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JADE LAÍSA GORDILIO ZAGO

INCORPORAÇÃO DE NANOHIDROXIAPATITA À INFILTRANTE RESINOSO EXPERIMENTAL E SEU DESEMPENHO NA ESTABILIDADE DE COR E REFORÇO NO ESMALTE DESMINERALIZADO: ESTUDO *IN VITRO*

INCORPORATION OF NANO-HYDROXYAPATITE INTO EXPERIMENTAL RESIN INFILTRANT AND ITS PERFORMANCE ON COLOR STABILITY AND REINFORCEMENT IN DEMINERALIZED ENAMEL: *IN VITRO* STUDY

Piracicaba, 2023

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Dissertação apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Mestra em Clínica Odontológica, na Área de Dentística.

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Orientador: Profª. Drª. Giselle Maria Marchi

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RESUMO

O objetivo do estudo foi avaliar a influência da incorporação de nanohidroxiapatita 10% em infiltrante resinoso experimental, na estabilidade de cor e microdureza transversal, quando comparado ao infiltrante experimental, ao infiltrante resinoso comercial Icon® e às superfícies de esmalte não-tratadas. Foram obtidos 135 blocos de esmalte de dentes bovinos no padrão 4x4 mm, foram submetidos a ciclos desmineralização/remineralização (des-re) durante 8 dias e, posteriormente, divididos em 5 grupos: H: hígido; MB: mancha branca; I: Icon®; E: experimental; EH: experimental contendo nanohidroxiapatita 10%. Em seguida, os grupos foram infiltrados ou não de acordo com os respectivos grupos. Para avaliação de cor (n=15) foram obtidos valores CIEL*a*b* por meio de espectrofotômetro nos tempos: T0 (antes da imersão), T1 (14 dias após imersão em café) e T2 (28 dias após). Para o manchamento foi utilizado 1,5l de solução de café, trocado diariamente durante 28 dias e os dados obtidos foram aplicados na fórmula CIEDE2000. Para a microdureza Knoop transversal foram obtidas metades dos espécimes (n=12) e realizadas 30 leituras em cada amostra: 3 colunas distantes 200 μm entre si, e cada uma contendo 10 leituras a cada 30 μm a partir da superfície. Os dados obtidos de microdureza nos grupos MB, I, E e EH foram aplicados na fórmula de perda mineral (ΔS). Para a Microscopia Óptica de Luz Polarizada foram utilizadas 5 metades de cada grupo (n=5) e foram polidas até atingirem a espessura de 0,1mm e levadas para a Microscopia. Para estabilidade de cor e perda mineral foram aplicados o teste Shapiro-Wilk, análise de variância (ANOVA one-way), seguido pelo teste Post Hoc de Bonferroni e Tukey, respectivamente, e nível de significância estabelecido em 5%. Nos resultados de estabilidade de cor, independente do tempo, não houve diferença significativa entre os grupos H e MB; em 14 dias, H e MB diferiram estaticamente dos grupos infiltrados I, E e EH e, em 28 dias, MB diferiu de todos os grupos infiltrados, e H diferiu de E e EH. A variação entre 14 e 28 dias não foi significativamente diferente para nenhum grupo. Na perda mineral (ΔS), o grupo MB apresentou diferença significativa em relação aos grupos I e EH, porém não diferiu do grupo E. I e EH não diferiram estatisticamente. Nas imagens obtidas, foi possível observar as lesões iniciais no grupo MB e deposição superficial de material nos grupos infiltrados. Em conclusão, os grupos experimentais de infiltrantes resinosos tiveram desempenho similar ao do comercial quanto à variação de cor, porém os três tiveram uma variação maior do que a de superfícies não tratadas; na microdureza transversal, foi possível notar que os grupos I e EH apresentaram menor perda mineral, sugerindo um aumento de dureza na estrutura dental, enquanto os grupos MB e E apresentaram maior perda mineral.

Palavras-chave: Cárie dentária. Desmineralização. Materiais dentários.

