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Evaluation of polymerization characteristics and penetration into enamel caries lesions of experimental infiltrants

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ABSTRACT

Objectives: To evaluate the properties of experimental infiltrant blends by comparing them with the commercial infiltrant Icon® and penetration homogeneity into enamel caries lesions.

Methods: Groups were set up as follows: G1 (TEGDMA 100%); G2 (TEGDMA 80%, Ethanol 20%); G3 (TEGDMA 80%, HEMA 20%); G4 (TEGDMA 75%, BisEMA 25%); G5 (TEGDMA 60%, BisEMA 20%, Ethanol 20%); G6 (TEGDMA 60%, BisEMA 20%, HEMA 20%); G7 (TEGDMA 75%, UDMA 25%); G8 (TEGDMA 60%, UDMA 20%, Ethanol 20%); G9 (TEGDMA 60%, UDMA 20%, HEMA 20%) and Icon®. Ten specimens were comprised by each group for the following tests ($n = 10$): degree of conversion (DC), elastic modulus (EM), Knoop hardness (KH), and softening ratio (SR). Infiltrant penetration was evaluated using confocal microscopy (CLSM). Data were subjected to two-way ANOVA and a Tukey's test (5%). Data comparing experimental materials and Icon® were analysed using ANOVA and Dunnett's test (5%).

Results: The highest DC values were found in G1, G7, G8, and G9. The lowest DC values were found in G2, G4, G5, and G6. EM and KHN were significantly lower in HEMA and with ethanol addition for all blends, except for G9. There was no significant difference among the groups regarding SR, and it was not possible to take KHN readings of G2, G5, and G8 after storage. There was no significant difference among groups for infiltrant penetration into enamel lesions.

Conclusions: The addition of hydrophobic monomers and solvents into TEGDMA blends affected DC, EM, and KHN. UDMA added to TEGDMA resulted in an increase in DC, EM, and KHN. Overall, solvents added to monomer blends resulted in decreased properties. The addition of hydrophobic monomers and solvents into TEGDMA blends does not improve the penetration depth of the infiltrants.

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1. Introduction

Minimum intervention dentistry (MID) is the modern medical approach to the management of caries lesions. It has been shown that it is a good approach, since over time, sealing of carious dentine results in lower levels of infection[1] and can allow higher dental tissue preservation than traditional dentine caries removal.[2] MID is based on caries risk assessment and focusing on the early prevention and interception of disease.[3–5] The remineralization of an early enamel lesion could be achieved by an improvement in the patient's oral hygiene and by local fluoridation.[6] However, remineralizing conditions are difficult to reach and depend on good oral hygiene.[7]

While healthy enamel microstructure reveals regular periodicity of prisms of hydroxyapatite[8,9] a promising approach to arrest early caries lesions might be the infiltration of enamel subsurface lesions with low-viscosity light-curing resins.[10] The ability of resins to penetrate into porous enamel lesions was firstly described more than 30 years ago.[11]

Regarding lesion progression, there are some clinical studies in the literature.[12–15] The evaluation of the progression of the lesion is performed in patients with high, medium and low caries risk, comparing control and experimental (sealed lesions) and showed that infiltrated/sealed groups have increased therapeutic effect when compared to nonsealed/noninfiltrated lesions.[12,13] However, it is known that in low caries risk patients, many of these lesions will remain in enamel for at least twelve months and do not require treatment.[9] In addition, studies have shown that in low caries risk patients receiving regular topical fluoride therapy, progression could take forty months.[16] Consequently, the time for evaluating caries progression plays an important role in clinical studies comparing techniques or therapeutics.

Studies conducted by some authors[12,13] showed a range from 25% to 37.8% on therapeutic effect of infiltration technique, depending on the age group and material (infiltrant or adhesive system). It should be considered that the therapeutic effect can be directly related with the material physic-chemical and mechanical properties. In order to increase the therapeutic effect, materials properties should be improved, since ideally, an infiltrant should present low viscosity, low surface tension, and acceptable mechanical properties that support dental abrasion and oral degradation.[10]

TEGDMA-based materials show appropriate characteristics for an infiltrant material, including low viscosity and high degree of conversion. However, this monomer is highly hydrophilic and may undergo degradation in an oral environment, reducing the clinical performance.[17] Thus, the addition of UDMA or BisEMA, which are considered more hydrophobic monomers with low viscosity than TEGDMA (BisEMA – 0.03 Pa s; UDMA – 1.23 Pa s),[18] could be interesting.

