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Effect of iodonium salt and chitosan on the physical and antibacterial properties of experimental infiltrants

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Abstract: Resinous infiltrants are indicated in the treatment of incipient carious lesions, and further development of these materials may contribute to greater control of these lesions. The aim of this study was to analyze the physical and antibacterial properties of experimental infiltrants containing iodonium salt and chitosan. Nine experimental infiltrants were formulated by varying the concentration of the diphenyliodonium salt (DPI) at 0, 0.5 and 1 mol%; and chitosan at 0, 0.12 and 0.25 g%. The infiltrants contained the monomeric base of triethylene glycol dimethacrylate and bisphenol-A dimethacrylate ethoxylate in a 75 and 25% proportion by weight, respectively; 0.5 mol% camphorquinone and 1 mol% ethyl 4-dimethylaminobenzoate. The degree of conversion was evaluated using Fourier transformer infrared spectroscopy, and the flexural strength and elastic modulus using the three-point bending test. Sorption and solubility in water, and antibacterial analysis (minimum inhibitory concentration and minimum bactericidal concentration) were also analyzed. Data was analyzed statistically by two-way ANOVA and Tukey's test ($p<0.05$), with the exception of the antibacterial test, which was evaluated by visual inspection. In general, the infiltrant group containing 0.5% DPI and 0.12% chitosan showed high values of degree of conversion, higher values of elastic modulus and flexural strength, and lower sorption values in relation to the other groups. Antibacterial activity was observed in all the groups with DPI, regardless of the concentration of chitosan. The addition of DPI and chitosan to experimental infiltrants represents a valid option for producing infiltrants with desirable physical and antibacterial characteristics.

Keywords: Composite resins; Onium Compounds; Chitosan.

Introduction

Recently, the production of dental materials that can prevent and/or treat carious lesions has grown, considering that the treatment of caries disease consequences is still one of the most common health-related concerns.[1,2] Dental caries can be defined as a chronic disease of slow progression. Its first clinical manifestation is a white-spot lesion in enamel, characterized by the formation of tiny pores at the subsurface layer of enamel, whereas the surface of this tissue remains relatively intact.[3]

The first option for management of these lesions in permanent teeth usually includes remineralization strategies,[4] whereas strategies such as fluoride therapy are not considered effective at more advanced stages of the disease. Conversely, conventional fillings that remove the enamel to access affected dentin lead to substantial concomitant healthy tissue loss.[5]

Although a minimally invasive approach for carious lesions still remains a challenge,[6] the current principles of Minimally Invasive Dentistry seek conservative solutions to restore small and localized defects.[7] Therefore, micro-invasive approaches, such as the infiltration of low-viscosity, hydrophobic resin-based materials into porous enamel, have been proposed as an alternative among invasive and non-invasive options, and have become a frequent choice for treatment of incipient carious lesions.[5,6,8] The literature suggests that the sealing of carious lesions with an inert material at their early stages might promote the control and arrest of lesions, inducing a change in the arrangement of the dentin tissue, with partial or complete obliteration of the dentin tubules.[5] Hence, this treatment has been an alternative for inhibiting demineralization, since the diffusion pathways for cariogenic acids can be blocked by sealing the lesions.[6] In the search to find materials with ideal properties to infiltrate carious lesions, experimental composites have been studied, including those based on triethylene glycol dimethacrylate (TEGDMA), known as "infiltrating agents," which present good results in reducing carious lesion progression.[9] Clearly, what is needed is an infiltrant with acceptable mechanical properties to stabilize the fragile structure of carious lesions. Accordingly, several formulations of infiltrating agents have been developed, which include different combinations of monomers, diluents, and solvents used to obtain a material with low viscosity, rigid state after polymerization, and high penetration capacity.[9] However, it is difficult to develop a composition with satisfactory flowability and adequate mechanical strength.

The addition of bisphenol-A ethoxylate dimethacrylate (BisEMA) is a feasible alternative to replace bisphenol-A glycidyl methacrylate (BisGMA), and also reduce the viscosity of a resin-based material.[10] BisEMA has a molecular structure similar to BisGMA; consequently, both monomers present similar properties. Nevertheless, the absence of hydroxyl groups in the BisEMA molecules renders this monomer less viscous, and less susceptible to water sorption than BisGMA,[11] thus providing important features that meet the requirements for promising infiltrant components.

