Influence of Monomers and Solvents on the Contact Angle, Penetrability and Knoop Hardness of Experimental Resin Infiltrants into Caries-Like Lesions

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Received: 02-Sep-2022, Manuscript No. OHDM-22-17916; **Editor assigned:** 05-Sep-2022, Pre QC No. OHDM-22-17916 (PQ); **Reviewed:** 21-Sep-2022, QC No. OHDM-22-17916; **Revised:** 03-Oct-2022, Manuscript No. OHDM-22-17916 (R); **Published:** 10-Oct-2022, DOI: 10.35248/2247-2452.22.21.1021.

ABSTRACT

Objective: To evaluate the influence of monomers Urethane Dimethacrylate (UDMA), Ethoxylated Bisphenol A Glycidyl Dimethacrylate (BisEMA), and solvents Tetrahydrofuran (THF) and Dimethyl Sulfoxide (DMSO) on the contact angle, penetrability and Knoop microhardness of resin infiltrants into caries-like lesions.

Methods: Eleven groups were evaluated: 1) Icon; 2) 75% TEGDMA (T)+25%UDMA (U); 3) T+U+0.5%DMSO; 4) T+U+5%DMSO; 5) T+U+0.5%THF; 6) T+U+5%THF 7) 75% T+25%BisEMA(B); 8) T+B+0.5%DMSO; 9) T+B+5%DMSO; 10) T+B+0.5%THF; 11) T+B+5%THF. The contact angle measurement was performed in a viscosimeter (n=5). The resin infiltrants penetrability (n=5) by Confocal Laser Scanning Microscopy by qualitative analysis and Knoop microhardness (n=10) was evaluated in caries-like lesions produced on the enamel of bovine teeth. Contact angle data were submitted to one-way ANOVA and Tukey's test. Knoop hardness was evaluated by two-way ANOVA and Tukey's test. It was used 5% significance level.

Results: The icon showed a contact angle significantly lower (11.4(2.4)) than the other groups. T+U+5%DMSO (16.7(3.3)) showed significantly lower contact angle with statistical difference ($p<0.001$) when compared with T+U (29. 8(6.3)); T+U +0.5%DMSO (29.5(5.5)); T+U+5%THF (31.8(3.7)); T+B+0.5%DMSO (32.3(5.7)); and T+B+0.5%THF (29.8(3.6)) ($p=0.0751$). Confocal Laser Scanning Microscopy showed good penetration into the demineralized area of infiltrants with TEGDMA+UDMA blend base and Icon. Caries-like lesions infiltrated by Icon showed Knoop hardness significantly except for the T+U+0.5%DMSO and T+U+5%THF groups. Thus, the Knoop hardness significantly increased in deeper sites.

Conclusion: The solvent with a lower concentration (0.5%) associated with the BisEMA monomer did not provide greater penetration of the experimental infiltrants. Higher solvent concentrations are not recommended.

Key Words: Contact angle, Knoop hardness, Confocal microscopy, Solvent, Infiltrant.

Introduction

The resin infiltration technique is based on masking the lesion by infiltrating the porous enamel subsurface with a hydrophobic composite resin that has a refraction index closer to sound enamel [1]. Combining this ultraconservative restorative approach (which is considered microinvasive) with a substantial caries remineralization program may provide therapeutic benefits and significantly reduce both long-term restorative needs and costs, thus complementing the concept of minimal intervention dentistry [2]. This caries infiltration technique also aims to occlude the pores impeding the continuous diffusion of acids and dissolved minerals through the lesion, so it can hamper caries progression [3].

Resin infiltration of enamel caries lesions requires materials optimized for penetration into the capillary structures of the lesion body. Therefore, coefficient penetration increases the outcomes of penetration and caries-inhibiting properties of low-viscosity composite resins can also be improved [4]. The penetration of infiltrants into the pores of the lesion body is mainly driven by capillary forces and depends on the penetration time, the capillary radius, and the penetration coefficient of the liquid [5]. The addition of fillers to infiltrant resin might combine the high penetration with higher applicability of composite resins [6]. Materials based on methacrylate have been developed and studied to penetrate the lesion body and reinforce the weakened structure of enamel caries lesions [7].