Although studies[19] using confocal microscopy show that TEGDMA neat monomer blends demonstrate satisfactory penetration, TEGDMA reduction and adding BisEMA or UDMA in blends could result in satisfactory curing properties. On the other hand, a high penetration coefficient, which describes the penetration of liquids into porous solids driven by capillary forces[20] can also be achieved through the addition of diluents. Paris et al.[21] found that mixtures containing HEMA and ethanol showed the highest penetration coefficient; however, in some cases, the polymerization was deficient, and the final material was rubbery or even liquid. Therefore, the addition of a solvent such as ethanol increases the penetration coefficient, but it could jeopardize the mechanical properties, such as degree of conversion, flexural strength, elastic modulus, hardness and cross-link density. Nevertheless, although the DC is an important factor, it does not provide a complete characterization of the network structure. Cross-linking density test indicate pendant double bonds that are tied into the polymer network. Cross-linking density is an important factor for good network formation and physical properties.[22] Cross-linking density has been indirectly assessed by polymer softening after exposure to ethanol.[23]

The first aim of this study was to evaluate the effect of hydrophobic monomers and solvents on properties (degree of conversion, Knoop hardness, softening ratio, elastic modulus) of experimental infiltrant blends and comparing them to a commercially available infiltrant, Icon® (DMG, Germany). The second aim was to evaluate the penetration depth of the materials as well as their homogeneity into enamel caries lesions.

2. Materials and methods

2.1. *Infiltrant preparation*

The following monomers were used in different combinations, as described in Table 1: triethyleneglycol dimethacrylate (TEGDMA) (Sigma–Aldrich Inc., St. Louis, MO, USA, Batch #01612M), ethoxylated bisphenol A glycidyl dimethacrylate (BisEMA) (Sigma–Aldrich, Inc., St. Louis, MO, USA, Batch #03514HF), diurethane dimethacrylate (UDMA) (Sigma–Aldrich, Inc., St. Louis, MO, USA, Batch #09405BJ), 2-hydroxy-etilmetacrylate (HEMA) (Sigma–Aldrich, Inc., St. Louis, MO, USA, Batch #MKBF2452V), and ethanol (Sigma–Aldrich, Inc., St. Louis, MO, USA, Batch #51496AM). The light-curing initiator system selected for photoinitiation was camphorquinone (CQ) (Sigma–Aldrich, Inc., St. Louis, MO, USA, Batch #532604), and dimethyl aminoethyl methacrylate (DMAEMA)

Table 1 – Infiltrant blends composition.

Infiltrant icon	Composition Methacrylate-based resin matrix
G1	TEGDMA 100%
G2	TEGDMA 80%, Ethanol 20%
G3	TEGDMA 80%, HEMA 20%
G4	TEGDMA 75%, BisEMA 25%
G5	TEGDMA 60%, BisEMA 20%, Ethanol 20%
G6	TEGDMA 60%, BisEMA 20%, HEMA 20%
G7	TEGDMA 75%, UDMA 25%
G8	TEGDMA 60%, UDMA 20%, Ethanol 20%
G9	TEGDMA 60%, UDMA 20%, HEMA 20%

(Sigma–Aldrich, Inc., St. Louis, MO, USA, Batch #BCBF8391V) was used as the co-initiator (proportion 1:2 by weight; 0.5% CQ/1% DMAEMA). Also, the inhibitor BHT (butylated hydroxytoluene – Sigma–Aldrich Inc., St. Louis, USA, Batch # 04416KD) was added to the resin blends at a concentration of 0.1 wt%.

2.2. Properties assessment

Ten bar specimens (5 mm × 2 mm × 1 mm) from each group were produced with polyvinylsiloxane moulds (Aquasil LV, Dentsply DeTrey, Denver, USA). The specimens were light-cured at 1000 mW/cm^2 for 60 s using Free Light 2 (3M/ESPE, St Paul, USA) under a polyester strip (Airon, Maquira Dental Products Industry, Maringá, Brazil). Specimens were dry stored for 24 h in lightproof containers at 37 °C. Then, degree of conversion, elastic modulus, Knoop hardness, and softening ratio evaluations were performed in the same specimen.

2.3. Degree of conversion (DC)

DC was measured by Fourier Transform Infrared Spectroscopy (FTIR) (Spectrum 100, Perkin Elmer, Beaconsfield, UK) and was determined from the aliphatic C=C and carbonyl C=O peaks for unpolymerized and polymerized resin, according to the standard baseline technique.[24] The remaining unconverted double bonds were determined by comparing the ratio of the aliphatic C=C absorption peak at 1638 cm^{-1} to the carbonyl group C=O peak at 1716 cm^{-1} between the polymerized and unpolymerized specimens.[25] The absorption of the carbonyl C=O stretching band served as an internal standard, as it remains constant during the polymerization reaction. The monomer conversion was determined by subtracting the percentage of residual aliphatic C=C bonds from 100%.