Moreover, the combined use of an ionic salt with camphorquinone might promote salt decomposition, thus enhancing free radical polymerization of dimethacrylates.[12] There is evidence that the addition of ionic salts in resinous materials can improve their reactivity and polymerization potential, allowing the formation of polymers that are more resistant to water degradation, even in the presence of high concentrations of TEGDMA.[13] In addition, the inclusion of an antibacterial agent in the infiltrating agent has been proposed, considering that restorative materials which show antibacterial activity are useful for eliminating the harmful effects caused by bacterial residues or bacterial microleakage.[14]

Chitosan is a polysaccharide generally considered non-toxic, biocompatible, and biodegradable, as well as a natural antibacterial agent.[14,15] One of its antibacterial mechanisms is based on its positively-charged cellular characteristic that alters its permeability upon interaction with the bacterial cell, thus leading to the escape of intracellular components, and to cell death. In recent years, this polysaccharide has been widely used in dentistry, possibly because of its action as a barrier against acid penetration, thereby interfering with dental enamel demineralization.[14]

Considering all the aforementioned information, new studies that propose and evaluate improvements in the composition of infiltrating agents are necessary. A primary line of investigation would be to determine the influence of different infiltrant compositions on the physicochemical properties of these infiltrants, in an attempt to minimize the risk of failure associated with the use resin-based materials of low viscosity. Thus, the aim of the present study was to add diphenyliodonium salt (DPI) and chitosan to experimental infiltrants, in different concentrations, to evaluate their physical, mechanical,

and antibacterial properties. The null hypothesis was that the addition of iodonium salt and chitosan to experimental infiltrants would not alter their physical, mechanical, and antibacterial properties.

Methodology

Infiltrant formulation

Based on previous studies,[16,17] nine groups of experimental infiltrants were tested (Table 1). The monomeric base of all the tested materials was composed of 75% TEGDMA (Sigma Aldrich, St. Louis, USA) and 25% BisEMA (Esstech, Essington, USA). The diluent monomer used was HEMA (Esstech, Essington, USA), at a 10% concentration (used as a solvent for the base monomers). The photoinitiator system was composed of 0.5% camphorquinone (Esstech Inc., Essington, USA), and 1% EDAB (Sigma Aldrich, St. Louis, USA). The concentrations of the salt added to the base of the infiltrants was based on a previous study by Ogliari et al.,[12] in which the authors used 0.5 and 1 mol% diphenyliodonium hexafluorophosphate salt (DPI; Sigma-Aldrich, St. Louis, USA).

The chitosan (Sigma-Aldrich, St. Louis, USA) was prepared by dissolving 2 g of its powder in 1 L of 1 vol% acetic acid. The resulting preparation was then diluted in distilled water to obtain the desired ratio (0.12% and 0.25%, both by weight, according to the best results from Elsaka[14]). The materials were weighed on a high-precision analytical scale (Chyo JEX-200, YMC Co, Tokyo, Japan), and handled in a yellow-light environment with controlled humidity and temperature.

Table 1. Groups of experimental infiltrants according to composition in relation to diphenyliodonium salt (DPI) and chitosan concentration.

DPI (mol%)	Chitosan (w%)		
	0	0.12	0.25
0	G1	G2	G3
0.5	G4	G5	G6
1	G7	G8	G9

Basic composition of infiltrant: BisEMA (25%), TEGDMA (75%), HEMA (10%), CQ (0.5%), EDAB (1%).

The resulting composites were homogenized using a magnetic mixer (Model M089, Piracicaba, Brazil) and packed in amber glass bottles covered with insulation tape, under refrigeration at 4ºC. The light-curing device used to perform all the tests was the polywave, 3rd-generation VALO light-emitting diode (LED) (Ultradent Products, S. Jordan, USA), with an irradiance of 1000 mW/cm^2.

Degree of conversion

The degree of conversion of the experimental infiltrants (n = 5) was measured using Fourier transformer infrared spectroscopy (FTIR, Vertex 70, Bruker Optics, Germany). The wavelength ranged from 4000 cm^{-1} to 9840 cm^{-1}, with 6 cm^{-1} resolution. Polyvinylsiloxane matrices (Express XT, 3M ESPE, St. Paul, USA) were used to prepare the specimens, handled according to the manufacturer's instructions. Sample preparation consisted of pressing the matrix between two glass slides to a 1-mm thickness, as measured by a digital caliper (Mitutoyo, Tokyo, Japan). After the pre-reaction of polyvinylsiloxane, a 5-mm diameter hole was made at the center of the matrix, and the glass slide was secured to the machine readout with adhesive tape.

