The Effect of Incorporating Different Concentrations of Octenidine Dihydrochloride on the Degree of Conversion of an Experimental Flowable Resin Composite

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Abstract

BACKGROUND: One of the important parameters in assessing the definitive physical, mechanical, and biological characteristics of resin composites is the degree of conversion (DC), as composite qualities have been proven to improve with increasing the DC after photo-polymerization. Besides, fracture or secondary caries are the most common causes of composite resin failure. Accordingly, this reflects the need of formulating dental restorative materials possessing antibacterial activity.

AIM: This study was designed to incorporate different concentrations of a new antibacterial agent (Octenidine dihydrochloride [OCT]) into an experimentally formulated flowable resin composite and evaluate its DC.

MATERIALS AND METHODS: Four groups were tested in this study; group I was used as the control group, it's a commercially available flowable composite "Herculite Ultra Flowable". Group II was an experimental flowable composite with no antibacterial agent. During the preparation of the experimental flowable resin composite material, OCT antibacterial agent was added to the filler in special dark containers at a concentration of 1% wt. and 1.5% wt. respectively, in groups III and IV. The DC was measured and compared to the commercially available resin composite using the Fourier Transform Infrared spectroscopy method.

RESULTS: Results of the current study showed that the mean values of DC ranged between (70.37 and 48.7), where Group1 showed the highest mean value, followed by Group 2 than Group 3, Group 4 specimens had the lowest mean value. The data showed that there is a statistically significant difference between all the tested groups. However, the DC was still within the accepted ranges for dental use.

CONCLUSION: Based on the results obtained within the experimental conditions of this study it may be stated that the inclusion of the antibacterial OCT 1% and 1.5% wt., into the flowable resin composite showed satisfactory results for the DC as it met the ADA requirements for clinical use.

Introduction

Esthetic restorations that have the properties of matching the natural tooth color are in exceedingly high demand by both patients and dentists. Hence, many trials have been made to find the highest quality of esthetic dental restorations [1]. Resin composites have undergone numerous improvements along the years to achieve more durable esthetic restorative materials with similar mechanical and physical properties to that of the natural tooth [2], [3].

One of the important parameters in assessing the definitive physical, mechanical, and biological characteristics of resin composites is the degree of conversion (DC), as resin composite qualities have been proven to improve with increasing the DC achieved after photo-polymerization [4]. Since dental caries is an infectious disease introduced by cariogenic bacteria, attempts to develop antibacterial restorative materials became a popular issue in dental materials science [5].

Recent antimicrobial agents, such as Octenidine dihydrochloride (OCT) have been investigated as alternatives to other antibacterial agents because of their superior microbicidal activity and low cytotoxicity [6]. Accordingly, this study is designed to incorporate different concentrations of OCT into experimentally formulated flowable composite and measure their DC.

Materials and Methods

Four groups were tested in this study:

1. Group 1: The control group was a commercially available resin composite by Kerr Corporation with the brand name "Herculite Ultra Flowable"
2. Group 2: Experimental resin composite with 0 wt% OCT

3.   Group 3: Experimental resin composite with 1% OCT

4.   Group 4: Experimental resin composite with 1.5% OCT

The materials used in this study are shown in Table 1.

Table 1: Materials, presentation, manufacturer, and batch number of chemicals used in this study