ABSTRACT

The aim of the study was evaluate the influence of 10% nanohydroxyapatite penetration in an experimental resinous infiltrant, on color stability and cross-sectional microhardness, when compared to experimental infiltrant, Icon® commercial resin infiltrant and untreated enamel surfaces. 135 enamel blocks of bovine teeth were obtained in the 4x4 mm pattern. They were subjected to demineralization/remineralization (des-re) cycles for 8 days and subsequently divided into 5 groups: H: healthy; MB: white spot; I: Icon®; E: experimental; EH: experimental containing 10% nanohydroxyapatite. Then, the groups were infiltrated or not according to respective groups. For color evaluation (n=15), CIEL*a*b* values were obtained using a spectrophotometer at times: T0 (before immersion), T1 (14 days after immersion on coffe) and T2 (28 days after). For staining, 1.5l of coffee solution was used, changed daily for 28 days and data obtained were applied in CIEDE2000 formula. Cross-sectional Knoop microhardness was performed in halves obtained from the specimens (n=12) and 30 readings were performed on each sample: 3 columns 200 μm apart from each other, and each containing 10 readings every 30 μm from the surface. The hardness data obtained in groups MB, I, E and EH were applied in the mineral loss formula (∆S). For Optical Microscopy of Polarized Light, 5 halves of each group (n=5) were used and polished until they reached a thickness of 0.1mm and taken to Microscopy. Shapiro-Wilk was applied for color stability and mineral loss, then analysis of variance (one-way ANOVA), followed by the Post Hoc test by Bonferroni and Tukey, respectively, and the significance level set at 5%. In the results of color stability, regardless of time, there was no significant difference between groups H and MB; in 14 days, H and MB differed statically from infiltrated groups I, E and EH and, in 28 days, MB differed from all infiltrated groups, and H differed from E and EH. The variation between 14 and 28 days was not significant for any group. In terms of mineral loss, MB group showed a significant difference in relation to groups I and EH, but it did not differ from group E. I and EH did not differ statistically. Images obtained was possible to observe initial lesions in the MB group and superficial deposition of material in the infiltrated groups. In conclusion, the experimental groups of resinous infiltrants performed similarly to the commercial ones in terms of color variation, but they had a greater variation than that of untreated surfaces; in the cross-sectional microhardness, it was possible to notice that groups I and EH presented smaller mineral loss, suggesting a reinforcement in dental structure, while the groups MB and E presented greater mineral loss.

Keywords: Caries. Demineralization. Dental materials.

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1.INTRODUÇÃO

Estima-se que de 3 a 5 bilhões de pessoas na população global sejam acometidas por doenças bucais, sendo a doença cárie a mais recorrente (Watt et al., 2019). No Brasil, a prevalência de lesões cariosas não tratadas em 1990 era de 38,17% e em 2017 esse número passou a ser de 37,16%, sendo uma redução pouco expressiva considerando o tempo (Crescente et al., 2022) e, por isso, é muito importante que o cirurgião-dentista seja capaz de diagnosticar precocemente essas lesões e se atentar aos determinantes sociais e comportamentais que podem estar associados ao seu desenvolvimento (Warreth, 2023).

A lesão de cárie dentária é o resultado de uma dissolução química da superfície dentária frente aos eventos metabólicos que ocorrem no biofilme (Fejerskov, 2017). Esse desenvolvimento é dependente de alguns fatores que determinam a doença, como: a microbiota presente, hábitos e comportamentos de higiene bucal, composição da dieta e frequência e a capacidade tampão da saliva (Fejerskov, 2017; Warreth, 2023; Malcangi et al., 2023). Há um equilíbrio entre a desmineralização e a remineralização na superfície dental, quando o pH cai abaixo de certo nível ocorre a desmineralização, e quando ele sobe ocorre uma nova deposição mineral (remineralização) entre o biofilme e a estrutura, e quando há um descompasso dessa dinâmica ocorre maior desmineralização do que remineralização, surgindo assim os primeiros sinais da lesão cariosa (Fejerskov, 2017).

As lesões cariosas se desenvolvem a partir de eventos metabólicos dos biofilmes na superfície dental, havendo uma perda de substância mineral do dente tornando-o poroso, reduzindo a sua translucidez e, assim, caracterizando o primeiro sinal clínico da lesão de cárie: a mancha branca (El-Zankalouny et. al, 2016; Fejerskov, 2017). Essas lesões podem estar ativas, com a doença e progressão ainda em curso, ou inativas, onde estão paralisadas, porém o sinal clínico permanece, como uma cicatriz na superfície do esmalte dental, na forma de mancha branca ou com coloração alterada (Fejerskov, 2017). O sinal clínico da mancha branca antecede a cavitação do esmalte, e esse sinal ocorre por meio da diferença de refração que existe entre o esmalte sadio, onde a região mais desmineralizada vai apresentar uma maior captação de luz, tornando mais visível a discrepância da coloração translucida para opaca (Warreth, 2023).