Next, the infiltrants were carefully placed in the matrix hole, and the matrix-infiltrant-glass slide assembly was positioned in the FTIR, so that the laser locator and the infrared source beams passed through the center of the sample. Spectra of uncured (control) and light-cured material were obtained. Each spectrum was subjected to 32-scan readings. Light-curing was carried out for 40 seconds. Data analysis was performed by Opus software v.6 (Bruker Optics, Germany).

The degree of conversion was calculated according to the ratio of the double aliphatic and aromatic carbon bonds used as the internal standard in the light-cured and the uncured states. The baseline technique was used to make the calculation [19], and was performed by the software itself. Then, the degree of conversion (%) was calculated as follows: 1st Equation - Residual double bond rate (cured sample/uncured sample x 100); 2nd Equation - Degree of conversion (%) - 100 - residual double bonds (%).

Elastic modulus and flexural strength

Twelve samples (7 mm x 2 mm x 1 mm) of each group were made in polyvinylsiloxane matrices (Express XT, 3M ESPE, St. Paul, USA), according to ISO 178: 2001 specifications, except for length. Light-curing of the samples was conducted for 40 seconds, under a mylar strip, and the specimens were stored for 24 hours at 37°C.

The three-point bending test was performed in a universal testing machine (Instron, Model 4111, Instron, Canton, USA), with a 5-mm distance between the holders, at 0.5 mm/min speed and 50 N load, until the fracture of the samples. Prior to the test, the dimensions of each specimen were obtained with a digital caliper (Mitutoyo, Tokyo, Japan), and then transferred to Bluehill 2 software (Instron, Canton, MA, USA) to calculate the elastic modulus in GPa and the flexural strength in MPa, according to dimensions and strain.

Water sorption and solubility

Sorption and solubility tests were based on ISO 4049: 2009 specifications, except for the sample size, and the water volume (mL) used. Disc samples of the infiltrants were prepared (5 mm x 1 mm, n = 5) in polyvinylsiloxane molds (Express XT, 3M ESPE, St. Paul, USA) that had been previously made in a Teflon matrix.

Right after light-curing, the samples were stored in a desiccator containing silica gel at 37°C. The specimens were weighed repeatedly at 24-hour intervals for 4 days until a constant initial mass (m1) was obtained, with a variation of less than 0.1 mg. The thickness and the diameter of the samples were measured using a digital caliper, and the measurements were then used to calculate the volume (V) of each specimen (in mm^3).

Afterwards, the specimens were stored individually at 37°C in Eppendorf vials containing 2 mL of distilled water. After seven days of water storage, the samples were removed from the incubator and left at room temperature for 30 minutes. The specimens were then washed in running water, dried with absorbent paper, and weighed on an analytical scale (m2). The samples were further dried in a desiccator containing silica gel, and weighed daily until a constant mass (m3) was obtained, a process that took 6 days in this study. The sorption (So) and solubility (SL) values were calculated using the following formulas: So = (m2-m3) / V; SL = (m1-m3) / V.

Antibacterial activity

Microorganisms and microbial sensitivity tests

The test microorganisms used were *Streptococcus mutans* UA159 and *Lactobacillus acidophilus* LYO50DCU-S procured from the Laboratory of Microbiology and Immunology of the Piracicaba Dental School (University of Campinas, Piracicaba, Brazil). These species were chosen because they are indicative of the presence of caries, and are commonly associated with white spot lesions.[19] The preparation of *S. mutans* and *L. acidophilus* strains was performed using a microdilution method, following the recommendations of the M7-A619 protocol, with modifications. The microorganisms were maintained in a BHI medium (brain heart infusion - Difco Laboratories, Detroit, USA) with 20% glycerol at -20°C. Each species was reactivated on BHI plates, and incubated at 37° C and 10% CO_2 for 24 hours for *S. mutans*, and for 48 hours for *L. acidophilus*.

Immediately after the growth period, absorbance was adjusted in a spectrophotometer (10mV Genesys, Thermo Electron, USA) to obtain an inoculum concentration equivalent to 1.5 x 10^8 cells / mL. Serial dilution was performed to attain a 1.0 x 10^6 cell / ml concentration in BHI broth (Difco Laboratories, Detrotit, USA). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays were performed to evaluate the bacteriostatic and bactericidal activities of the experimental infiltrants, respectively.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The strains of both microorganisms were adjusted to 0.05 and 625 nm absorbance in a spectrophotometer (Genesys 10mV, Thermo Electron, Waltham, USA) to determine the MIC of the groups. The MIC and MBC determinations were performed according to the recommendations of the Clinical and Laboratory