Material	Presentation	Manufacturer	Batch Number
Bisphenol A glycerolate dimethacrylate, (Bis-GMA) ($C_{29}H_{36}O_8$) Mol. Wt: 512.59	Viscous liquid	Sigma Aldrich, USA	494356
Triethylene glycol dimethacrylate (TEGDMA)	Liquid	Sigma Aldrich, USA	261548
Trimethoxysilane ($C_3H_{10}O_3Si$), Mol. Wt: 122.2	Liquid	Sigma Aldrich, USA	282626
Silicon dioxide nano-powder (spherical) (SiO_2), Mol. Wt: 60.08	Powder (size: 5-15 nm)	Sigma Aldrich, USA	637246
Ethyl 4-(dimethylamino) benzoate ($C_{11}H_{15}NO_2$) Mol. Wt: 193.24	Crystalline Powder	Sigma Aldrich, USA	E24905
Camphorquinone, ($C_{10}H_{14}O_2$)Mol. Wt: 166.22	Powder	Alpha Aesar, USA	124893
Acetic Acid Glacial	Liquid	PioChem, Egypt	AC0121
Ethanol 70%	Liquid	PioChem, Egypt	Et0012
Buffer solution DURACAL (pH 4.01)	Liquid	Sigma Aldrich, USA	242142
Potassium Bromide (KBr)	Powder	Sigma Aldrich, USA	221864
OCT	Powder	MedChem Express (MCE), USA	23549
Flowable composite, (Herculite Ultra Flowable)	Paste	Kerr Corporation	35408

OCT: Octenidine dihydrochloride.

For the experimental groups, the flowable composite was formulated according to the following steps:

Preparation of the experimental flowable resin composite material

Preparation of the resin matrix

Preparation of the resin matrix was done by mixing the viscous monomer bisphenol A-glycidyl methacrylate (Bis-GMA) with the liquid monomer TEGDMA in a ratio of 70:30% weight [7].

Preparation of the photo-initiator system

A ratio of 1:1 of the crystalline camphorquinone powder and ethyl 4-(dimethyl-amino) benzoate were used for the preparation of the photo-initiator system. The prepared photo-initiator powder was then gradually added to the dark glass beaker containing the mixture of the unfilled resin matrix. A small magnet on a magnetic stirrer (MS-300HS, Human Lab Instrument Co., Korea) was used to stir the mixture constantly for 2 h to ensure homogenization of all components and complete dissolution of the monomers [8].

Preparation and hydrolysis of the silane coupling agent

Ethanol solution with a concentration of 70% was proportioned in a small glass beaker and covered with a filter paper. Drops of acetic acid were gradually added to ethanol to improve the hydrolysis rate of the silane coupling agent until the pH decreased to 3–4 [9], that was measured using a bench pH meter (Jenway,3505, UK). Afterward, 3% wt of the liquid silane coupling agent Trimethoxysilane was added to the solution then stirred in a glass beaker covered with a paraffin film for 1 h using a magnetic stirrer.

The commercial silicon dioxide nanopowder was sintered at 1300 °C for 20 min using an electric fast sintering dental furnace sintered by (TEGRA SPEED, Teknik Dental, Istanbul Turkey) The final shape of the particles after sintering appeared as white clusters. The sintered clusters were then ground using agate mortar and pestle then sieved using a stainless steel 400 mesh sieve [10].

Sintered silicone dioxide nanoparticles were immersed directly in the prepared hydrolyzed silane coupling agent solution and stirred using the magnetic stirrer for 2 h. The mixture was then centrifuged for 30 min, at a temperature of 25°C, with a speed of 6000 rotation/min using a centrifugation machine (SIGMA 3-16KL, Sigma Laborzentrifugen GmbH, Germany). The nanoparticles were deposited at the base of the clear plastic Eppendorf tube after centrifugation. The precipitate was washed with ethanol and mixed in a vortex mixer (Stuart, SA7, UK) at 1800 rpm to ensure proper homogenous mixing, and then centrifuged again for 5 min using the same centrifugation apparatus. This process was repeated three times and the remaining ethanol was removed. The precipitate was then dried in a small petri dish using a hot air drying and heating oven (BINDER, FD 23, 20L, Germany). The heating temperature was maintained constant at 105°C for 60 min [11]. The petri dish was then removed from the oven and covered with aluminum foil and placed in a desiccator for a further 10 min.

Addition of the OCT to the silanized nano filler particles

The OCT antibacterial agent was added to the silanized nanofillers in special dark containers at a concentration of 1% wt. and 1.5% wt., respectively. These concentrations were determined based on a pilot study conducted prior to the test.

Addition of the fillers containing the antibacterial agent to the unfilled resin matrix

The nanofillers mix containing the antibacterial agent was incrementally added to the experimentally prepared resin matrix [11]. The uncured resin composite mix was placed in a dark-tinted bottle, then stored in a dark place overnight, and mixed manually the next day.