2.4. Elastic modulus (EM)

The three point-bending flexural test was performed to assess EM in a universal testing machine (INSTRON, model 4111, Instron Corp., OH, USA). The test was performed with a crosshead speed of 0.5 mm/min and a cell load of 50 N until fracture. The distance between supports was 3 mm. EM was calculated using Bluehill 2 software (Illinois Tool Works, Inc., IL, USA) coupled to the universal testing machine.

2.5. Knoop hardness number (KHN) and softening ratio (SR)

KHN measurements were taken on the irradiated surface using an indenter (HMV-2, Shimadzu, Tokyo, Japan), under a load of 490 N (equivalent to 50 g) for 15 s. Three readings were performed for each specimen. The initial Knoop hardness number (KHN 1) was the average of the three indentations. Thereafter, the specimens were stored in absolute ethanol for 24 h at room temperature, and hardness was again determined (KHN2). The rate of softening was determined using following equation: 100 − (KHN2/KHN1 × 100). This softening test is considered an indirect estimation of the cross-link density.

2.6. Penetration into artificially produced enamel caries lesions

2.6.1. Artificial caries-like lesion formation

Fifty-five caries and restoration free third human molars were collected. Approval was given by the Ethic Committee in Research of the Dental School of Piracicaba – UNICAMP, 046/2006 protocol. Teeth roots were removed and discarded. The sound surfaces, including occlusal surfaces, were covered with two layers of acid-resistant nail varnish (Colorama®, São Paulo, Brazil), leaving a window of sound enamel on the occlusal surface (5 mm × 5 mm). Each enamel was put individually into 50 mL (2.0 mL/mm^2 of exposed enamel) of 0.05 M acetate buffer solution, pH 5.0, at 50% hydroxyapatite saturation, for 10 h at 37 °C.[26] The enamel surfaces were randomly distributed in nine experimental groups (n = 5) and one commercially-available material, Icon® group, as discussed below.

2.7. Lesion infiltration and preparation for confocal laser scanning microscopy (CLSM)

Caries-like lesions were etched with 37% phosphoric acid gel Magic Acid (Vigodent, Rio de Janeiro, Brazil) for 60 s,[27] washed with water spray, and dried for 15 s. Infiltrants were impregnated with 0.1% rhodamine B isothiocyanate (RITC) (Sigma–Aldrich, St. Louis, USA), applied onto the caries-like lesion using a microbrush, and left to penetrate for 60 s.[28] All groups were light cured for 60 s with Free Light 2 (3M/ESPE, St Paul, MN, USA) with 1000 mW/cm^2 irradiance. Next, tooth sections with 0.5 mm thickness were obtained perpendicularly to the lesion surface, impregnated with the materials using a diamond saw (Isomet 1000, Buhler, Lake Bluff, IL, USA), and polished in a SiC papers series. To visualize the porous structure (not infiltrated lesion parts), specimens were immersed in a 50% ethanolic solution of 100 μM sodium fluorescein (NaFl) (Sigma–Aldrich) for 3 min and washed in deionized water for 10 s.

2.8. CLSM evaluation

Specimens were observed with a confocal laser scanning microscope (Leica, TCS NT; Leica, Heidelberg, Germany) using a 10× objective in dual fluorescence mode as described previously.[19]

Measurements were made using LAS AF Lite tool software (CLSM – Leica, TCS SP5, Mannheim, Germany). The evaluator was blind with regard to the group allocation of the teeth. CLSM was validated previously as a method to evaluate caries infiltration with excellent inter- and intra-examiner reproducibility in natural caries lesions.[19]

2.9. Statistical analysis

The null hypotheses[29] in this study were as follows: Addition of monomers (UDMA and BisEMA) or diluents (HEMA and ethanol) to TEGDMA will not affect infiltrant's properties or the infiltrant penetration depth and homogeneity in the caries-like lesion; association of hydrophobic monomers (BisEMA and UDMA) and diluents (HEMA and ethanol) will not affect

Table 2 – Mean values of degree of conversion (%), elastic modulus (GPa), Knoop hardness (KHN), and softening ratio (%) comparing a commercial infiltrant (Icon) and the experimental infiltrants.

