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Original Article

Testing mechanical properties and degree of conversion of resin-based composite material containing contact killing antibacterial agent in comparison with fluoride composite resin

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A R T I C L E I N F O

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A B S T R A C T

Background: A major drawback of resin composites is their tendency to accumulate microbial biofilms that can lead to secondary caries. The objective of this study was to compare the mechanical properties and the degree of conversion of commercial resin-based composite materials containing a contact-killing antibacterial agent, dimethylaminohexadecyl methacrylate (DMAHDM), at different concentrations, with a fluoride-releasing composite material.
Materials and methods: Four groups were tested: Tetric N Ceram composite material (G1), Tetric Evo Ceram (G2), and Tetric N Ceram with the addition of contact-killing antibacterial agent DMAHDM at concentrations of 3% (G3) and 5% (G4). The mechanical properties, including flexural strength, elastic modulus, and Vickers microhardness and the degree of conversion were investigated.
Results: Adding 3 % and 5 % DMAHDM resulted in flexural strength values that were comparable to Tetric Evo Ceram. Tetric N Ceram was comparable to the group containing 3 % DMAHDM ($p > 0.05$). However, it was significantly greater when compared to Tetric Evo Ceram (93.3 $\pm$ 9.4) and 5 % DMAHDM ($p < 0.05$). Both the elastic modulus and Vickers microhardness values of Tetric N Ceram were significantly higher than those of the other groups ($p < 0.05$). Furthermore, the elastic modulus of Tetric Evo Ceram showed similar results to groups with 3 % and 5 % DMAHDM. Nevertheless, the Vickers microhardness value is significantly higher when compared to 5 % DMAHDM (0.394 $\pm$ 0.021) ($p < 0.05$) while it was comparable to that of 3 % DMAHDM (0.484 $\pm$ 0.016) ($p > 0.05$). There was no statistically significant difference in the degree of conversion between the groups ($p > 0.05$).
Conclusion: Adding 3% DMAHDM to Tetric N Ceram resulted in flexural strength values that were similar to those of Tetric N Ceram and Tetric Evo Ceram. DMAHDM did not affect the degree of conversion of Tetric N Ceram composite.

1. Introduction

Since resin-based composites were introduced into conservative dentistry (Bowen, 1963), patients have favored these tooth-colored restorations because of their excellent aesthetic appearance (Ferracane, 2011; Wolff et al., 2010). In addition to their excellent aesthetic properties, resin materials result in minimal tooth destruction owing to their bonding capabilities (Roulet, 1997; Wolff et al., 2010), eradicating the concept of “extension for prevention” (Roulet, 1997).

However, resin-based composites have some disadvantages. They lack antimicrobial and remineralization properties that play an important role in the protection against dental caries. *Streptococcus mutans* and *Lactobacilli* are the main oral pathogens involved in caries formation. Specific proteins attach to the surface of resin-based restorative

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materials. These proteins aid in the accumulation of bacteria and their by-products, resulting in secondary caries formation and tooth demineralization (Do et al., 2013; Polizzi et al., 2019).

The inclusion of antimicrobial agents is a strategy to control and/or eliminate infectious diseases resulting from biofilm attachment to restorative materials, thus enhancing the longevity of restorations (Mitwalli et al., 2020a, 2020b). One of the various antibacterial strategies available is the use of contact-based antibacterial materials (Cloutier et al., 2015). Contact-killing antibacterial agents use covalent bonds to chemically bond to the core dental monomers. Therefore, they offer long-lasting antibacterial effects through contact killing without leaching. Unlike fluoride-containing materials where fluoride ions exhibit a burst release effect that tends to fade over time. Contact-killing antibacterial materials are especially beneficial for resin-based materials because they tend to accumulate bacterial biofilms on their surfaces (Balhaddad et al., 2019, 2020).

