evidence evaluating the superior efficacy of higher filled sealants in preventing demineralization with optimal wear resistance and without affecting the original enamel color.

Spectrophotometry specifies color by recording accurate quantitative measurements. The difference between two colors (ΔE) can be calculated according to a formula ($\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ that was established by the Commission Internationale de l'Eclairage CIE (CIE, 1978). Most studies approved a ΔE value less than 3.7 units to be a threshold for color matching. Color differences exceeding this threshold are clinically visible (Eliades, Kakaboura, Eliades and Bradley, 2001). Spectrophotometers showed high repeatability and accuracy in measurements (Chu, Trushkowsky, and Paravina, 2010; Ragain, 2016). This in-vitro study was conducted to test the null hypothesis that there is no significant difference between using highly filled and lightly filled orthodontic sealants in preventing demineralization or in wear resistance.

MATERIALS AND METHODS

A sample size of 50 teeth per group was the enough required sample to detect an effect size of 27% between the effectiveness of the sealants (Lucchese & Gherlone, 2013; Øgaard, 1989; Willmot, 2008), as statistically significant (Killen, 2005) with 80% power and at a significance level of 0.05. The sample size was calculated using G*Power software (version 3.1.9.2.) (Faul, Erdfelder, Lang and Buchner, 2007). One hundred human premolars extracted for orthodontic purposes were collected from orthodontic patients in the Department of Orthodontics, Faculty of Dentistry, Alexandria University.

Inclusion criteria: 1- sound teeth 2- free from caries, hypoplastic lesions, or visible cracks 3- did not receive any

chemical treatment. All the teeth were cleansed of debris then stored in distilled water at room temperature for a period not more than two weeks before conducting the experiment in order to avoid changes in the microstructural elements of the teeth (Santana, Pereira, Pereira, Neto and Soares, 2008; Secilmis, Dilber, Gokmen, Ozturk and Telatar, 2011).

Specimen preparation:

Teeth were prepared by setting the roots in black acrylic blocks to prevent any light scattering during the spectrophotometric evaluation. Blocks were numbered from 1 to 100 for identification purposes. The teeth were kept in an artificial saliva solution (20 mmol/l $NaHCO_3$, 3 mmol/l NaH_2PO_4 and 1 mmol/l $CaCl_2$) at neutral pH for the duration of the study to simulate the oral environment. The solution was changed daily (Jayarajan et al., 2011; Zaher et al., 2012). A black acrylic mold was fabricated especially for the study with a round opening from one side with the same dimensions of the spectrophotometer tip. The other side of the mold was designed to receive the teeth in the acrylic blocks (Fig. 1). The buccal surfaces were oriented to face the round opening to meet the spectrophotometric tip every time at the exact working area for standardized measurements.

Procedures:

Baseline (T0) reading was taken for all teeth via a spectrophotometer (Vita EasyShade, model # V505h, VITA Zahnfabrik H. Rauter GmbH & Co. KG, Bad Säckingen, Germany) according to the (CIE) L*a*b* system (Fig. 2) (CIE, 1978).

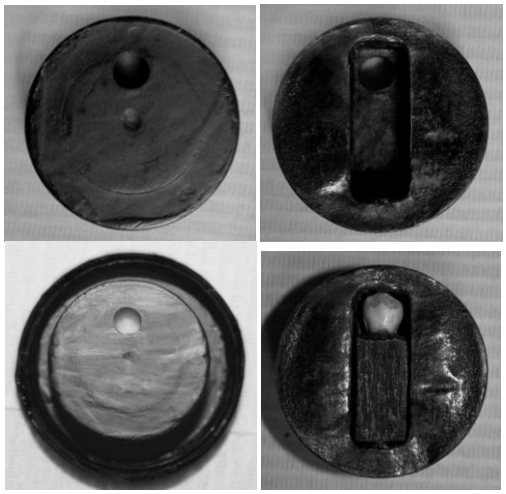

Fig. 1. The mold.

The measurements were taken by a single operator according to the manufacturer's instructions. The steel probe of the spectrophotometer was placed perpendicular and flushed to wet enamel surfaces (Abdel-Raouf et al., 2014).

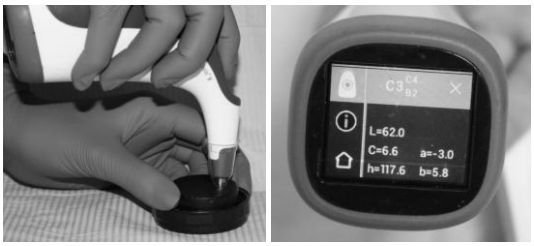

Fig. 2. Enamel color reading via spectrophotometer

Then teeth were randomly allocated into two groups by closed envelope technique. All buccal surfaces of teeth were etched with a 37% phosphoric acid gel etchant (Meta Etchant, Meta Biomed Co. Ltd, Korea) for 30 seconds, rinsed for 20 seconds then dried with oil & moisture-free compressed air. The first group received Opal® Seal™ (Opal Orthodontics by Ultradent, South Jordan, UT, USA) while the second group PRO SEAL® (Reliance Orthodontic Products, Itasca, IL, USA) according to the manufacturers' instructions. (T1) color measurements were taken after sealants application.

Acidic challenge

Both groups were immersed in an artificial caries solution that contains (2.2 mmol/l KH_2PO_4, 2.2 mmol/l $CaCl_2$, 50 mmol/l acetic acid) at pH 4.5 for 96 consecutive hours to induce enamel demineralization (Buren, Staley, Wefel and Qiand, 2008; Jayarajan et al., 2011; Zaher et al., 2012). The solution was changed daily. Enamel colors were recorded by the spectrophotometer (T2).