Standards Institute.[20] Bacterial cultures of each tested species were grown in BHI medium for 24 h and 48 h. Three to five colonies were selected from these cultures, and placed in 5 mL of BHI medium. This new broth culture was incubated at 35°C until obtaining an optical turbidity comparable to the standard 0.5 McFarland solution (spectrophotometer absorbance reading, λ = 625 nm, 0.05 absorbance), resulting in an inoculum containing approximately 1 to 2 x 10^8 CFU / mL (colony forming units / mL). Serial dilutions (2X) of the infiltrant samples were placed in 96-well plates. The infiltrant concentration of subsequent wells was halved; thus, the first well had 50 μL, the second 25 μL, and so on until the infiltrant concentration was equal to 0 (zero) μL. Afterwards, the samples were light-cured, and the wells were filled with 100 μL of bacterial inoculum. When this inoculum achieved the desired optical turbidity, it was diluted to contain 5 x 10^5 CFU / mL; 50 μL of the new bacterial suspension was removed from this concentration and added to each well of the plate, resulting in a final concentration of 5 × 10^4 CFU / well. The plates were incubated for 24 h in an oxygenated environment and analyzed visually for presence or absence of turbidity in the medium. The lowest concentration of the compound that did not show turbidity was considered MIC. MBC was determined by transferring a 10 μL aliquot of each well where no bacterial growth was present to a Petri dish containing BHI agar medium, and incubating for 24 h. The antibacterial test (MIC and MBC) was evaluated by visual inspection. The plate referring to the lowest concentration, where there was no bacterial growth, was considered the MBC. The growth of microorganisms was examined after 24 and 48 hours.

Statistical analysis

Data were analyzed regarding normality and homogeneity of variances, and passed both the Kolmogorov-Smirnov and Shapiro-Wilk tests, with the exception of the antibacterial tests, which were analyzed by visual inspection. The results concerning the degree of conversion, flexural strength, elastic modulus, sorption, and solubility were analyzed by two-way ANOVA, considering the factors "DPI concentration" and "chitosan concentration," followed by Tukey's multiple comparison test. All analyses were performed at a significance level of 5% (α = 0.05).

Results

Degree of conversion

Since there was no statistical interaction between chitosan and DPI factors, they were evaluated separately, according to their respective general averages in the tested concentrations.

DPI at 0.5% showed no statistical difference in the degree of conversion of the infiltrant, compared with the concentration of 0% (Figure 1), indicating higher degree of conversion values, whereas 1% DPI plus chitosan showed a negative effect on the degree of conversion, from the lowest concentration (0.12%) to the maximum concentration tested (0.25%).

Elastic modulus

The chitosan-only groups had the lowest elastic modulus values (Figure 2). When DPI was added to the infiltrant formulation at a concentration of 0.5%, the optimal concentration of chitosan was 0.12%. However, when the salt concentration was increased to 1%, the best result was observed with the mixture containing 0.25% chitosan.

Flexural strength

Flexural strength results are described in Figure 3. The highest mean flexural strength was found with the combination of 0.5% DPI + 0.12% chitosan (Group 5). High flexural strength rates were also observed in groups: 7 (1% DPI, 0% chitosan) and 9 (1% DPI, 0.25% chitosan).

Water sorption

The lowest water sorption rates were observed in Group 5 (0.5% DPI + 0.12% chitosan), and were statistically similar to those of groups 6 (0.5% DPI + 0.25% chitosan) and 8 (1% DPI + 0.12% chitosan) (Figure 4). The highest water sorption rates were observed in the group with 0.5% DPI and no addition of chitosan (Group 4), and in the group with 1% DPI and 0.25% chitosan (Group 9).