Quaternary ammonium methacrylates (QAMs) are a group of contact-killing antibacterial agents (Mitwalli et al., 2020a, 2020b). An interaction between their positively charged surfaces and the negatively charged bacterial membrane results in an electrical imbalance across the cell membrane, causing a balloon-bursting effect on the bacterial cell wall (N. Zhang et al., 2018). The length of the QAMs alkyl chain affects its antibacterial response, and the most potent antibacterial properties against caries-related pathogens was demonstrated in a QAM known as dimethylaminohexadecyl methacrylate (DMAHDM) with an alkyl chain of 16 units (Li et al., 2013). Incorporating 3 % DMAHDM into resin composites showed significant reduction in biofilm growth that was more effective than other QAMs without affecting the mechanical properties of the material (K. Zhang et al., 2016). In addition, as a result of increasing the concentration, the surface charge density was also increased, which may explain the enhanced antibacterial response (Wu et al., 2015).

DMAHDM exhibits long-lasting antibacterial effects when added to different materials. However, to the best of our knowledge, the impact of adding DMAHDM at different concentrations to Tetric N Ceram against the mechanical properties and degree of conversion in comparison to a fluoride-releasing resin-based composite, Tetric Evo Ceram, has never been investigated.

The paper tested the following hypotheses: (1) Adding DMAHDM to the Tetric N Ceram composite would not compromise the mechanical properties compared to Tetric N Ceram and Tetric Evo Ceram; (2) Adding DMAHDM to the Tetric N Ceram composite would not affect the degree of conversion when compared to Tetric N Ceram and Tetric Evo Ceram.

2. Materials and methods

2.1. Commercial resin-based composite

Two nanohybrid composite materials were included in the study: Tetric N Ceram (Ivoclar Vivadent, Mississauga, ON, Canada) and Tetric Evo Ceram (Ivoclar Vivadent, Mississauga, ON, Canada). Tetric Evo Ceram was added as a fluoride-releasing material.

2.2. Synthesis of DMAHDM

DMAHDM was synthesized following a modified Menschutkin chemical reaction (Zhou et al., 2013). In summary, 10 mmol of 2-(dimethylamino) ethyl methacrylate (Millipore Sigma, Burlington, MA, USA), 10 mmol of 1-bromohexadecane (TCI America, Portland, OR, USA), and 3 g of ethanol were added into the reaction vessel. The mixture was stirred for 24 h at 70 °C. DMAHDM was collected after evaporation of the solvent and removal of any impurities. DMAHDM was finely ground using a mortar and pestle. In one group, DMAHDM was added to Tetric N Ceram at a concentration of 3 %, and in the other group, it was added at a concentration of 5 %. The mixture was mixed using a spatula and glass slab until it was homogenous (Mitwalli et al., 2022). The tested materials were divided into the following groups.

1- Group I: Tetric N Ceram
2- Group II: Tetric Evo Ceram
3- Group III: Tetric N Ceram + 3 % DMAHDM
4- Group IV: Tetric N Ceram + 5 % DMAHDM

2.3. Testing of mechanical properties

2.3.1. Specimen preparation and testing for flexural strength and elastic modulus

A rectangular metal mold with dimensions of 2 × 2 × 25 mm^3 (width × height × length) was used to prepare the specimens. The material was then placed in a mold. Glass slides and Mylar strips were then placed below the material and on top of it to form a smooth surface, as illustrated in Fig. A. Specimens were cured for 60 s from all sides following the manufacturer's instructions utilizing a light-curing unit at 1110 nm (Ivoclar Vivadent Bluephase N cordless light cure, Mississauga, ON, Canada). The Light intensity was measured through a Bluephase Meter II (Ivoclar Vivadent, Mississauga, ON, Canada). Afterwards, the specimens were stored dry in an incubator for a minimum of 24 hrs at 37 °C. The incubated bar specimens were water-aged for another 24 h (Mitwalli et al., 2022). Eight specimens were prepared from each group (n = 8) (Mitwalli et al., 2022).