Abrasive challenge

The acidic challenge was extended for another 96 hours with a second solution then all the teeth were placed in toothbrushing simulator machine. A medium bristled brush heads were centered over the buccal surfaces. Each tooth was subjected to 15,000 strokes with a force of 280g applied to simulate a period of two years of normal patient hygiene (Buren et al., 2008). A 1:3 slurry of non-fluoridated toothpaste and water was applied to the specimens (Buren et al., 2008). After that, the teeth were examined by the spectrophotometer (T3).

Evaluation of color change difference

The differences between the CIE-L*a*b* coordinates (ΔL*, Δa* and Δb*) were calculated for each specimen at all time points. The color difference (ΔE) values were calculated for each tooth according to the formula: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. Intragroup and intergroup comparisons of the color changes (ΔE) between T0, T1, T2 and T3 were done (Yacout, 2016; Abbas, 2018).

Evaluation of wear resistance

Standardized digital photographs of each tooth fluorescing under ultraviolet light were taken to record the sealant coverage and intensity of fluorescence (Buren et al., 2008; Chau, Campbell, Deljavan, Taylor & Buschang, 2015; Tüfekçi et al., 2014). The teeth were photographed at baseline and after challenges at T3 (Fig 3). In order to capture standardized photographs with a 1:1 magnification ratio, each tooth was positioned 31.2 cm away from the camera lens according to the lens manufacturer instructions. An ultraviolet light source was fixed to a special frame and mounted over the camera lens. A Canon DSLR 700D camera (Canon Inc., Ōta, Tokyo, Japan) with a Sigma 105 mm F2.8 DG OS HSM macro lens (Kawasaki, Kanagawa Prefecture, Japan) was used to capture the images (exposure time: 1/4 second; ISO speed: 100; F-stop: f/6.3). Then the photographs were entered in image analysis computer software (ImageJ 1.46r, Wayne Rasband, National Institute of Health, USA). The fluorescing areas were morphometrically traced automatically in square pixels by color and brightness thresholding of the images by the software. Integrated density of the images was recorded by

the software to calculate the corrected total fluorescence CTF for each image through the following formula:

CTF = Integrated Density – (Area of selection x Mean fluorescence of background readings) (Burgess, 2011; Burgess et al., 2010; McCloy et al., 2014).

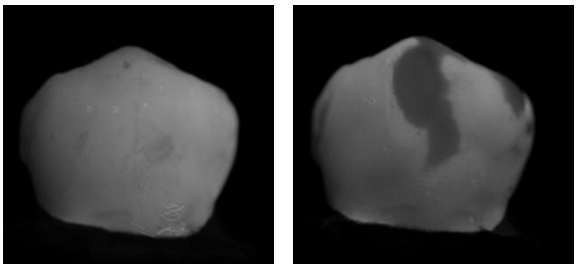

Fig. 3. Fluorescing tooth at T1 and T3

Statistical methodology

Data were entered into the computer using SPSS program (Statistical Package for Social Science, IBM SPSS Statistics, Version 21 Armonk, NY) for statistical analysis (IBM Corp, 2010). Data were entered as numerical or categorical, as appropriate. Kolmogorov-Smirnov test of normality revealed significance in the distribution of most of the variables, so the non-parametric statistics were adopted (Field, 2013). Data were described using minimum, maximum, mean, standard deviation and 95% CI of the mean, (Snecdecor& Cochran, 1991) median and inter-quartile range for not-normally distributed data. Comparisons were carried out between two studied independent not-normally distributed subgroups using Mann-Whitney U test (Mann & Whitney, 1947). Box and Whiskers plot was used accordingly. Comparisons were carried out among related samples by Friedman's test (Friedman, 1937). Pair-wise comparison when Friedman's test was significant was carried out using Dunn-Sidak method (Dunn, 1964). Intragroup points of comparisons were 6 points as follows; ΔE (T0-T1), ΔE (T0-T2), ΔE (T0-T3), ΔE (T1-T2), ΔE (T1-T3), and ΔE (T2-T3). An alpha level was set to 5% with a significance level of 95%, and a beta error accepted up to 20% with a power of study of 80%.

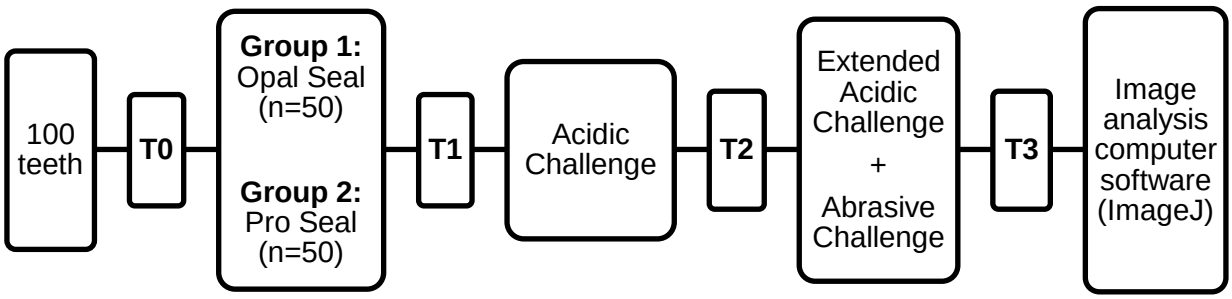

Fig 4. Flow chart showing summarized procedures

RESULTS

I- Effectiveness of the sealants:

A- Intragroup comparisons:

1- Opal Seal

The ΔE in Opal Seal group changed significantly among the 6 points of intragroup comparisons ($X^2_{(Fr)}$ = 76.480) (Fig. 5). There was a significant difference between the following ΔEs assessments:

1- ΔE (T1-T2) and ΔE (T0-T1) where ($Z_{(MW)}$=3.635, *p*= 0.004)
2- ΔE (T1-T2) and ΔE (T2-T3) where ($Z_{(MW)}$=-3,795, *p*=0.002)
3- ΔE (T1-T2) and ΔE (T1-T3) where ($Z_{(MW)}$=-6.628, *p*= 0.000)
4- ΔE (T1-T2) and ΔE (T0-T3) where ($Z_{(MW)}$=-7,109, *p*= 0.000)
5- ΔE (T0-T2) and ΔE (T1-T3) where ($Z_{(MW)}$=5.025, *p*= 0.000)
6- ΔE (T0-T2) and ΔE (T0-T3) where ($Z_{(MW)}$=-5.506, *p*= 0.000)
7- ΔE (T0-T1) and ΔE (T1-T3) where ($Z_{(MW)}$=-2.993, *p*= 0.041)
8- ΔE (T0-T1) and ΔE (T0-T3) where ($Z_{(MW)}$=-3.474, *p*= 0.008)
9- ΔE (T2-T3) and ΔE (T0-T3) where ($Z_{(MW)}$=-3.314, *p*= 0.014)

There was no significant difference between the ΔE of other times (table 1)

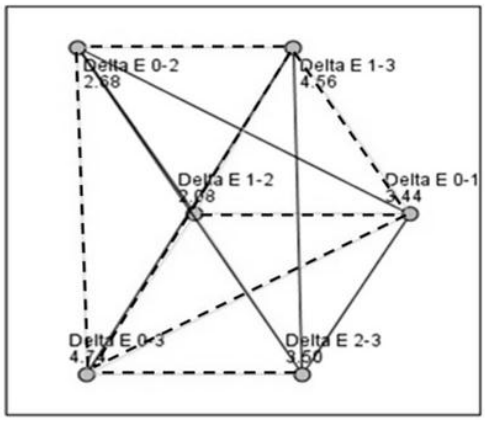

Fig. 5. Opal Seal Dunn-Sidak graph.

Table 1. Dunn-Sidak pairwise comparisons of ΔE in Opal Seal group.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Delta E 1-2-Delta E 0-2	-.600	.374	-1.604	.109	1.000
Delta E 1-2-Delta E 0-1	1.360	.374	3.635	.000	.004
Delta E 1-2-Delta E 2-3	-1.420	.374	-3.795	.000	.002
Delta E 1-2-Delta E 1-3	-2.480	.374	-6.628	.000	.000
Delta E 1-2-Delta E 0-3	-2.660	.374	-7.109	.000	.000
Delta E 0-2-Delta E 0-1	.760	.374	2.031	.042	.634
Delta E 0-2-Delta E 2-3	.820	.374	2.192	.028	.426
Delta E 0-2-Delta E 1-3	1.880	.374	5.025	.000	.000
Delta E 0-2-Delta E 0-3	-2.060	.374	-5.506	.000	.000
Delta E 0-1-Delta E 2-3	-.060	.374	-.160	.873	1.000
Delta E 0-1-Delta E 1-3	-1.120	.374	-2.993	.003	.041
Delta E 0-1-Delta E 0-3	-1.300	.374	-3.474	.001	.008
Delta E 2-3-Delta E 1-3	1.060	.374	2.833	.005	.069
Delta E 2-3-Delta E 0-3	-1.240	.374	-3.314	.001	.014
Delta E 1-3-Delta E 0-3	-.180	.374	-.481	.630	1.000

2- Pro Seal

The ΔE in Pro Seal group changed significantly among the 6 points of intragroup comparisons ($X^2_{(Fr)}$ = 46.823). As illustrated in the Dunn-Sidak pairwaise comparison graph (Fig. 6); there was a significant difference between the following ΔEs assessments:

1- ΔE (T0-T1) and ΔE (T0-T3) where ($Z_{(MW)}$= -4.330, *p*= 0.000)

2- ΔE (T0-T1) and ΔE (T1-T3) where (Z(MW)= -5.987, p= 0.000)

3- ΔE (T2-T3) and ΔE (T0-T3) where (Z(MW)= -3.261, p= 0.017)

3- ΔE (T2-T3) and ΔE (T0-T3) where (Z(MW)= -3.261, p= 0.017)

4- ΔE (T2-T3) and ΔE (T1-T3) where (Z(MW)= 4.918, p= 0.000)

5- ΔE (T1-T2) and ΔE (T1-T3) where (Z(MW)= -3.474, p= 0.008)

6- ΔE (T0-T2) and ΔE (T1-T3) where (Z(MW)= 3.207, p= 0.020)

There was no significant difference between the ΔE of other times (table 2).

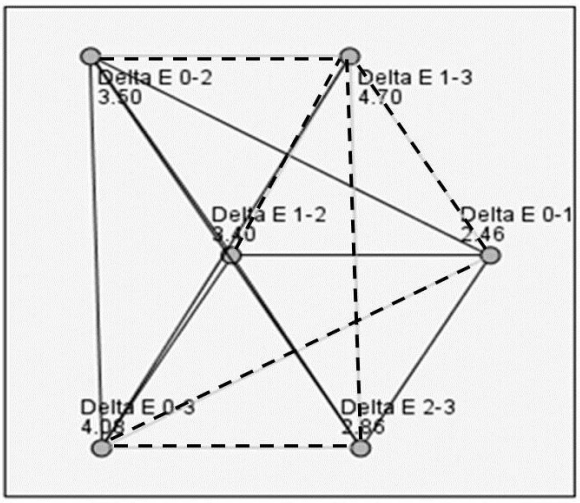

Fig. 6. Pro Seal Dunn-Sidak graph.