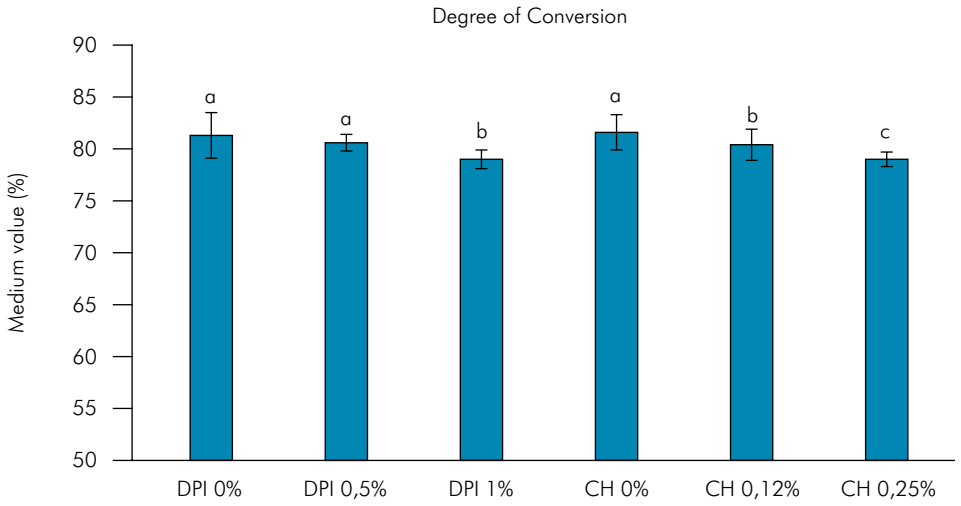

Figure 1. Mean values of degree of conversion (%) of experimental infiltrants, according to DPI (mol%) and chitosan (w%) concentrations.

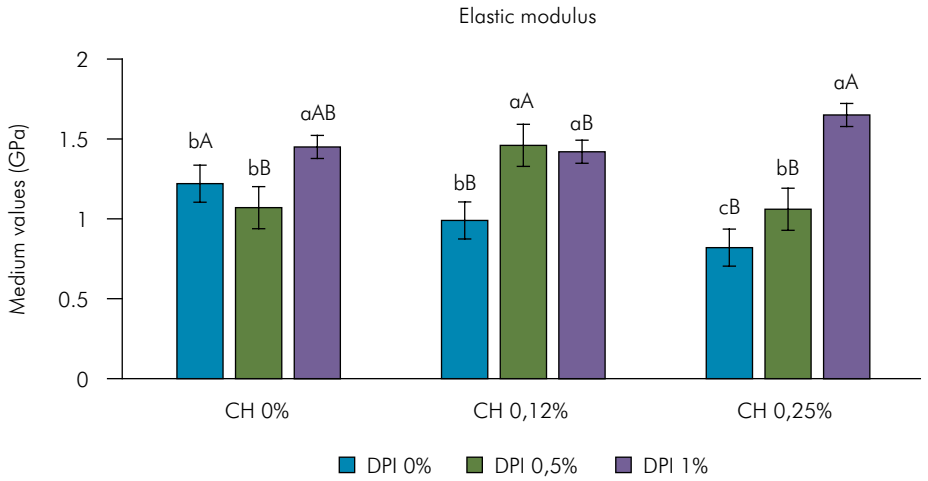

Figure 2. Mean values of elastic modulus (GPa) of experimental infiltrants, according to DPI (mol%) and chitosan (w%) concentrations.

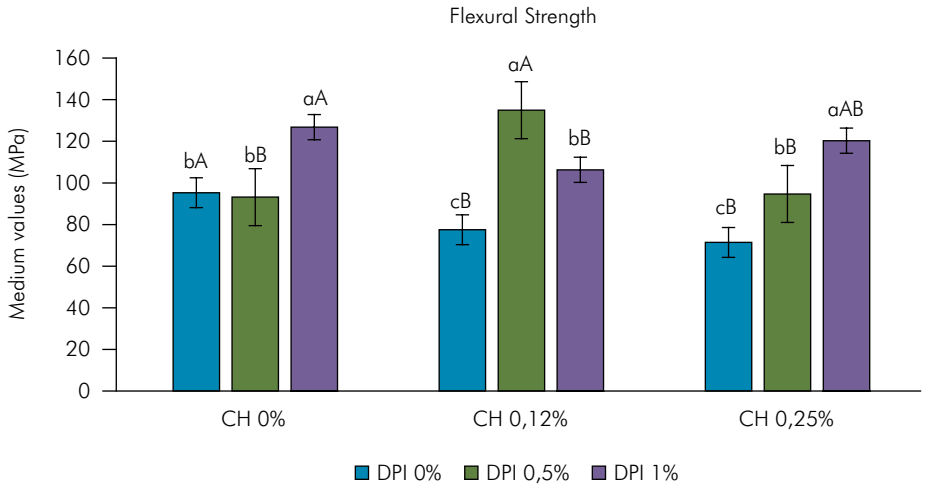

Figure 3. Mean values of flexural strength (MPa) of experimental infiltrants, according to DPI (mol%) and chitosan (w%) concentrations.