The Flexural strength and elastic modulus tests were performed via a three-point flexural test using 10 mm span and a crosshead speed of 1 mm/min on a Universal Testing Machine (Instron 5965, Norwood, MA, USA) (ISO, 2019; Xu et al., 2011). Flexural strength (S) was calculated using the following formula: $S = 3P_{max}L/(2bh^2)$. P_{max} is fracture load, L is span, b is specimen width and h is specimen thickness. The elastic modulus (E) was calculated using the following formula; $E=(P/d)\,(L^3/[4bh^3])$, where P divided by d is the slope in the linear elastic region of the load–displacement curve. Three readings were recorded for each specimen (Zhou et al., 2013).

2.3.2. Specimen preparation and testing for Vickers microhardness

A circular rubber mold with dimensions of 2 × 10 mm^2 (height × diameter) was used to prepare the specimens. The material was packed into the mold. Mylar strips and glass slides then placed below and on top of the material were used to form a smooth surface. After that, the samples were cured for 60 s on both sides according to the manufacturer's recommendation using a light-curing unit at 1110 nm (Ivoclar

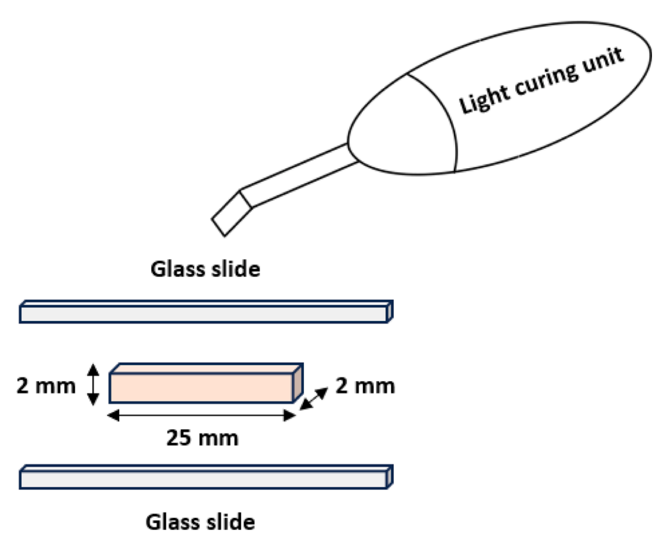

Fig. A. Schematic presentation of specimen preparation for flexural strength and elastic modulus tests.

Vivadent Bluephase N cordless light cure, Mississauga, ON, Canada). Afterwards, the specimens were stored dry in an incubator for a minimum of 24 hrs at 37 °C. Five specimens were prepared for each group ($n = 5$).

Vickers microhardness indentations at a load of 100 g and dwelling time of 10 s were performed (Abuelenain et al., 2015). The test was performed using the INNOVATEST software (Maastricht, Netherlands). The indentation sizes were measured using lens with 10 × and 40 × objectives. Three measurements were performed for each specimen.

2.3.3. Degree of conversion

All groups were submitted Fourier transform infrared spectroscopy (FTIR) utilizing (Thermo Scientific Nicolet iS20 FTIR Spectrometer, Waltham, Massachusetts, USA) (Balagopal et al., 2021). Two millimeters of each material were maintained in a dark room for FTIR analysis. The material was subjected to light-cure for 60 s, following the manufacturer's instructions, using a light-curing unit at 1110 nm (Ivoclar Vivadent Bluephase N Cordless Light Cure, Mississauga, ON, Canada). One minute after curing, the material was subjected to FTIR spectroscopy. The ratio of the absorbance intensities of aliphatic C═C (peak at 1637 cm^{-1}) was utilized to establish the percentage of unreactive carbon–carbon double bonds (% C═C) before and after curing the specimen in reference to an internal standard of aromatic C—C (peak at 1608 cm^{-1}) (Ribeiro et al., 2012). The degree of conversion (DC) was calculated following the formula below (Ribeiro et al., 2012):

$$DC(\%) = \left(1 - \frac{(A_{1637}/A_{1608})_{postcure}}{(A_{1637}/A_{1608})_{beforecure}}\right) \times 100$$

2.4. Statistical analysis

All data were analyzed via one-way analysis of variance (ANOVA). Post-hoc multiple comparisons were performed using Tukey's honest significant difference test. IBM SPSS version 23 (SPSS Inc., IBM, Chicago, IL, USA) at 5 % significance level was used to perform the statistical analysis.