Table 2. Dunn-Sidak pairwise comparisons of ΔE in Pro Seal group.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Delta E 0-1-Delta E 2-3	-.400	.374	-1.069	.285	1.000
Delta E 0-1-Delta E 1-2	-.940	.374	-2.512	.012	.180
Delta E 0-1-Delta E 0-2	-1.040	.374	-2.780	.005	.082
Delta E 0-1-Delta E 0-3	-1.620	.374	-4.330	.000	.000
Delta E 0-1-Delta E 1-3	-2.240	.374	-5.987	.000	.000
Delta E 2-3-Delta E 1-2	.540	.374	1.443	.149	1.000
Delta E 2-3-Delta E 0-2	-.640	.374	-1.710	.087	1.000
Delta E 2-3-Delta E 0-3	-1.220	.374	-3.261	.001	.017
Delta E 2-3-Delta E 1-3	1.840	.374	4.918	.000	.000
Delta E 1-2-Delta E 0-2	-.100	.374	-.267	.789	1.000
Delta E 1-2-Delta E 0-3	-.680	.374	-1.817	.069	1.000
Delta E 1-2-Delta E 1-3	-1.300	.374	-3.474	.001	.008
Delta E 0-2-Delta E 0-3	-.580	.374	-1.550	.121	1.000
Delta E 0-2-Delta E 1-3	1.200	.374	3.207	.001	.020
Delta E 0-3-Delta E 1-3	.620	.374	1.657	.098	1.000

B- Intergroup comparisons between Opal Seal and Pro Seal:

1- After sealant application

Table 3. The color difference ΔE between the enamel color at T0 (baseline) and T1 (after initial sealant application) in Opal®Seal™ and PRO SEAL® groups.

Δ E (T0-T1)	Group	n	Min-Max	Mean ± Std. Dev.	95% CI for mean	Median (IQR)	KS test of normality	Test of significance *p* value
	Opal Seal	50	0.444-19.413	6.570± 4.023	5.427-7.713	5.785 (4.139-7.605)	D=0.194, *p*=0.000*	$Z_{(MW)}$=1.055 *p*=0.292 NS
	Pro Seal	50	0.534-17.234	6.104± 4.358	4.865-7.342	5.0947 (2.905-8.443)	D=0.159, *p*=0.003*	

There was no statistically significant difference between the ΔE (T0-T1) of the two sealant groups when compared to each other ($Z_{(MW)}$=1.055, *p*=0.292 NS). Both changed the initial enamel color similarly.

2- After acidic challenge:

Table 4. The color difference ΔE between the T0 and T2 (after acid challenge).

Δ E (T0-T2)	Group	n	Min-Max	Mean ± Std. Dev.	95% CI for mean	Median (IQR)	KS test of normality	Test of significance *p* value
	Opal Seal	50	0.702-15.138	5.943± 3.503	4.947-6.938	4.889 (3.579-7.354)	D=0.187, *p*=0.000*	$Z_{(MW)}$=2.930 *p*=0.003*
	Pro Seal	50	1.453-18.483	8.441± 4.652	7.118 - 9.762	7.293 (4.922 - 11.692)	D=0.133, *p*=0.027*	

There was a statistically significant difference between the ΔE (T0-T2) of the two groups when compared to each other ($Z_{(MW)}$=2.930, *p*=0.003*). The change in color difference was greater in Pro Seal group than Opal Seal group.

Table 5. The color difference ΔE between the enamel color at T1 and T2.

Δ E (T1-T2)	Group	n	Min-Max	Mean ± Std	95% CI for mean	Median (IQR)	KS test of normality	Test of sig. *p* value
	Opal Seal	50	0.403-17.025	4.356± 3.038	3.492-5.219	3.641 (2.413-5.275)	D=0.144, *p*=0.011*	$Z_{(MW)}$=4.784 *p*=0.000*
	Pro Seal	50	2.073-18.560	8.116± 4.584	6.813-9.419	6.616 (4.528-11.604)	D=0.167, *p*=0.001*	

There was a statistically significant difference between the ΔE in the Opal Seal and Pro Seal groups when compared to each other ($Z_{(MW)=}$4.784, *p*=0.000*). Less color change was seen in Opal seal group.

3- After abrasive and extended acidic challenge:

Table 6. The color difference ΔE between the enamel color at T0 and T3.

Δ E (T0-T3)	Group	n	Min-Max	Mean ± Std	95% CI for mean	Median (IQR)	KS test of normality	Test of sig. *p* value
	Opal Seal	50	1.521-25.619	9.808± 5.759	8.171-11.445	9.518 (5.418-12.636)	D=0.084, *p*=0.200 NS	$Z_{(MW)}$=0.152 *p*=0.879 NS
	Pro Seal	50	0.992-18.889	9.317± 4.407	8.065-10.569	9.183 (6.487-11.677)	D=0.076 *p*=0.200 NS	

There was no statistically significant difference between the ΔE of the two groups when compared to each other ($Z_{(MW)}$=0.152, *p*=0.879 NS).

Table 7. The color difference ΔE between the enamel color at T1 and T3.

Δ E (T1-T3)	Group	n	Min-Max	Mean ± Std	95%CI for mean	Median (IQR)	KS test of normality	Test of sig. *p* value
	Opal Seal	50	0.885-29.973	9.641± 6.387	7.8257 - 11.456	7.846 (5.392-11.731)	D=0.155, *p*=0.004*	$Z_{(MW)}$=1.965 *p*=0.049*
	Pro Seal	50	2.025-23.296	10.64± 4.622	9.331-11.958	10.726 (6.541-13.251)	D=0.083, *p*=0.200 NS	

There was a statistically significant difference between the ΔE of the two sealant groups when compared to each other ($Z_{(MW)}$=1.965, *p*=0.049*). Opal Seal group had less color change difference than Pro Seal group.

Table 8. The color difference ΔE between the enamel color at T2 and T3.