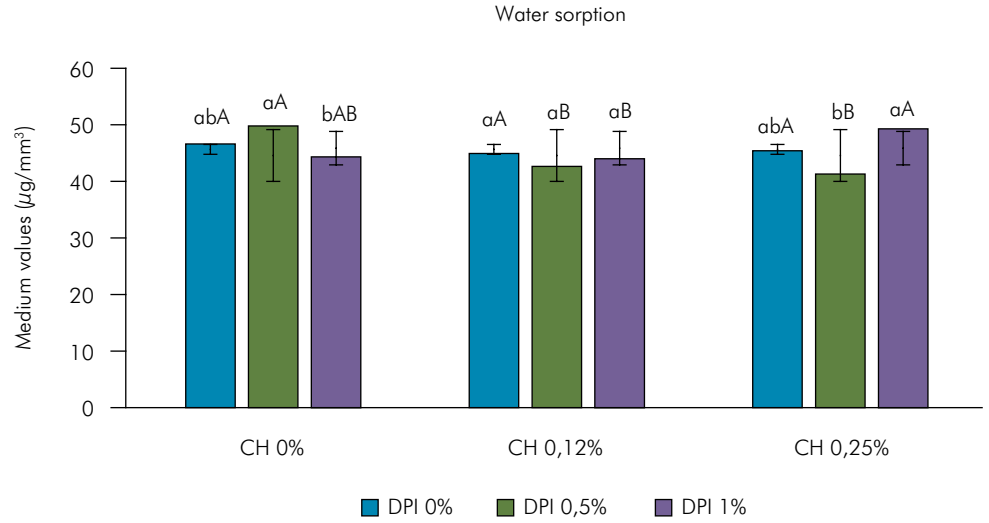

Figure 4. Mean values of water sorption (µg/mm³) of experimental infiltrants, according to DPI (mol%) and chitosan (w%) concentrations.

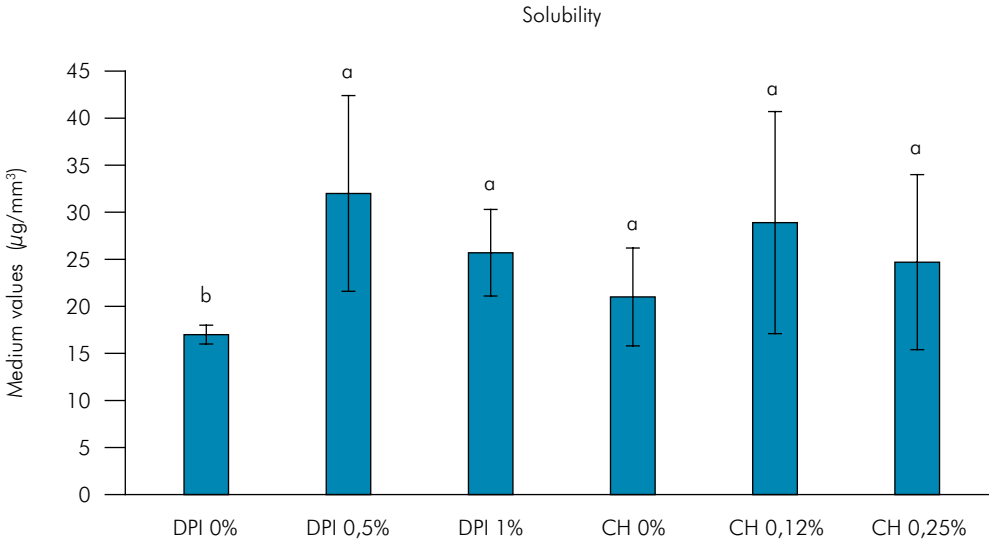

Figure 5. Mean values of solubility (µg/mm³) of experimental infiltrants, according to DPI (mol%) and chitosan (w%) concentrations.

Solubility

Like the results for the degree of conversion test, those for the solubility test showed no interaction between chitosan and DPI. Thus, these factors were evaluated separately, according to their respective general averages in the tested concentrations.

The lowest solubility values were observed without the addition of DPI (Figure 5). The solubility test indicated no statistical differences regarding the concentration of chitosan ($p \geq 0.05$). Only DPI exhibited a statistical variation as an isolated factor. DPI increased the solubility of the material at both 0.5% and 1% concentrations, regardless of the concentration of chitosan involved.

Antibacterial activity

In regard to the antibacterial activity test (Table 2), groups 1, 2, and 3 (all with 0% salt) did not show any antibacterial activity, thus they exhibited bacterial growth, either when light-cured or left uncured. The

Table 2. Antibacterial activity (MIC: minimal inhibitory concentration / MBC: minimal bactericidal concentration).