The penetration coefficient combines the liquid properties viscosity, surface tension, and contact angle to the solid. The time required for a sealant to penetrate a specific distance is highly dependent on the penetration coefficient of the sealant [8]. Materials with different penetration coefficient and different concentrations of ethanol were tested in natural caries lesions in permanent teeth and deepest and most homogeneous infiltration was obtained with a solvent-free composite resin. A solvent-free resin mainly consisting triethylene glycol dimethacrylate seems to be preferable [4]. Also was found that the addition of solvent ethanol or HEMA into TEGDMA blends does not improve the penetration depth and homogeneity of the infiltrants [9].

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Was introduced in the dental market with the infiltrant material Icon (DMG, Hamburg, Germany) to infiltrate the incipient carious lesions. This material based on TEGDMA has been studied and shown success [10]. After evaluation over 18 months showed satisfactory results, thereby demonstrating that progression of lesions *in vivo* interproximal caries was interrupted. The current results corroborate that caries infiltration is an efficacious method to hamper the progression of non-cavitated proximal lesions which extended radiographically into the inner half of enamel [11]. The addition of alternative solvents can improve the chemical stability of infiltrating as well as adhesives improving the miscibility and diffusion of the monomers [12]. The solvent Dimethyl Sulfoxide (DMSO) is used in multiple biochemical and pharmacological practices due to its high polarity [13]. Tetrahydrofuran (THF) was reported promising as an alternative solvent due to its advantageous volatile properties [14]. These are promising solvents that can decrease the viscosity of infiltrants and promote higher lesion infiltration. Currently, little has been reported in the literature about the association and its benefits on the physical-mechanical properties of resin infiltrants.

The present this *in vitro* study aims to evaluate the influence of solvents THF and DMSO used in infiltrants materials experiments on properties such as Knoop hardness, contact angle, and penetration of the materials into enamel caries lesions. The null hypothesis will be no difference between resinous infiltrants and Icon on the variables tested.

Materials and Methods

Formulation of Infiltrants

The monomers used to prepare the infiltrants were Triethylene Glycol Dimethacrylate (TEGDMA), Ethoxylated Bisphenol A Glycidyl Dimethacrylate (BisEMA), Urethane Dimethacrylate (UDMA), Dimethyl Sulfoxide (DMSO), tetrahydrofuran (THF) in different ratios, as described in *Table 1*. All reagents were purchased from Sigma-Aldrich Inc. (St. Louis, USA). For all blends, the photoinitiator system selected was camphorquinone (CQ) (Sigma-Aldrich) and the co-initiator system selected was Dimethyl Aminoethyl Methacrylate (DMAEMA) (Sigma-Aldrich Inc.) (1:2 weight ratio). The light-curing initiator was completely dissolved in the monomer matrix with a concentration of 1.5 wt% (0.5% CQ/1% DMAEMA). Also, the inhibitor Butylated Hydroxytoluene (BHT) (Sigma-Aldrich Inc.) was added to the composite resin blends with a concentration of 0.1 wt% to avoid the spontaneous polymerization of the monomers and the composite resin blends were stored at 4°C until its use to avoid the premature polymerization as well. For each experimental group, the monomers were blended in light-protected glass jars.

Table 1: *Blend composition of low viscosity composite resin materials.*

Infiltrant	Composition (wt,%)
Icon®	Methacrylate resin
TU	75% TEGDMA+25%UDMA
TU-0.5%DMSO	T+U+0.5%DMSO
TU-5% DMSO	T+U+5%DMSO
TU-0.5%THF	T+U+0.5%THF
TU-5%THF	T+U+5%THF
TB	75% TEGDMA+25%BisEMA
TB-0.5%DMSO	T+B+0.5%DMSO
TB-5% DMSO	T+B+5%DMSO
TB-0.5%THF	T+B+0.5%THF
TB-5%THF	T+B+5%THF
Note: T-Triethyleneglycol dimethacrylate, B-Ethoxylated bisphenol A glycidyl dimethacrylate, UDMA-Urethane dimethacrylate, DMSO-Dimethyl Sulfoxide, THF-Tetrahydrofuran.	