Experimental infiltrants	Degree of conversion	Elastic modulus	Knoop hardness	Softening ratio	Penetration depth
Icon	98.41	0.90	6.54	45.59	170.6 (30.7)
G1	98.58 A	1.15 A	12.01* A	32.72 A	98.2 (30.2) A
G2	60.12* C	0.16* C	1.92 C	[a]	97.5 (22.9) A
G3	76.76* B	0.70 B	8.86 B	42.25 A	124.1 (35.3) A
G4	69.03* C	0.95 B	13.08* A	43.25 A	157.9 (50.8) A
G5	56.55* C	0.05* C	2.09 C	[a]	120.6 (32.8) A
G6	68.14* C	0.76 B	10.32* B	51.13 A	141.5 (43.10) A
G7	78.30* A	1.12 A	16.04* A	49.27 A	164.8 (60.5) A
G8	80.40* A	0.30* C	3.77 C	[a]	120.5 (32.7) A
G9	81.01* A	1.01 A	14.74* B	56.53 A	117.6 (8.3) A

Different letters indicate statistically significant difference ($p < 0.05$).
[a] Indicate that it was impossible to measure Knoop hardness.
* Indicate statistically significant difference from the control (Icon) ($p < 0.05$), according to Dunnet's test.

the infiltrant properties or infiltrant penetration depth and homogeneity in the caries-like lesion.

Data from DC, EM, KHN, CLD and penetration depth were subjected to two-way ANOVA and Tukey's test for post hoc comparisons between groups, and the significance level was set at $\alpha = 0.05$. Additionally, data from all experimental materials were compared to a commercial material, Icon®, using Dunnett's test, and the significance level was set at $\alpha = 5\%$. Statistical analyses were performed by Assistat software (Campina Grande, Brazil).

3. Results

3.1. *Evaluation of polymer properties and mechanical properties of experimental infiltrants*

The results of polymer properties are presented in Table 2. The lowest DC was showed by all BisEMA blends and the one containing TEGDMA + ethanol. The UDMA addition did not jeopardize the DC. For EM, the ranking was as follows: G1 = G7 = G9 > G3 = G4 = G6 > G2 = G5 = G8. The addition of any solvent caused a significant reduction in KHN values.

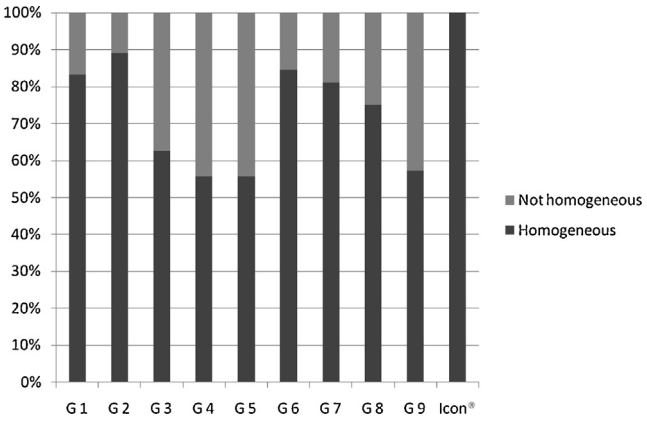

Fig. 1 – Qualitative analysis of the groups concerning percentage of material penetration on enamel caries-like lesion (not homogeneous and homogeneous classification).

The addition of ethanol to base monomers resulted in the lowest KHN values for all experimental groups, and HEMA-containing blends presented intermediary results. There was no significant difference in the softening ratio among the groups. The addition of HEMA/Ethanol did not affect the softening ratio. In addition, for ethanol-containing blends, it was not possible to take hardness readings after ethanol storage. The specimens became rubbery, hindering a reliable assessment of hardness.

3.2. *Evaluation qualitative analysis of depth penetration*

A qualitative analysis was employed to evaluate the homogeneity degree of the penetrated materials into the lesion body (homogeneous/non-homogeneous), and the results are presented in Fig. 1. There was no significant statistical difference among all experimental groups ($p > 0.05$) for both quantitative and qualitative analyses.

3.3. *Comparative analysis infiltrants: experimental × commercial*

Table 2 shows that the TEGDMA-based experimental infiltrant showed a DC similar to the commercial infiltrant Icon®. The other experimental blends showed a lower DC than Icon®. Regarding EM, the experimental infiltrants—diluent free and those containing HEMA—showed similar results to Icon®. It was also observed that the KHN of all diluent-free blends and those containing TEGDMA + UDMA + HEMA or TEGDMA + BisEMA + HEMA were significantly higher than those observed for Icon®. There was no difference between the experimental and commercial infiltrants in softening ratio and penetration depth. Only Icon® showed all samples with homogeneous penetration, but it did not show a statistically significant difference from experimental groups.