3. Results

The mechanical properties and degree of conversion of the tested groups are listed in Table A.

Flexural strength and elastic modulus of all groups (mean ± SD, $n = 8$) are demonstrated in Fig. B. The flexural strength was significantly higher in Tetric N Ceram (115.8 ± 7.9) when compared to Tetric Evo Ceram (93.3 ± 9.4) and 5 % DMAHDM (79.6 ± 18.6) ($p < 0.05$) while it is similar to 3 % DMAHDM (97.6 ± 10.9) ($p > 0.05$). However, the flexural strength of Tetric Evo Ceram (93.3 ± 9.4) matched those of 3 % (97.6 ± 10.9) and 5 % DMAHDM (79.6 ± 18.6) ($p > 0.05$).

The elastic modulus value of Tetric N Ceram (15.9 ± 0.81) was significantly higher compared to all other groups ($p < 0.05$), while other groups had similar elastic modulus values ($p > 0.05$).

The Vickers microhardness values of all groups (mean ± SD, $n = 5$) are demonstrated in Fig. C. The Vickers microhardness value of Tetric N Ceram (0.557 ± 0.036) was significantly higher than all other groups ($p < 0.05$). The Vickers microhardness value of Tetric Evo Ceram (0.508 ± 0.044) was significantly higher in comparison to 5 % DMAHDM (0.394 ± 0.021) ($p < 0.05$). However, it was similar to 3 % DMAHDM (0.484 ± 0.016) ($p > 0.05$).

Degree of conversion of the test groups is presented in Fig. D. Among all groups, there was no statistically significant difference ($p > 0.05$).

4. Discussion

In this study, a contact-killing antibacterial agent (DMAHDM) was incorporated to a commercial resin-based composite, Tetric N Ceram, and the mechanical properties and degree of conversion were investigated and compared with a fluoride-releasing resin-based composite, Tetric Evo Ceram. DMAHDM was added in 3 % concentration, which is the minimum concentration that produces antibacterial effect (Mitwalli et al., 2020a, 2020b). Similarly, we added DMAHDM in 5 % concentration, as this is the maximum acceptable concentration that does not jeopardize the material properties with regard to the minimum requirements of the International Organization for Standardization (ISO) standard 4049:2019 (ISO, 2019). DMAHDM lowered the mechanical properties when higher concentrations were used. Nevertheless, the flexural strength values of DMAHDM groups, (97.6 ± 10.9) and (79.6 ± 18.6) for 3 % and 5 % DMAHDM respectively, were within the acceptable range of ISO standard 4049:2019 (ISO, 2019). Furthermore, 3 % DMAHDM showed mechanical properties that were comparable to those of Tetric Evo Ceram. The addition of DMAHDM at either concentration did not influence the degree of conversion of the resin-based composite. This could be attributed to the fact that DMAHDM was simply added to the commercial resin-based composite. To date, most studies on DMAHDM have investigated the effects of adding DMAHDM to experimental composite formulations (Balhaddad et al., 2020; Zhang et al., 2016).

The contact-killing antibacterial agent DMAHDM is added to the resin-based composite material to improve the antimicrobial quality of the composite material in an attempt to decrease the possibility of secondary caries at the interface between the tooth and the restoration (Mitwalli et al., 2020a, 2020b). In a previous study, it was shown that the addition of 3 % DMAHDM to resin composites reduced the growth and activity of biofilms more effectively than other QAMs without having an impact on the mechanical characteristics of the material (K. Zhang et al., 2016). In another study, the addition of a higher concentration (5 %) of DMAHDM to resin composite increased the antibacterial activity while maintaining acceptable mechanical properties (Balhaddad et al., 2020). However, in this study, the addition of DMAHDM decreased the elastic modulus and Vickers microhardness of the Tetric N Ceram composite material. While the flexural strength of the composite material was reduced with increased concentration of DMAHDM (5 %). Meanwhile, when compared to Tetric Evo Ceram, a fluoride releasing composite, adding DMAHDM at 3 % and 5 % showed comparable

Table A
The flexural strength, elastic modulus, Vickers microhardness, and degree of conversion of the tested groups.