Contact Angle

The contact angle measurements were performed on polished glass slides. Also, the viscometer GBX (Instrumentation Scientifique France No, 04230711-Contact Angle Meter-Digitrop) was used to measure the contact angle of the resin infiltrants. Droplets of liquid materials (about 1 µl) were placed on a glass slide *via* a micro syringe. After 10 sec, an image was recorded and analyzed using a software drop shape analysis ImageJ. For each group (n=5), the average of three measurements was calculated. Each measurement was performed on a new glass slide. Data were statistically analyzed by one-way ANOVA and Tukey's multiple comparison test (α=0.05).

Knoop Hardness (KHN) and Confocal Laser Scanning Microscopy (CLSM)

Specimen preparation: One hundred and sixty-five bovine incisors without cracks or caries were collected. The teeth were cleaned and stored in 0.1% thymol solution for 1 month after extraction. The roots were sectioned 1 mm below the cementum-enamel junction using a diamond saw in a cutting machine (Isomet, Buehler Ltd., Lake Bluff, USA). The teeth were cut using the diamond saw to obtain blocks of enamel (5 mm × 5 mm). The enamel surfaces of fifty-five teeth (n=5) were ground flat with a water-cooled mechanical grinding machine using 340- and 600- grit silicon carbide abrasive papers (Aropol E, Arotec S.A. Ind.&Com., São Paulo, Brazil) for analyses in CLSM. The other one hundred and ten teeth were used to assess the Knoop Hardness (n=10).

Artificial enamel caries-like lesion formation: Each enamel block was covered with double coats of acid-resistant nail varnish (Colorama®-São Paulo, Brazil) except for the polished enamel area (5 mm × 5 mm). Then the caries-like lesions were produced in the sound enamel surface by immersion of each enamel surface into 50 mL of a demineralizing solution containing 0.05% M acetate buffer 50% hydroxyapatite saturated from enamel powder, pH 5.0, for 16 hours at 37°C. To prepare the solution, enamel powder was agitated into 0.05% sodium acetate buffer, pH 5.0, for 96 hr at 37°C (0.50 g/L). The solution was used in a ratio of 2.0 mL/mm² of exposed enamel area. The immersion period of 16 hours was determined in a previous study, by analyzing thin enamel slices with polarized light microscopy. It was clearly observed the presence of caries-like lesions on the enamel subsurface. Calcium concentration into the solution was 66.3 µg/mL, which was determined by atomic absorption spectrometry with flame spectrophotometer model 506 (Perkin Elmer), phosphorus concentration was about 32 µg/mL, with UV-Vis spectrophotometer adjusted at 660 nm. Such solution was employed to induce artificial white-spot lesions in enamel surfaces by pH CYCLING.

Lesion infiltration and preparation for CLSM: The enamel blocks with caries-like lesions were randomly distributed into eleven groups *(Table 1)* (n=5). The enamel was etched with 16% hydrochloric acid gel (Icon Etch, DMG, Munich, Germany) for 120 sec (19), washed with water spray and dried for 30 sec. The infiltrants were impregnated with 0,1% rhodamine B (Sigma-Aldrich), applied onto the caries-like lesion using a microbrush, and left to penetrate for 60 s. Block surface was air-dried for 15 sec to evaporate the solvent. All groups were light cured for 60 sec with Ultralume 5 (Ultradent, South Jordan, Utah, USA) with 1000 mW/cm^2 irradiance. 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 with wet SiC papers series (#600, #1200, #2000). To visualize the porous structure (not infiltrative lesion parts) the specimens were immersed in a 50% ethanolic solution of 100 mM sodium fluorescein (NaFl) (Sigma Aldrich) for 3 min and washed in deionized water for 10 sec.

CLSM evaluation: The specimens were observed with a confocal laser scanning microscope (Leica, TCS NT; Leica, Heidelberg, Germany) with 10x magnification in dual fluorescence mode. The excitation light had two wavelength maxima, at 488 nm and 568 nm. The emitted light was split by a 580 nm reflection short-pass filter and was passed through a 525/50 nm band-pass filter for rodhamine B and a 590 nm long-pass filter for rodhamine B detection. Images with a lateral dimension of 1.0 × 1.0 mm^2 and a resolution of 1024 × 1024 pixels were recorded and analyzed by Leica SP2 CLSM software (Zeiss, Oberkochen, Germany). The analysis of group allocation of the teeth was blind. A qualitative analysis was employed to evaluate the penetration of materials into the lesion body.