4. Discussion

Overall, this study found that ethanol negatively affected the curing characteristics and mechanical properties, confirming the hypothesis that the addition of solvents reduces the polymer's mechanical properties. These results corroborate

those obtained by other authors.[19,22] However, in this study for UDMA blends, it was not really true since UDMA blends did not show a significant difference concerning DC. Adding a solvent to monomer blends can prompt the formation of microgels near the centres of initiation, which become sites of low mobility of radicals, thus decreasing polymerization. These chains form a heterogeneous distribution of chain mobility. The greater the amount of solvent, the greater the formation of microgels and the greater the accuracy of heterogeneity can be verified.[30–32] In addition, a previous study[14] considered a valid attempt to add ethanol and HEMA to infiltrants to reduce viscosity and increase the depth of penetration; the results of this study indicate that adding a solvent to infiltrant blends, except for UDMA blends, even in small quantities, damaged the DC, EM, softening ratio, and KHN. However, the addition of solvents/diluents did not show a difference among the groups in homogeneity of penetration, so the second null hypothesis can be partially rejected. Considering the clinical application of infiltrants, this addition can be more prejudicial than beneficial, producing polymers with unsatisfactory properties.

This study also evaluated the addition of higher-molecular-weight monomers to TEGDMA. The addition of BisEMA to TEGDMA was observed to significantly reduced DC. This may be due to its high molecular weight (540 g/mol), lower chain flexibility than TEGDMA, and, consequently, lower DC.[33] However, it is important to state that BisEMA increases the hydrophobic characteristics of the infiltrant, which could be interesting because more hydrophobic material tends to show reduced degradation in the oral environment.[34]

The addition of UDMA resulted in an increase in DC, EM, and KHN, confirming the hypothesis that the addition of UDMA to TEGDMA could improve the mechanical properties of infiltrants. This may have occurred due to the similarity in the characteristics of the chains between the monomers. The monomer UDMA, as well TEGDMA, presents linear chains. They have flexible cores and two aliphatic urethane linkages that are capable of forming hydrogen bonds. UDMA has a small size, low viscosity (1.23 Pa s), and a high amount of double bonds. In addition, it shows flexible linkage, which characterizes the increase EM when UDMA is added to a mixture.[26]

According to the CLSM analysis, variation in the blend composition through the addition of high molecular monomers did not significantly affect the homogeneity of penetration. This was observed maybe due to the high standard deviation observed in this study. A high standard deviation was also observed by the study conducted by Paris et al.[19] However, it can be inferred that the association of hydrophobic monomers and diluents could result in materials with satisfactory properties, and homogeneous penetration.

In this study, the experimental infiltrants were compared to Icon®. Concerning DC, the experimental infiltrant most similar to the commercial was the neat TEGDMA blend. Regarding the EM, experimental infiltrants that presented similar results to the commercial material were those that did not contain ethanol in its composition. The softening ratio was not significantly different between the commercial and experimental materials. Regarding penetration depth, the Icon® group presented high homogeneity in all groups (100% homogeneous) while in the experimental materials, homogeneity varied between homogeneous (100%) and non-homogeneous (about 70%). Although there were no significant differences regarding the homogeneity of penetration among the experimental blends, it could be claimed that the addition of solvents harms properties. With time, these results could lead to further degradation; thus, studies are necessary to investigate the behaviour of these materials with greater longevity.

From these findings, other assumptions can be explored. First, the evaluation of penetration coefficient and bond strength to enamel should be explored to indicate the ideal infiltrant. Further, it would be relevant to verify the clinical significance of these chemical and mechanical property variations of polymers since, in the oral cavity, the sum of thermal, mechanical, and chemical stimuli occur concurrently and repeatedly.

5. Conclusions

The results of this study show that the addition of hydrophobic monomers and solvents into TEGDMA blends affected DC, EM, and KHN. UDMA added to TEGDMA resulted in an increase in DC, EM, and KHN. Overall, solvents added to monomer blends resulted in decreased properties, mainly when ethanol was added to TEGDMA neat monomer and TEGDMA + BisEMA. Moreover, the addition of hydrophobic monomers and solvents into TEGDMA blends does not improve the penetration depth and homogeneity of the infiltrants.

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