Δ E (T2-T3)	Group	n	Min-Max	Mean ± Std	95% CI for mean	Median (IQR)	KS test of normality	Test of sig. *p* value
	Opal Seal	50	0.782-19.541	7.382 ± 4.452	6.117-8.647	6.947 (3.543-9.797)	D=0.100, *p*=0.200 NS	$Z_{(MW)}$=0.076 *p*=0.940 NS
	Pro Seal	50	1.047-16.389	7.150 ± 3.755	6.083-8.217	6.156 (4.868 -9.098)	D=0.143, *p*=0.012*	

There was no significant difference between the two sealant groups in ΔE (T2-T3) ($Z_{(MW)}$=0.076, *p*=0.940 NS).

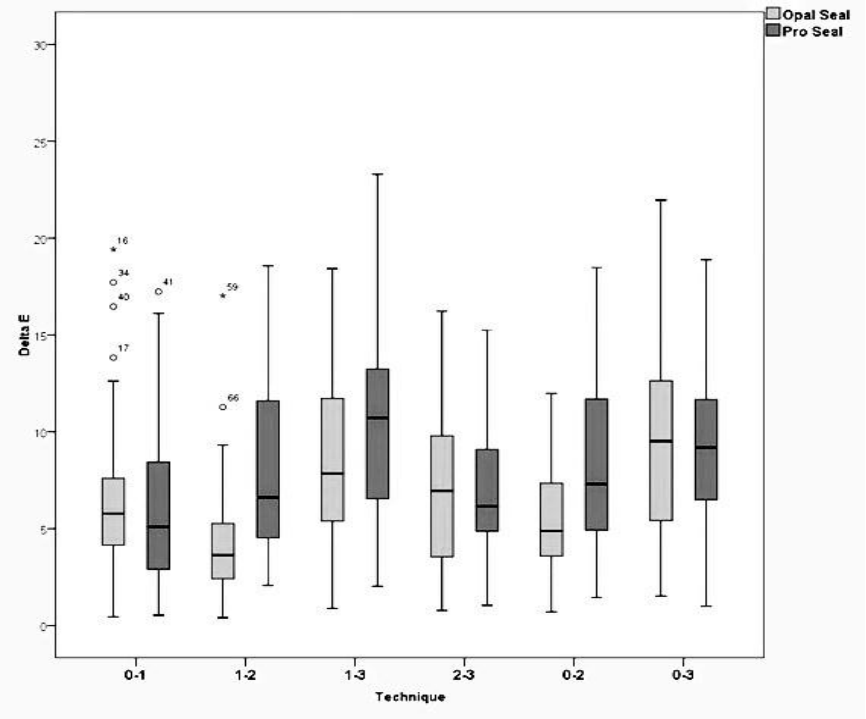

Fig. 7. Box and whisker graph of ΔE of the sealant groups in all time points; the thick line in the middle of the box represents the median, the box represents the inter-quartile range (from 25th to 75th percentiles), the whiskers represents the minimum and maximum, after excluding outliers (circle) and extremes (asterisks).
(Numbers indicate serial number of teeth in the original master table).

Table 9. ΔE changes in comparison to the critical threshold of clinical detection.

Sealant		Mean ΔE	SD	SE	Critical value	t	*p* value
ΔE (T0-T1)	Opal Seal	6.570	4.023	0.568	3.7	5.045	0.000*
	Pro Seal	6.104	4.358	0.616		3.900	0.000*
ΔE (T1-T2)	Opal Seal	4.355	3.038	0.429		1.526	0.134 NS
	Pro Seal	8.115	4.584	0.648		6.812	0.000*
ΔE (T1-T3)	Opal Seal	9.640	6.387	0.903		6.577	0.000*
	Pro Seal	10.644	4.621	0.653		10.624	0.000*
ΔE (T2-T3)	Opal Seal	7.381	4.452	0.629		5.847	0.000*
	Pro Seal	7.150	3.755	0.531		6.497	0.000*
ΔE (T0-T2)	Opal Seal	5.942	3.503	0.495		4.527	0.000*
	Pro Seal	8.440	4.651	0.657		7.207	0.000*
ΔE (T0-T3)	Opal Seal	9.807	5.759	0.814		7.499	0.000*
	Pro Seal	9.317	4.406	0.623		9.013	0.000*

In all ΔE values that exceeded the critical value of clinical detection the change in enamel color was clinically visible to the naked eye. Only ΔE (T1-T2) of Opal Seal group did not exceed the critical value.

II- Wear resistance of the sealants:

1- Sealant coverage (surface area)

Table 10. Comparison between area loss percentages of the sealants.

Area Loss Percentage	Technique		Test of significance *p* value
	Opal Seal	Pro Seal	
- n	50	50	
- Min-Max	-69.36 – -0.78	-96.61 – -34.13	
- Mean ± SD	-30.00 ± 15.48	-68.78 ± 15.54	$Z_{(MW)}$=7.914
- 95% CI for mean	-34.3948 – -25.5979	-73.1926 – -64.3602	*p*=0.000*
- Median (IQR)	-29.12 (-40.18 – -21.12)	-67.42 (-80.91 – -58.77)	
- KS test of normality	D=0.062, *p*=0.200 NS	D=0.085, *p*=0.200 NS	

There was a statistically significant difference between the sealant material loss between Opal Seal and PRO SEAL. Opal Seal group showed about 30% ± 15.48 loss of sealant coverage, while PRO SEAL revealed a much greater loss percentage of about 68.78% ± 15.54 as illustrated in table 10.