Lesion infiltration and preparation for KHN: The enamel blocks with caries-like lesions were randomly distributed into eleven groups *(Table 1)* (n=10) according to the composition of low viscosity composite resin materials. The previously determined area (5 mm × 5 mm) on enamel surface blocks was etched with 16% hydrochloric acid gel (Icon Etch, DMG, Munich, Germany) for 120 sec, rinsed for 30 sec and air-dried for 15 sec. The experimental infiltrants were applied using micro-brush for 60s to improve the penetration into the etched enamel. Block surface was air-dried for 15 sec to evaporate the solvent. Infiltrants were then light cured for 60 sec with Ultralume 5 (Ultradent, South Jordan, Utah, USA) with 1000 mW/cm^2. Thereafter, the enamel blocks were stored in 100% humidity for 24 hr at 37°C. Afterwards, each enamel block was longitudinally cut into slices of 2 mm thickness using water-cooled diamond blade (Isomet 1000-Buehler Ltda, Lake Bluff, IL, USA). The slices of all groups were embedded in acrylic resin in a Polymethyl methacrylate resin (PVC) matrix and polished with water-cooled silicon carbide papers (600-, 1200-, and 2000- grit).

KHN evaluation: The longitudinal KHN measurement was performed through three sequences of four indentations at distances of 10 µm, 30 µm, 50 µm and 100 µm from the surface under a load of 490 N (50 g) for 10 sec using the microhardness tester (HMV-2, Shimadzu, Tokyo, Japan). The measures of hardness were calculated in each distance (kgf/mm^2). The results were statistically analyzed using two-way ANOVA and Tukey's test (α=0.05) *(Figures 1-3) (Table 2).*

Table 2: *Contact angle among the groups with significant differences (p<0.00).*

Group	Contact angle (*)
Icon®	11.4(2.4)c
T+U	29.8(6.3)a
T+U+0.5%DMSO	29.5(5.5)a
T+U+5%DMSO	16.7(3.3)b
T+U+0.5%THF	23.0(1.3)ab
T+U+5%THF	31.8(8.7)a
T+B	26.4(3.5)ab
T+B+0.5%DMSO	32.3(5.7)a
T+B+5%DMSO	24.5(6.8)ab
T+B+0.5%THF	29.8(3.6)a
T+B+5%THF	25.4(0.5)ab
Note: a,b,c indicates the significant differences between groups (p<0.001).	

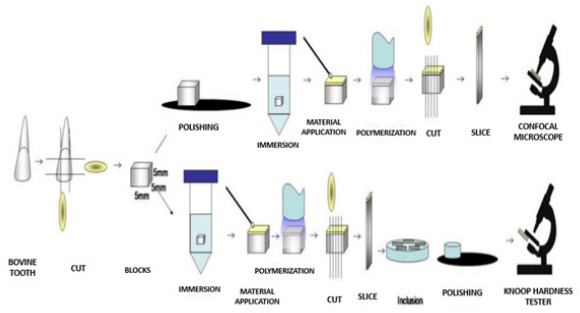

Figure 1: *Representative scheme showing the specimen preparation for CLSM and KHN.*

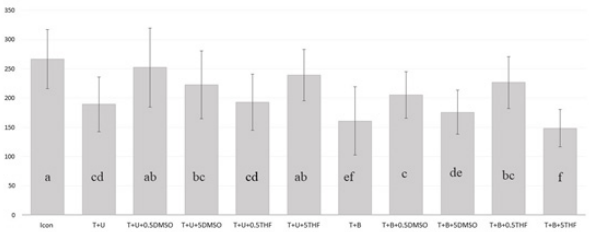

Figure 2: *Knoop hardness outcomes with means (standard deviation) of infiltrated lesions. Different letters indicate statistically significant differences (p<0.001).*