Groups	Materials	Flexural Strength (mean ± SD, n)	Elastic Modulus (mean ± SD, n)	Vickers Microhardness (mean ± SD, n)	Degree of Conversion (mean ± SD)
Group I	**Tetric N Ceram**	(115.8 ± 7.9, 8) [a]	(15.9 ± 0.81, 8) [a]	(0.557 ± 0.036, 5) [a]	(50.1 ± 2.84) [a]
Group II	**Tetric Evo Ceram**	(93.3 ± 9.4, 8) [b]	(13.4 ± 1.03, 8) [b]	(0.508 ± 0.044, 5) [b]	(49.3 ± 2.07) [a]
Group III	**Tetric N Ceram + 3 % DMAHDM**	(97.6 ± 10.9, 8) [a,b]	(13.2 ± 0.65, 8) [b]	(0.484 ± 0.016, 5) [b]	(49.5 ± 4.7) [a]
Group IV	**Tetric N Ceram + 5 % DMAHDM**	(79.6 ± 18.6, 8) [b]	(11.6 ± 0.81, 8) [b]	(0.394 ± 0.021, 5) [c]	(48.6 ± 4.25) [a]

The flexural strength was measured in (MPa), the elastic modulus was measured in (GPa), the Vickers microhardness was measured in (GPa), and the degree of conversion was measured in (%).
The different letters indicate significant differences.