Varibles	S. mutans			L. acidophilus		
	MIC (μg/mL)	MBC (μg/mL)	Proportion MBC:MIC	MIC (μg/mL)	MBC (μg/mL)	Proportion MBC:MIC
G1 (0% DPI; 0% CH)	-	-	-	-	-	-
G2 (0% DPI; 0.12% CH)	-	-	-	-	-	-
G3 (0% DPI; 0.25% CH)	-	-	-	-	-	-
G4 (0.5% DPI; 0% CH)	1.56	50	32:1**	0.78	0.78*	1:1***
G5 (0.5% DPI; 0.12% CH)	3.13	3.13	1:1***	0.78*	0.78*	1:1***
G6 (0.5% DPI; 0.25% CH)	3.13	3.13	1:1***	0.78*	0.78*	1:1***
G7 (1% DPI; 0% CH)	0.78*	3.13	4:1***	0.78*	3.13	4:1***
G8 (1% DPI; 0.12% CH)	0.78*	6.25	8:1 **	0.78*	1.56	2:1***
G9 (1% DPI; 0.25% CH)	0.78*	3.13	4:1***	0.78*	0.78*	1:1***

*Maximum at the tested concentrations; **Bacteriostatic; ***Bactericidal.

other groups induced antibacterial activity, both when light-cured and left uncured.

The following interesting observation can be made: the higher the concentration of salt involved, the greater the bacteriostatic and bactericidal activities analyzed. When light-cured, Group 4 was bacteriostatic for *S. mutans* and bactericidal for *L. acidophilus*, whereas groups 5, 6, 7, and 9 were found to be bactericidal for both strains, indicating that the salt at 0.5% obtained superior results when added to chitosan at 0.12 or 0.25%. On the other hand, when the salt was at the maximum tested concentration (1%), high antibiotic capacity was observed regardless of the presence of chitosan.

Discussion

Based on the results presented above, the null hypothesis was rejected, since the addition of both DIP and chitosan salts to the experimental infiltrants altered the physicochemical and antibacterial properties of these materials. The present study indicates that the association between DIP salt and chitosan contributes to improving the formulation of experimental infiltrants. These two components presented a synergistic action, favoring the physical properties of the experimental infiltrating agents, in addition to an antibacterial effect.

The presence of both DPI and chitosan led to a reduction in the degree of conversion of the experimental materials (Figure 1), in contrast with the findings of previous studies by Ogliari, et al.,[12] Gonçalves, et al.,[13] and Dressano et al.,[21] who obtained an increase in the degree of conversion in their results associated with the presence of the ion salt. However, Elsaka[14] showed that chitosan at 0.12% and 0.25% concentrations in an adhesive, despite inducing significant antibacterial improvements, caused a decrease in the degree of conversion of the evaluated polymer. The degree of conversion

is a determining factor for the physical-mechanical resistance of a polymer.[22] However, no resin-based material is capable of achieving a complete degree of conversion, and even low conversion rate values have been accepted for composites and adhesives.[23]

In addition to low mechanical resistance, a low degree of conversion has been associated with higher permeability, higher water sorption, and lower biocompatibility of dental polymers due to higher leaching of uncured monomers.[22,24] Nonetheless, the current literature does not accurately establish an acceptable minimum degree of conversion for composites. However, a negative correlation has been found [25] between the values for abrasion wear in composites and degree of conversion at 55-65%. Thus, degree of conversion values lower than 55% have been contraindicated for dental composites.[25]

According to the results shown in Figure 1, the infiltrating agent with DPI salt at 0.5% concentration did not result in any statistical difference when compared with this agent without DPI, in which case there was actually a higher degree of conversion. This indicates that the use of DPI at a concentration of 0.5% can maintain a satisfactory degree of conversion for experimental infiltrants. This corroborates the findings by Gonçalves et al.,[13] who demonstrated a higher degree of conversion with the addition of 0.5% DPI to experimental dental cements.

DPI salt has been associated with the improvement of the polymerization potential of resin-based materials.[12,13] DPI is capable of improving the photopolymerization kinetics of methacrylate free radicals up to twofold.[13] Moreover, this salt may react with camphorquinone or EDAB amine, and with the inactive camphorquinone radicals produced in the photoactivation process,[12] even if it does not absorb the known blue wavelength of current LEDs.