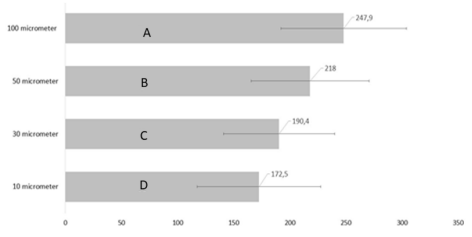

Figure 3: *Knoop hardness outcomes with means (standard deviation) in different depths into the caries-like lesions. The values increased significantly from the depth of 10 micrometers up to 100 micrometers. Different letters indicate statistically significant differences (p<0.001).*

Results

Contact Angle

The contact angle results are described in *Table 2*. Icon® (11.4) was significantly lower than all experimental infiltrants which showed significant differences between groups ($p<0.001$). Furthermore, the T+U+5%DMSO (16.7(3.3)) experimental composite resin infiltrant showed significantly lower contact angle with statistical difference ($p<0.001$) when compared with T+U (29.8(6.3)); T+U+0.5%DMSO (29.5(5.5)); T+U+5%THF (31.8(3.7)); T+B+0.5%DMSO (32.3(5.7)); and T+B+0.5%THF (29.8(3.6)) ($p=0.0751$). Intermediate values were presented by the groups T+U+0.5%THF (23.0(1.3)); T+B+5%DMSO (24.5 (6.8)); T+B+5%THF (25.4 (0.5)); T+B (26.4(3.5)).

Confocal Laser Scanning Microscopy (CLSM)

Confocal micrographs are presented in *Figures 4-6*. The penetrability of the commercial infiltrant (Icon®) and the demineralized area are represented in *Figure 4*. Indeed, the full extent of the lesion was penetrated by the commercial infiltrant: Icon® *(Figure 4)*. Sequentially, the overlapping of the demineralized areas and red areas were demonstrated, indicating the infiltrant impregnated with rhodamine b into the lesion body.

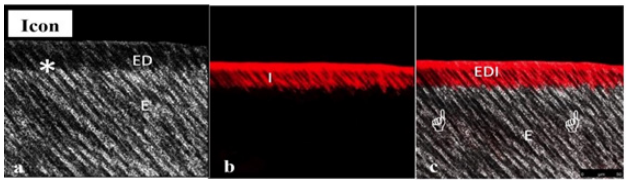

Figure 4: *Confocal micrographs showing the commercial material (Icon) penetration. The red areas show the infiltrant impregnated with rhodamine b into the lesion body (b). Pointers (c) indicate the overlapping of the demoralized areas and red areas, indicating the materials impregnated with rhodamine b into the lesion body. E-Sound enamel, ED-Enamel area demineralized, I-Experimental infiltrant, EDI-Enamel area demineralized and infiltrated with experimental infiltrant.*

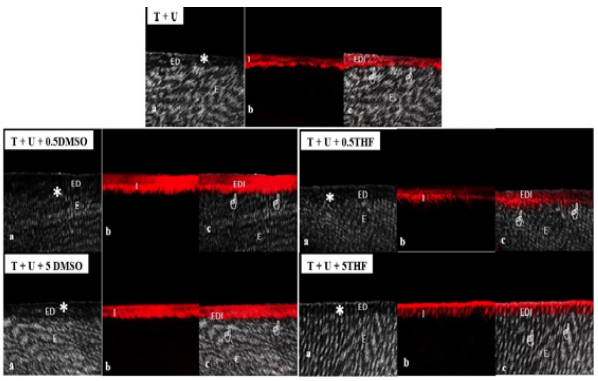

Figure 5: *Confocal micrographs showing the penetration of the experimental materials with monomer UDMA. Note the demineralized area (asterisks in a for all groups). Sequentially, red areas show the infiltrants impregnated with rhodamine b into the lesion body (b for all groups). The pointers (c for all groups) indicate the overlapping of the demoralized areas and red areas, indicating the materials impregnated with rhodamine b into the lesion body. E-Sound enamel, ED-Enamel area demineralized, I-Experimental infiltrant, EDI-Enamel area demineralized and infiltrated with experimental infiltrant.*