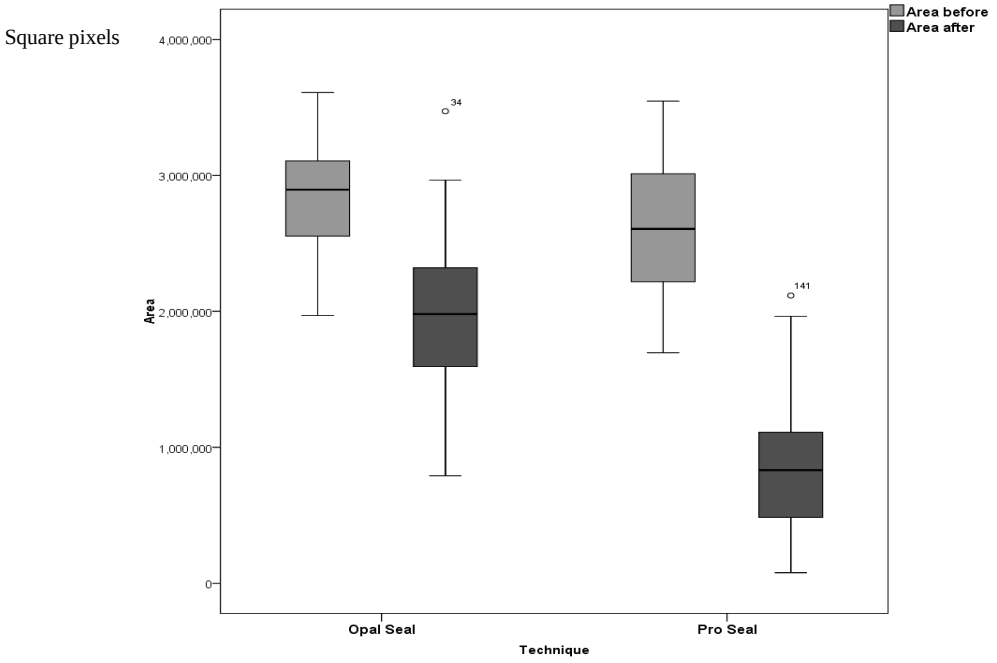

Fig 8. Box and whisker plot of surface area of the sealants covering the enamel surface before and after the challenges.

2- Corrected total fluorescence CTF (integrated intesity of fluorescence)

Table 11. Comparison between the total fluorescence percentage loss.

CTF percentage loss	Opal Seal	Pro Seal	Test of sig.
- n	50	50	
- Min-Max	-78.26 – -1.34	-98.56 – -43.67	
- Mean ± SD	-33.30 ± 17.75	-82.46 ± 11.04	$Z_{(MW)}$=8.362
- 95% CI for mean	-38.3432 – -28.2524	-85.6011 – -79.3254	*p*=0.000*
- Median (IQR)	-29.60 (-41.81 – -22.07)	-83.52 (-90.25 – -76.06)	
- KS test of normality	D=0.101, p=0.200 NS	D=0.087, p=0.200 NS	

There was a statistically significant difference between the two sealants regarding the percentage of the total fluorescence loss after exposure to acidic and abrasive challenges ($Z_{(MW)}$=8.362, *p*=0.000*). Opal Seal group showed about 33.30% ± 17.75 fluorescence loss under ultraviolet light, while PRO SEAL had an aggressive fluorescence loss of about 82.46% ± 11.04.

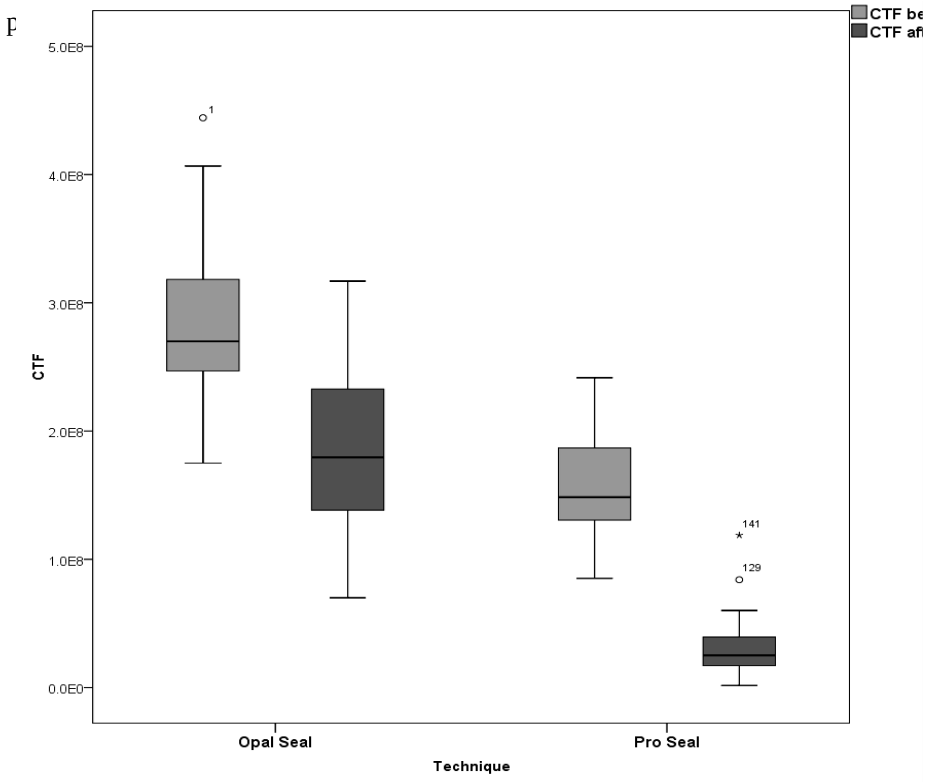

Fig. 9. Box and whisker plot of the corrected total fluorescence of the sealants before and after challenges.