Measuring the DC using the Fourier transform infrared (FTIR) spectroscopy

According to specification n. 27 of ANSI/ADA, in 1993 [12], the cylindrical specimens with dimensions (5 mm in diameter × 4 mm thickness) were fabricated. The total number of specimens used in this study was 32 specimens, divided equally among the four groups where eight specimens were used for each group (n = 8), which was further subdivided into two groups where n = 4(cured) and n = 4 (uncured) for each testing group.

Photo-activation was performed by placing the light curing device (Premium Plus light cure C02-D, Premium PlusUK, England) on the resin composite specimens' upper surface through each side of the mold, the specimens were irradiated for 40 s. After photo-activation, the specimens were taken from the mold and stored for 24 h in dry, dark containers at 37°C (±1°C) for 24 h [4].

24 h after the photoactivation, the polymerized specimens from each group were milled into a fine powder using a mortar and pestle. 5 mg of the ground powder were mixed meticulously with 100 mg of the KBr powder salt. This grounded mixture was then positioned into a pelleting device (SCHIMADZU, Kyoto, Japan) and the mix was then pressed with a pressure of 86KN for 1 min to obtain a pellet [13]. FTIR (SCHIMADZU IRAffinity-1S, Kyoto, Japan) was used to assess the DC.

The diffuse-reflection mode of the FTIR was used to record the absorbance peaks under the following conditions: 32 scans, over a wavelength of 400–4000 cm^{-1} and a resolution of 4 cm^{-1} [4], [13]. For the unpolymerized specimens (n = 4) of each group, the uncured material was smeared onto thin KBr discs, and placed into a cell holder in the spectrophotometer, to obtain a range with the same parameters as for the polymerized samples [13].

Finally, the DC was calculated by comparing the peak height ratios of the absorbance intensities of the aliphatic C=C peak at 1638 cm^{-1} and an internal reference peak of aromatic C=C at 1608 cm^{-1} before and after curing the specimens [13].

DC% for each specimen was calculated using the following equation [13]

$$DC\% = \left\{ 1 - \frac{(1638\,cm^{-1}\,/\,1608\,cm^{-1})\,cured}{(1638\,cm^{-1}\,/\,1608\,cm^{-1})\,uncured} \right\} \times 100$$

Statistical analysis was performed using a commercially available software program (SPSS 18; SPSS, Chicago, IL, USA).

The mean, standard deviation (SD), and confidence intervals were used to present the data. The Kolmogorov-Smirnov test of normality was used to examine the data for normalcy. For parametric data, a one-way analysis of variance (ANOVA) was performed to compare groups, and if a significant difference was found, Tukey's *post-hoc* test was utilized. The significance level was chosen at p ≤ 0.05.

Results

The mean values of DC ranged between (70.37 and 48.7), where Group 1 showed the highest mean value, followed by group 2 than Group 3, Group 4 specimens had the lowest mean value as shown in Figure 1. ANOVA test revealed a statistically significant difference between all groups as shown in Table 2. Tukey's *post-hoc* test revealed a significant difference between each two groups as shown in Table 3.

Table 2: Descriptive statistics of the DC% and comparison between groups (ANOVA)

Groups	Mean	Std. Dev	Std. Error	95% Confidence Interval for Mean		Min	Max	F	p
				Lower bound	Upper bound				
Group 1	70.37a	3.50	1.24	67.44	73.29	65.22	75.82	67.97	0.00*
Group 2	56.74b	3.15	1.11	54.10	59.37	50.49	60.18		
Group 3	52.88c	2.86	1.01	50.49	55.27	49.89	59.02		
Group 4	48.70d	3.34	1.18	45.91	51.49	44.98	53.13		

Significance level p ≤ 0.05, *significant, ns: non-significant, Means with different superscript letters are significantly different, DC: Degree of conversion, ANOVA: Analysis of variance.