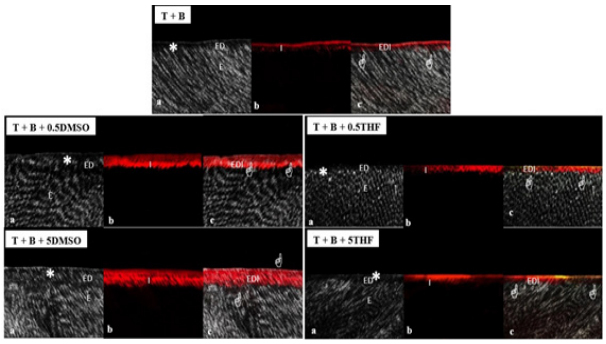

Figure 6: *Confocal micrographs showing the penetration of the experimental materials with monomer BisEMA. Note the demineralized area (asterisks in a) for all groups). Sequentially, red areas show the infiltrants impregnated with rhodamine b into the lesion body (b for all groups). The pointers (c for all groups) indicate the overlapping of the demoralized areas and red areas, showing the materials impregnated with rhodamine b into the lesion body. E-Sound enamel, ED-Enamel area demineralized, I-Experimental infiltrant, EDI-Enamel area demineralized and infiltrated with experimental infiltrant.*

The penetration of experimental infiltrants with TEGDMA+UDMA blend base and of all groups into the demineralized area by the artificial caries lesions are showed in the *Figure 5*. As a result, the groups with TEGMA+UDMA blend base performed good penetration into the lesion body. the penetration of TEGDMA+BisEMA, T+B; T+B+0.5%DMSO and T+B+0.5%THF is shown in *Figure 6*.

Knoop Microhardness

The two-way ANOVA microhardness showed significant differences for infiltrants ($p<0.001$) and for depth ($p=0.0003$) factors, but not for the interaction between these factors ($p=0.56$). The hardness outcomes (means and standard deviations) of different resin infiltrants is shown in *Figure 2*. The UDMA promoted significantly higher Knoop hardness compared to BisEMA, except for group 0.5%THF (valor). The mean values varied from the lowest Knoop hardness (148.9) to the highest (266.4) obtained from T+B+5%THF and Icon® respectively. The intermediate values were T+U+5%DMSO, T+B+0.5%THF similar to each other ($p=0.002$), T+B+0.5%DMSO, T+U and T+U+0.5%THF, while, lowest values for T+B+5%DMSO, T+B, T+B+5%DMSO.

Discussion

The present results demonstrated that the experimental infiltrants induced differences in the contact angle, Knoop hardness, and penetrability into the caries like lesions. Thus, the hypotheses must be rejected. Furthermore, this study demonstrated the influence of concentration and incorporation of Dimethyl Sulfoxide (DMSO) and Tetrahydrofuran (THF) in the physicochemical properties of experimental infiltrants. An infiltrating resin must have low viscosity whose composition penetrates inside the lesion by capillary forces and creates a diffusion barrier not only on the lesion's surface but in depth. Another relevant factor is its hydrophilicity for adequate wetting by water or aqueous solution, while hydrophobic solid denotes partial wettability by the aqueous phase [10].

The enamel wetting is evaluated by contact angle formed between the liquid and the solid substrate surface. This is determined by both the surface tension of the liquid and nature/condition of the substrate surface. The smaller the contact an-

gle and the lower the surface tension of the liquid, the greater the degree of wetting, that is, the droplet of liquid will spread across the substrate surface. If there is strong binding to the substrate surface and weak cohesion within the liquid, there is a higher degree of wetting, often termed lyophilic conditions. Conversely, a combination of weak binding and strong cohesion, referred to as lyophobic conditions, results in higher contact angles and poor wetting of the substrate surface [15].