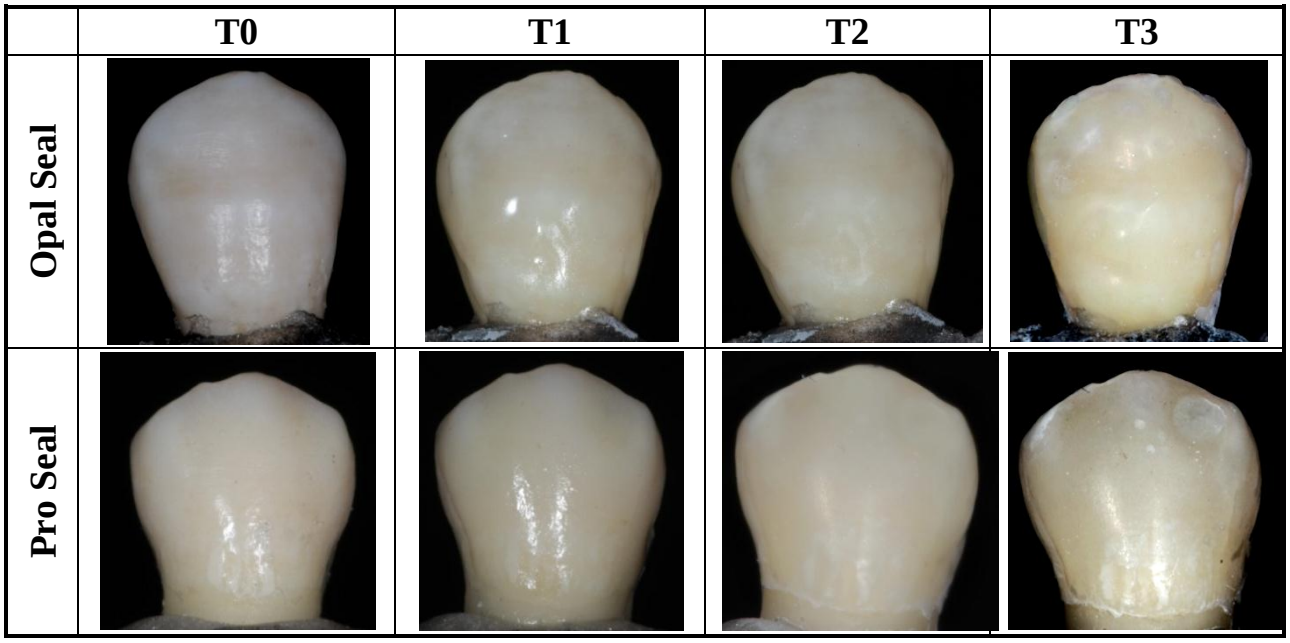

Fig. 10. Showing teeth after acid and abrasion challenges.

DISCUSSION

White spot lesions (WSLs) are inevitable during orthodontic treatment compromising the final esthetic result expected by the patient (Srivastava et al., 2013). They happen because of the acidic bacterial effect that jeopardizes the intraoral pH causing enamel demineralization around orthodontic attachments (Srivastava et al., 2013). Measures to prevent WSLs are better followed at starting fixed orthodontic treatment rather than treating them after they develop (Bishara and Ostby, 2008; Sundararaj, Venkatachalapathy, Tandon and Pereira, 2015).

Premaraj, Rohani, Covey and Premaraj (2017) compared between highly and lightly filled resin sealants on human teeth in terms of their acid penetration resistance but their sample size was only eight extracted teeth which was inadequate to detect an effect size between the two sealants (Pannucci et al., 2010). We increased the sample size in this study to a number that can detect an effect size of 27% between the two sealants used regarding assessment of WSLs formation (Pannucci et al., 2010).

The spectrophotometer was chosen to quantitatively record the enamel color changes that occur due to sealant application or decalcification (Chu et al., 2010; Eliadeset at., 2001; Ragain et al., 2016). The colors were measured in terms of the CIE L*a*b* values (Eliades et al., 2001; Jahanbin et al., 2009; Ragain et al., 2016; Zaher et al., 2012). The Vita Easyshade spectrophotometer was selected because of its high reliability and accuracy (Chu et al., 2010; Kim-Pusateri et al., 2009; Lagouvardos et al., 2009; Ragain et al.,

2016). Moreover, the high repeatability of the CIE L*a*b* measurements allowed its use for sequential and comparative measurements of the teeth (Chu et al., 2010; Lagouvardos et al., 2009; Ragain et al., 2016). A ΔE value of 3.7 units was used in this study as the threshold for clinical detection of color matching, beyond which color differences would be clinically visible to the naked eye (Eliades et al., 2001). Differences in colors were calculated for intragroup and intergroup comparisons.

The design of the black mold and specimen blocks was chosen to ensure creating a light-scatter-free room to eliminate the effect of any reflected light that could interfere with the measurements and for the standardization of the readings as reported by Kim-Pusateri et al. (2009) and Zaher et al. (2012). All the measurements were made on wet enamel surfaces to avoid alterations of enamel color caused by dehydration as recommended by other spectrophotometric studies (Zaher et al., 2007; Zaher et al. 2012).

Hu and Featherstone (2005) tested the protective effect of PRO SEAL after prolonged mechanical abrasion. They employed a 14-day pH cycling protocol to induce demineralization. They evaluated the sealant performance by measuring enamel microhardness. The enamel showed standard profiles at all measurements, indicating almost complete inhibition of demineralization. Their results came along with Buren et al. (2008), but the two experimental procedures were different. Buren et al. used polarized light microscopy to see the demineralized lesions directly and measure their depths. Despite the different methodology used, the results of both studies concluded that PRO SEAL can be used for long term enamel protection. In contrast, our results found that Pro Seal did not withstand the continuous acidic challenge (96 hours) used in our study without remineralization cycles as Hu and Featherstone did in their study. In addition, our study used a different methodology to assess demineralization by measuring the enamel color change from normal at baseline to whitish coloration that occurs due to loss of the mineral content after demineralization caused by simulated erosion and abrasion.