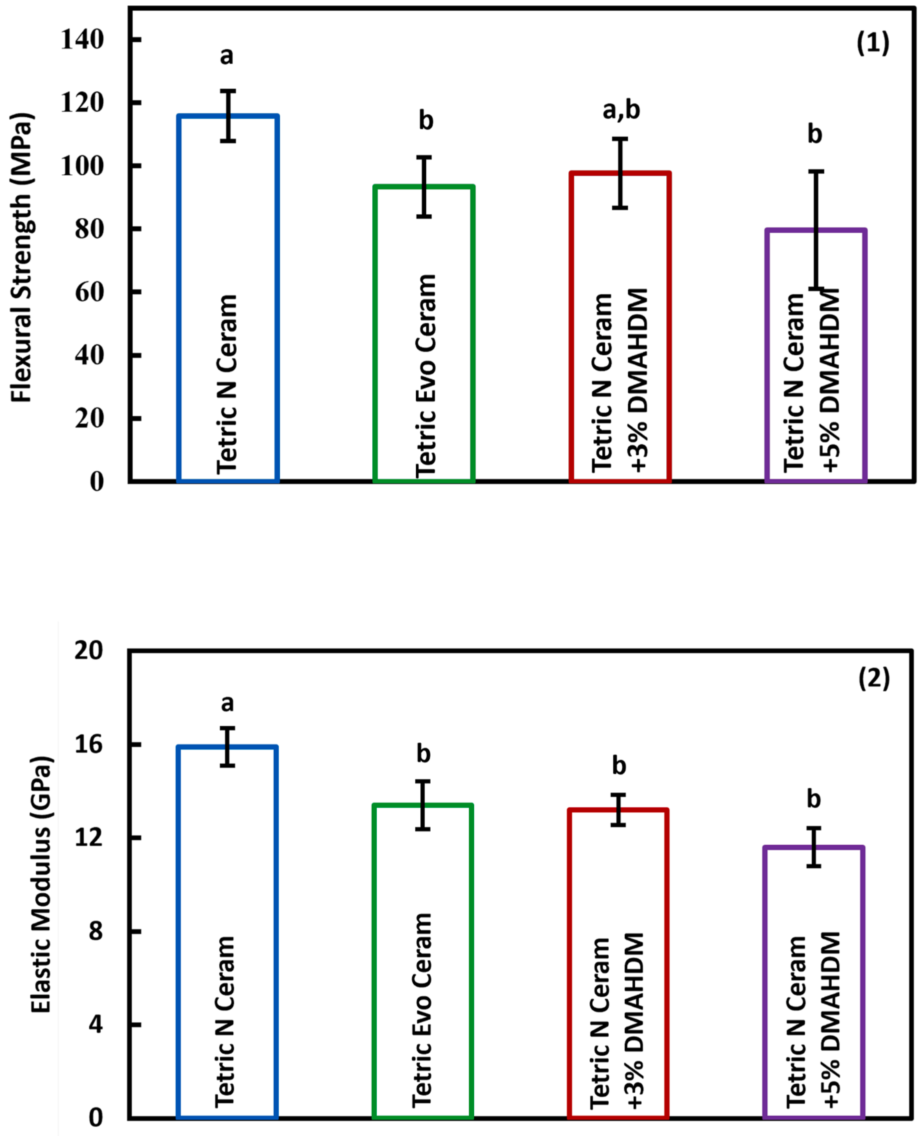

Fig. B. (1) Flexural strength (mean ± SD, n = 8). The flexural strength was significantly higher in Tetric N Ceram (115.8 ± 7.9) when compared to Tetric Evo Ceram (93.3 ± 9.4) and 5 % DMAHDM (79.6 ± 18.6) ($p < 0.05$). However, flexural strength of Tetric Evo Ceram (93.3 ± 9.4) matched those of 3 % (97.6 ± 10.9) and 5 % DMAHDM (79.6 ± 18.6) ($p > 0.05$). The group with 3 % DMAHDM (97.6 ± 10.9) matched that of Tetric N Ceram ($p > 0.05$). (2) Elastic modulus (mean ± SD, n = 8). The elastic modulus value of Tetric N Ceram (15.9 ± 0.81) was significantly greater than all other groups ($p < 0.05$), while other groups had comparable elastic modulus values ($p > 0.05$).

mechanical properties. Tetric Evo Ceram is a fluoride-releasing, resin-based composite. In addition to its biocidal effect, fluoride ions contribute to caries inhibition by forming fluoroapatite crystals, which reduce enamel solubility and remineralize decalcified tooth structure (Featherstone et al., 1990; Ten Cate, 1990; Zheng et al., 2015). Furthermore, fluoride reduces lactic acid production through neutralization, which may help inhibit recurrent caries (Cheng et al., 2012). However, fluoride release and recharge have only been observed over a short period (Naoum et al., 2011). Therefore, the sustained fluoride release and recharge ability of resin-based composites containing fluoride over long periods of time has not yet been assessed (Wiegand et al., 2007). In contrast, DMAHDM offers long-lasting antibacterial effects through contact killing without leaching (Balhaddad et al., 2019).

In this study, an antibacterial agent was added to a commercial resin-based composite, which could result in improper integration of DMAHDM into the ready-made material. Because DMAHDM may not have been chemically mixed into the resin composite material, this could compromise the mechanical characteristics of the composite material, as observed in this current study. This could be an area of further investigation, with the utilization of other techniques to finely grind DMAHDM particles to aid their incorporation. Future studies should focus on testing the antimicrobial effects of DMAHDM in vivo to better understand its clinical relevance and determine whether it causes long-term mechanical or biological adverse effects.

5. Conclusion

Although the addition of a contact-killing antibacterial material (DMAHDM) to Tetric N Ceram composite material negatively influenced the mechanical properties of the material, especially at higher concentrations (5 %) and lower concentrations (3 %), the mechanical properties that were comparable to Tetric Evo Ceram. Moreover, the mechanical properties of Tetric N Ceram containing DMAHDM were within the ISO-recommended ranges. The incorporation of DMAHDM does not influence the degree of conversion of Tetric N Ceram composite.