The lower values of degree of conversion associated with the increase in DPI concentration observed in this study could be attributed to the rapid polymerization that has been attributed to the DPI salt. This salt can act as an electron acceptor, leading to an increase in the initiation rate of new radicals generated from DPI fragmentation.[13] This speeds up polymerization, and could explain the difference observed in the degree of conversion of the materials evaluated.[26] On the other hand, according to the results of Figure 1, the presence of chitosan showed a negative effect on the degree of conversion of the experimental infiltrants, even at its lowest concentration (0.12%). However, it still presented a higher degree of conversion value than the 0.25% concentration of chitosan.

The properties of elastic modulus and flexural strength are related to the rigidity of the polymer formed, a rigidity determined by the extent of polymerization [28]. In this study, the addition of chitosan to DPI was found to increase the elastic modulus significantly. The presence of DPI salt and chitosan has resulted in more resistant polymers (Figures 2 and 3), despite a slightly lower degree of conversion associated with salt-containing groups. This can be explained by the ability of DPI to induce an increase in crosslinking density, leading to shorter polymer chains, and increased reaction speed and strength of the material.[13] Chitosan might act as a reinforcing framework for methacrylates,[28,29] by increasing the mechanical resistance of the infiltrants.

Figure 3 shows the flexural strength results, and reveals a possible correlation between the 0.5% DPI and the 0.12% chitosan concentrations (Group 5). The combination of these concentrations resulted in statistically higher flexural strength than that of all the other groups, indicating that the strength of the infiltrant is likely to increase by mixing these two elements (DPI and chitosan) at the above mentioned concentrations, thereby improving one of the main mechanical properties of dental materials.

Figures 4 and 5 show the results of the sorption and solubility tests.

In general, TEGDMA has been associated with high sorption and solubility values, resulting from a relatively hydrophilic behavior that may involve water diffusion inside the polymer.[22,30] Since the base of the experimental infiltrants used in the present study was composed of 75% TEGDMA, high sorption and solubility values were expected. Conversely, a beneficial effect of the interaction between DPI salt and chitosan in the water sorption of the resulting polymer could be perceived (Figure 4). This indicates that chitosan

plays a beneficial role in decreasing sorption values, provided it is used in the concentration of 0.12%, whereas DPI salt also decreases sorption values, provided it is used in the concentration of 0.5%. Gonçalves, et al.[13] also obtained favorable sorption results in experimental luting agents using this DPI salt concentration. However, the present study is the first to show a beneficial effect of the association between the ion salt and chitosan at the aforementioned concentrations, namely, lower water sorption in the resin matrix of the infiltrant, even at high concentrations of TEGDMA. On the other hand, regarding solubility (Figure 5), DPI has been found to have a negative influence at 0.5% and 1%, regardless of the concentration of chitosan involved; this is consistent with previous investigations.[13,21] The lower degree of conversion of the infiltrants containing ion salt and chitosan in their composition could have led to increased leaching of unreacted monomers during water storage.

Regarding the antibacterial evaluation (Table 2), a well-known concept determines that a substance is considered bactericidal when the MBC is the same or no more than fourfold greater than the MIC. On the other hand, it is considered bacteriostatic when the MBC is many-fold greater than the MIC.[31] Hence, a correlation between the DPI salt with greater antibacterial properties was hypothesized in relation to both *S. mutans* and *L. acidophilus*. In this study, chitosan might have played a secondary role in antibacterial action, since it showed an effect only when combined with DPI. There are previous studies[14,15,28] that indicate a positive outcome regarding the addition of chitosan to dental materials, by improving their antibacterial effect. However, as far as the authors know, there are no reports in the literature indicating the antibacterial potential of the ion salt in dental materials.

Important conclusions may be drawn from this study regarding the addition of different salt and chitosan combinations to experimental infiltrants. The known interaction between amino groups that become positively charged with chitosan[32] and the ionic nature of the diphenyliodonium salt are likely to produce mutually favorable mechanical and antibacterial properties that would improve the tested experimental infiltrants. If popularized, the use of infiltrating agents, coupled with public preventive measures, could lead to a decrease in dental caries, still considered a major public health problem.[33] The consequential benefits would promote an increase in the quality of life of the population as a whole. However, further studies are needed to better elucidate the advantages of the clinical use of the tested infiltrants.

Conclusions

This study indicates that the inclusion of diphenyliodonium salt at 0.5% concentration improves the physicomechanical properties of experimental infiltrating agents, such as elastic modulus, flexural strength, and water sorption, and the addition of 0.12% chitosan favors the behavior of the ionic salt, not only regarding mechanical, but also antibacterial properties. The addition of ionic salt and chitosan to the experimental infiltrants is a viable alternative for improving mechanical and antibacterial infiltrant properties.

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