Previous short-term studies subjected teeth to ten brush strokes, twice per day for the duration of their studies only, to simulate the average patient hygiene but this was insufficient time frame to assess the longevity of the sealants that were claimed by the manufacturers to withstand oral environment up to two years (Reliance Orthodontic Products, 2019; Schmitt et al., 2002; Todd, Staley, Kanellis, Donly&Wefel, 1999; Ultradent Products, 2019). In the current study, we extended the simulated toothbrushing protocol and continued the acidic

challenge to imitate the normal patient hygiene during the average fixed orthodontic treatment time that is approximately 24 months. All the teeth were subjected to 15,000 cycles in the brushing simulator that are equivalent to two years of patient brushing (Buren et al., 2008; Hu & Featherstone, 2005). In our study, to assess the wear resistance of the sealants, standardized digital photographs of each tooth were entered in image analysis computer software. The fluorescing areas were traced automatically and calculated in square pixels using color and brightness thresholding of the images by the software and not by free-hand tracing as done by Shungin et al. (2010) in their study that was more subjected to outlining errors. Corrected total fluorescence was calculated for each image according to the recommended method by Burgess (2011) and McCloy et al. (2014) that showed higher accuracy in measuring fluorescence than using the raw values of fluorescence only.

Blinding to the application of the sealants could not be applied to the operator because of their nature, use of different brushes, the difference in original sealants colors, and the different curing times for both sealants. However, the allocation of a sample assigned to both groups was concealed to the operator until the study procedures started. To ensure blinding during the image-analysis; all the images were given random serial numbers, and the renaming key was kept in a sealed envelope until the end of the analysis. In this way, tooth surfaces from both sealant groups were mixed up, and the researcher was blinded to know which image belonged to which group at which step (Shungin et al., 2010). The statistician was blinded.

In our study, the results showed that the enamel color in each group was changed after each experimental step. There was a statistically significant intragroup difference between each time point except in the Opal Seal group at ΔE (T1-T2). That means, the enamel color in Opal Seal group did not change after the exposure to the first acidic challenge and it protected the enamel against demineralization (table 5 & 9).

Looking up in the literature; we found only spectrophotometric studies investigating stability of filled sealants or staining of resin remnants on enamel surface after removal of sealants (Abdel-Raouf et al., 2014; Eliades et al., 2001; Jahanbin et al., 2009; Karamouzos et al., 2010; Zaher et al. 2012). In addition to the previous results of this study, a significant difference between the initial enamel color and its color after application of the sealants in both groups was found, i.e., ΔE both exceeded the critical value of clinical detection (3.7 units) and were clinically visible. Results showed that both sealants changed the enamel color (see ΔE (T0-T1) in table 9). However, there

was no significant difference between Opal Seal and Pro Seal in ΔE (T0-T1) and both affected the teeth similarly (table 3).

After the first exposure to the acidic challenge, the Opal Seal group did not show any color change of the enamel while the Pro Seal group was greatly affected (ΔE (T1-T2) in table 9). Enamel color was changed in the Pro Seal group indicating demineralization occurrence. There was a statistically significant difference between the two groups at ΔE (T0-T2) and ΔE (T1-T2) where p=0.003 and p=0.000 respectively (tables 4 & 5).

However, after the second exposure to the acidic challenge and brushing simulator, the enamel color in the Opal Seal group was affected as indicated by the increase in the ΔE values between T2 and T3 (table 9) i.e.; the Opal Seal could not withstand the second acidic exposure. This color change approaches the Pro Seal behavior after the challenges at T3. There was no significant difference between them when compared to each other at T3 (see ΔE (T2-T3) in table 8). Although the color change difference ΔE (T0-T3) was statistically insignificant, the ΔE (T1-T3) was statistically significant because the range of data from T0 to T3 was larger than from T1 to T3; so the significance did not show in the lager range data. This is due to the effect of changing the enamel color after sealant application.

After the simulated brushing, a significant material loss occurred in both sealant groups expressed in loss of surface area coverage to the enamel and fluorescence loss of the sealants. There was a statistically significant difference between both groups in wear resistance (p=0.000). Greater sealant loss (68.78% ± 15.54) and fluorescence loss (82.46% ± 11.04) were seen in the Pro Seal group as illustrated in tables (10 & 11). This means that increasing the filler content of the sealants helped it to withstand the induced challenges as shown in table (7).

Full simulation of the intraoral physiologic conditions was not achieved in this in-vitro designed study; however, evaluation of the enamel color was done under identical conditions. During sample preparation and before color measurement, all teeth were cleaned and polished with a brush mounted on a low-speed handpiece with slurry of non-fluoridated oil-free pumice then rinsed under running water and dried. The non-fluoridated pumice was used to prevent creating a hypermineralized enamel surface that is acid resistant, hinders the etching process and negatively affects the bond strength between the sealants and the enamel surface (Hosoya & Johnston, 1989; Kakaboura & Papagiannoulis, 2005).

Our results came along with Chau et al. (2015) who found 38.6% and 15.5% of Opal seal material loss in teeth treated with a self etching primer and a conventional 37% phosphoric acid etch

respectively. However, Tüfekçi et al. (2014) found that Opal Seal has some efficacy in preventing demineralization and its protective effect might diminish after 3 months. Despite the short time frame of their study that lasted for 3 months, only 50% of the sealant remained on teeth surfaces. Their assessment method was subjective as they only used a score system for determining sealant retention (Tüfekçi et al., 2014).

Finally, our quantitative results recommend the preventive use of highly filled sealants at the start of orthodontic treatment with proper patient selection. This would significantly reduce the occurrence of WSLs. Further research should be done to investigate the effect of the filler amount on the stability of the sealants color intraorally to determine the most suitable sealant composition according to the clinical situation.

CONCLUSIONS

Based on the results of the current study, it can be concluded that:

1. Increasing the filler content strengthens the sealant against acid and abrasion challenges, therefore, offering more protection against white spot lesions.

2. Both sealants degrade with increasing the challenges.

3. Both sealants change the initial enamel color similarly.

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