Discussion

New paradigms strive to formulate remedial resin composite materials exhibiting antibacterial effects. In this study, an antimicrobial agent OCT was incorporated into the fillers of an experimentally formulated resin composite and the DC was measured for all the tested groups.

Table 3: Detailed results of Tukey's post-hoc test for comparison of the DC%

I-Groups	J- Groups	Mean difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower bound	Upper bound
Group 1	Group2	13.62875*	1.610	0.000	9.232	18.025
	Group3	17.48500*	1.610	0.000	13.088	21.882
	Group4	21.66500*	1.610	0.000	17.268	26.062
Group 2	Group1	−13.62875*	1.610	0.000	−18.025	−9.232
	Group3	3.85625	1.610	0.101	−0.540	8.253
	Group4	8.03625*	1.610	0.000	3.640	12.433
Group 3	Group1	−17.48500*	1.610	0.000	−21.882	−13.088
	Group2	−3.85625	1.610	0.101	−8.253	0.540
	Group4	4.18000	1.610	0.067	−0.217	8.577
Group 4	Group1	−21.66500*	1.610	0.000	−26.062	−17.268
	Group2	−8.03625*	1.610	0.000	−12.433	−3.640
	Group3	−4.18000	1.610	0.067	−8.577	0.217

Significance level p ≤ 0.05, *significant, DC: Degree of conversion.

OCT is a Bis(pyridine) derivative that has been suggested as an alternative to chlorhexidine based on its antimicrobial effects and lower cytotoxicity. OCT exhibits high broad-spectrum antimicrobial efficacy on both gram-positive and gram-negative bacteria, fungi, and several viral species [14]. The enhanced antimicrobial efficacy of OCT is attributed to its cation-active structure that readily binds to the negatively charged bacterial

cell wall and thereby affects the vital functions of the cell membrane and causes cell death [15]. Based on a pilot study, the weight percentages of the OCT antibacterial agent used in this work were determined.

Because resin composite qualities have been shown to improve with increasing the DC obtained following photopolymerization, the DC is an important parameter for measuring the actual physical, mechanical, and biological features of resin composites [4].

The longevity of the resin composite restoration may also be affected by a lower DC that may result in unreacted monomers rendering them more soluble in the wet oral environment. Furthermore, reactive sites (double bonds) are also more vulnerable to hydrolyzation or oxidation and result in material degradation. Besides, uncured functional groups can also act as plasticizers, lowering the resin composite's final mechanical [4].

Furthermore, increased cure has been reported to result in a reduced quantity of leachable monomers, resulting in a more biocompatible restoration, because of the risk of biological responses associated with monomer release and pulp tissue affliction [4], [16].

The main methods commonly used to measure the DC of resin composites are the FTIR spectroscopy, the Raman and the FT-Raman spectroscopy [17]. FTIR spectroscopy was used to determine the DC of the resin composites in this study. The use of an interferometer to separate the spectral components and a mathematical technique to speed up the measurement is referred to as FTIR [17].

FTIR spectroscopy depends on the absorption or reflection of light whereas Raman spectroscopy investigates the light scattering processes. It is important to note that (a) radiation absorption is much more efficient (109 times) than scattering (b) IR absorption measurements are more appropriate for polar systems who have substantial transfers between atoms or molecules, such as the chemical bonds formed by polymerization of resin monomers. Based on these considerations the FTIR was the method of choice in this study as it is the simplest and most reliable technique [17]. FTIR detects the amount of unreacted C = C in the resin matrix and C = C stretching vibrations directly before and after curing of resin composite to determine changes in mechanical performance [18].

The obtained values for the DC in this study agreed with findings from other studies performed on similar systems and measured with the same method: The DC in this study ranged between 48.70% and 70.37%. In 1995 Ferracane [19], found that the DC ranged between 35% and 77%, whereas in 1997 Peutzfeldt [20]. stated that the DC of monomer to polymer in dental resin composites varied between 40% and 75%.

In the present study, all groups showed significantly different percentage values of DC. The addition of the antibacterial agent OCT significantly decreased the DC percentages in groups 2, 3, and 4, where Group 4 showed the lowest DC percentage among all tested groups. While the mean value for the control group 1 showed the highest DC percentage among all the tested groups.