The contact angle on the glass slides varied following the viscosity of the functional monomers used in the formulation of the standardized blend [16]. Thereby, the incorporation of solvents THF or DMSO did not influence the viscosity of the experimental infiltrants. The aspect shown in the study was the influence of hydrophobic monomers with higher molecular weight (UDMA and BisEMA). Consequently, the comparison between UDMA and BisEMA showed that the BisEMA has higher molecular weight, less flexibility (removal of the hydroxyl group in your molecule), higher viscosity and higher hydrophobicity and UDMA has lower viscosity and lower molecular weight comparing to BisEMA [17.18]. However, these features caused no significant change in contact angle of infiltrants even with the incorporation of DMSO and THF solvents. Icon® is composed predominantly by TEGDMA, presenting low molecular weight, high flexibility, high degree of conversion, low viscosity, which may be responsible for the improved performance of this material compared to the experimental infiltrants [19.20].

Icon's Contact Angle showed that the forces between glass slide and liquid are greater than the cohesive forces within the infiltrant. Therefore, a higher wetting can be achieved by decreasing the surface tension of the infiltrant. In the experimental infiltrants, the cohesion within the infiltrant may have exceeded the surface energy of the glass slide, such as poor wetting was noticed. Albeit, the hydrophilic composition of solvents DMSO and THF in the experimental infiltrants, the monomers UDMA and BisEMA are highly hydrophobic, balancing the overall hydrophobicity as well as yielding higher contact angle.

The results of this study indicated that adding a solvent to infiltrant blends, except for UDMA infiltrants, damaged the Knoop hardness. However, the addition of solvents/diluents did not show a difference among the groups inhomogeneity of penetration. The blends T+U+0.5%DMSO and T+U+5%THF resulted in an increased hardness. Thus, the association of UDMA to TEGDMA could improve the mechanical features of infiltrant [9].

The monomer UDMA shows lower viscosity than BisEMA, which comparatively could improve the penetration coefficient, there is also more flexible with urethane bonding, which can improve the mechanical properties of materials using this monomer, especially when compared to TEGDMA/BisEMA groups. The blends T+B; T+B+5%DMSO and T+B+0.5%THF showed the lowest hardness. The solvents tested (DMSO and THF) are aprotic solvents. However, the solvent THF has highly volatile character (143 mmHg of vapor pressure), demonstrating good water-removing capacity and enhanced evaporation, which may explain the optimal outcomes of hardness were added when higher percentages of THF in the experimental infiltrants association with the UDMA [21-23].

Conversely, DMSO has a low vapor pressure (0.42 mmHg), which hinders its evaporation resulting in structures with a residual solvent, showing lower performance KHN when used at a higher percentage (5%) regardless of the monomer used [24]. In association with UDMA to a lesser percentage (T+U+0.5% DMSO), it may not have influenced the properties of the experimental infiltrant, showing high values of KHN similar to Icon®. It was observed that the greater the depth, the higher values of hardness. This can be explained due to hardness is an indirect method for evaluating demineralization [25].

According to the CLSM images analysis, variation in the blend composition through the addition of solvents showed a good penetration into the demineralized enamel except for groups T+B; T+B+0.5%THF and T+B+0.5%DMSO. The monomer UDMA shows lower viscosity than BisEMA, which could improve the penetrability of the materials based on UDMA [26]. The association of monomer BISEMA with low percentage of solvents (0.5% THF or 0.5% DMSO) might not be sufficient to reduce the viscosity of infiltrants and promote good penetration into the demineralized area. The development of new formulations is a promising method for a significant improvement of the performance of these materials, increasing the penetration and establishment of new approaches. Further studies should be conducted to evaluate the effect of incorporation of solvents in experimental infiltrants the contact angle with dental structure (enamel) and the consequent bonding, since this study was conducted in glass slide, which varied the contact angle as a function of monomers used.

Conclusion

The results of this study showed that the addition of low concentrations of DMSO and THF in the experimental infiltrants may increase the hardness, which may reduce the wear. The blends with solvents in lower concentration (0.5%) associated with monomer BisEMA did not provide enhanced penetration of the experimental infiltrants. Likewise, the high concentration of DMSO (5%) decreased the contact angle only when it was added to the TEGDMA and UDMA infiltrant.

Acknowledgement

This research was supported by CNPq (grant 457931/2014-0) and Capes (grant 23038.006958/2014-96) grants (PI VPF). All authors are completely free of conflicts of interest regarding any material or technique presented in this manuscript. This manuscript is part of the PhD. thesis of T.G.F.A.

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