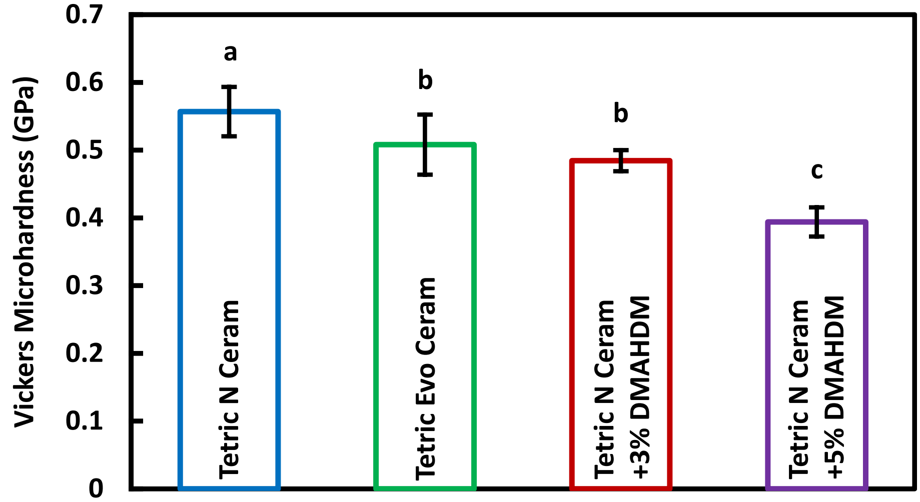

Fig. C. Vickers microhardness (mean ± SD, n = 5). The Vickers microhardness value of Tetric N Ceram (0.557 ± 0.036) was significantly greater than all other groups ($p < 0.05$). In addition, it was significantly higher in Tetric Evo Ceram (0.508 ± 0.044) when compared to 5 % DMAHDM (0.394 ± 0.021) ($p < 0.05$). However, the Vickers microhardness value of the group with 3 % DMAHDM (0.484 ± 0.016) was comparable to that of Tetric Evo Ceram (0.508 ± 0.044) ($p > 0.05$).

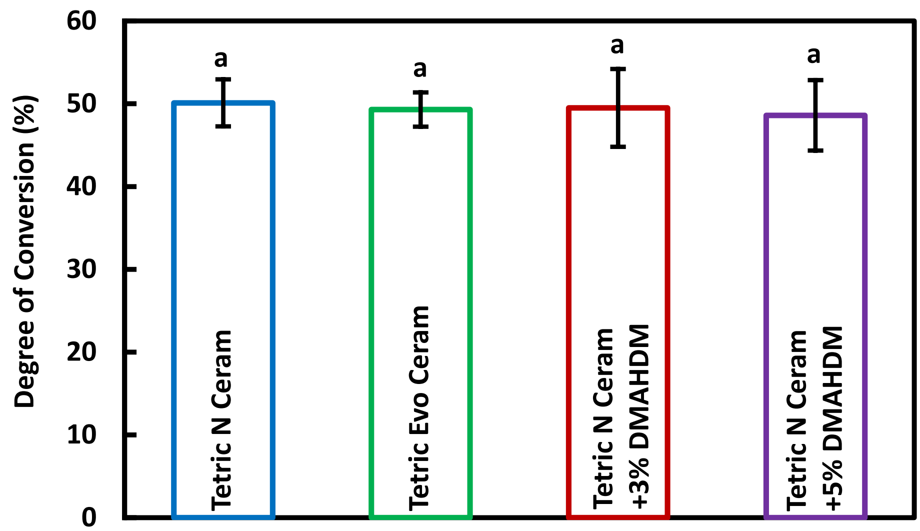

Fig. D. Degree of conversion of the four test groups. Among all groups, there was no statistical difference ($p > 0.05$).

6. Ethical consent

The authors declare that informed consent was obtained from each subject after receiving approval of the King Saud University. The authors also declare that this manuscript has not been submitted or published elsewhere.

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Author contributions

H.A.M. contributed to the design, data acquisition, and analysis, and revised the manuscript; B.H.B. contributed to the design, data analysis, and revised the manuscript; S.S.S. contributed to the design, data acquisition, and analysis, and drafted the manuscript; H.H.K.X. and M.D. W. revised the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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