The DC is regulated by a complex interaction of several factors, which during polymerization, affect the reactive species [21]. The differences in the percentages of the DC between the studied groups could be related to changes in chemical composition, because the sample sizes, curing procedure, light source, polymerization conditions, and method for measuring DC were all kept standardized between the various tested groups.

The above considerations are further complicated in the case of the experimental resin composites used in this study (groups 2, 3, and 4) could be due to the tendency of nanometre-sized silica particles to agglomerate into larger secondary particles [22], which makes it more difficult to control experimentally, and considering the fact that resin/ filler interactions vary depending on the level of filler silanization [23]. In addition, during preparation oxygen in agglomerates could inhibit free-radical polymerization in the specimen, this phenomenon has been observed in some experimental resin composite types [24].

Chemistry of filler matrix, filler particle sizes, and dispersion could interfere with the transmission of light through the material and thus interfere with proper polymerization and DC [25]. The reduction of DC values with increasing the antibacterial content in groups (3 and 4) could probably be related to the effect of light scattering by the antibacterial particles. Moreover, some reports also showed larger scattering when the particle size was approximately one half or close to that of the curing light wavelength [26].

Different sizes of the filler particles are another variable that could affect the DC of a resin composite. This is attributed to the scattering effect of tiny fillers which reduces the amount of light transmitted through the resin [18]. The commercially available (group 1) had a higher conversion rate than the other groups in this study, which could be due to unique filler combinations and different filler sizes in comparison to the other groups.

Moreover, the opacity of a substance has a considerable influence on light transmission through it [25]. The observed lower DC values corresponding to the increase in the antibacterial content in groups (3 and 4) may also be attributed to their increased opacity, reduced filler material can also help attain higher translucency between materials [25]. It has also been proven in other studies that increasing the filler-to-matrix ratio reduces conversion in experimental resin composites [27], this is also in accordance with findings in this study.
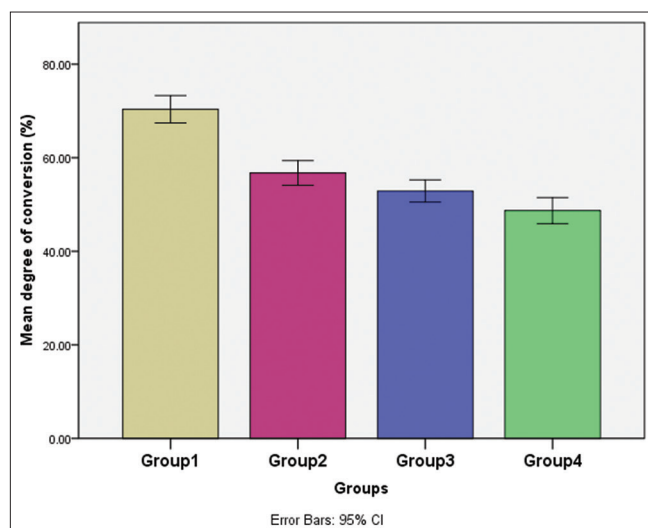
Figure 1: Bar chart illustrating mean degree of conversion % in different groups

The strengths of this study were first; the determination of the DC and the amount of residual monomers in dental resin composites are of great importance, as these parameters could be regarded as prognostic factors for the behavior of dental restorations under clinical conditions. Moreover, the antibacterial used in this study was previously used in the medical field but recently introduced into the field of dentistry and was not incorporated in flowable resin composites before, therefore it's a pioneering idea that also gave promising results.

Additionally, *in vitro* testing helps build a strong and original scientific record, to highlight the technological and competitive advantages of any new material. However, from the limitations of this study were that it failed to capture the inherent complexity of biological organ systems under clinical use. Adding to that, scarce literature was available for comparisons with other studies.

Conclusion

Under the limitations of the current study, adding 1% and 1.5% wt of OCT to the experimentally formulated composite resin showed satisfactory results for the DC that met the ADA requirements for clinical use.

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