Regiões onde o biofilme encontra-se protegido de ações mecânicas que o desorganizem são mais propensas a desenvolverem as lesões, como áreas proximais e de cicatrículas e fissuras. No entanto, também podem se desenvolver em outras regiões, desde que haja o acúmulo de biofilme (Fejerskov, 2017).

Por ser o primeiro sinal clínico, é de muita importância o diagnóstico dessas manchas brancas e que seja realizado o tratamento mais conservador possível para que a lesão seja paralisada. O seu correto diagnóstico se dá com a superfície limpa e seca, garantindo que seja possível uma correta visualização da lesão, pois caso a superfície porosa esteja úmida ela não irá apresentar o aspecto opaco e poroso, podendo ser incorretamente diagnosticada como uma lesão inativa ou até mesmo como superfície hígida (Fejerskov, 2017; Warreth, 2023).

A remineralização não é sinônimo de paralisar a lesão de cárie, visto que para sua paralisação é essencial agir na origem da doença: o biofilme cariogênico (Fejerskov, 2017). No entanto, a remineralização do esmalte é um tratamento ótimo para a estagnação das lesões iniciais em esmalte aliado com o controle da placa. O método mais amplamente difundido é a aplicação tópica de flúor, uma vez que quando o flúor está presente no fluido do biofilme e este não se encontra abaixo de pH 4,5, ocorre a formação de fluorapatita havendo então a diminuição da dissolução do esmalte (Cury, Tenuta, 2008).

Mesmo com uma lesão paralisada e remineralizada, o sinal clínico da mancha branca pode permanecer e causar incômodo estético dependendo de sua extensão e severidade. Uma alternativa é a microabrasão, podendo ser realizada somente em lesões inativas, que consiste em remover mecanicamente a mancha com o uso de ácidos, fluorídrico, clorídrico ou fosfórico, e com ou sem o uso de pastas e/ou pós abrasivos (Yetkiner et. al, 2014; Baratieri, 2018).

Em se tratando de lesões subsuperficiais não cavitadas e com manutenção da superfície do esmalte, terapêuticas com preparos não são indicadas, visto que tecido dental sadio seria removido desnecessariamente durante esse procedimento. Pensando nisso, foi desenvolvido o infiltrante resinoso como tratamento alternativo minimamente invasivo, que tem como mecanismo sua infiltração ao longo da superfície desmineralizada, impedindo a progressão da lesão e proporcionando a preservação da estrutura dentária (Paris, Meyer-Lueckel, 2010; Paris, Meyer-Lueckel, 2010; Araújo, et. al, 2013; Perdigão 2019).

O infiltrante resinoso também pode ser utilizado para o mascaramento da mancha branca, sendo a infiltração do material resinoso facilitada pelo condicionamento ácido prévio, tornando a superfície do esmalte mais permeável. Com a penetração do infiltrante no corpo poroso da lesão, as propriedades ópticas e mecânicas do esmalte dental são modificadas, devido à alteração do índice de refração mais próximo da superfície dental e consequente mascaramento da área esbranquiçada (Paris et. al, 2007; Paris, Meyer-Lueckel, 2010; Perdigão, 2019).

Os infiltrantes resinosos possuem uma natureza menos viscosa com monômeros de trietileno-glicol-dimetacrilato (TEGDMA), que possibilitam o material infiltrar nas microporosidades do esmalte, permitindo que essas áreas de fragilidade sejam reforçadas e impedindo sua cavitação e progressão (Paris, Meyer-Lueckel, 2010; Perdigão, 2019). Estudos prévios relatam que há bons resultados clínicos, principalmente considerando a paralisação da progressão da lesão (Paris, Meyer-Lueckel, 2010; Gelani et. al, 2014; Perdigão, 2019).

O infiltrante disponível no mercado é o Icon®, produzido pelo fabricante DMG e sua composição é a partir de TEGDMA. O TEGDMA é um monômero de baixo peso molecular e baixa viscosidade, que apresenta hidrofilicidade, fazendo com que o material absorva mais fluidos e pigmentos (Gelani et. al, 2014; Golz et. al, 2016). Embora o Icon® tenha ótimas vantagens, como impedir a progressão da lesão de cárie e melhorar a estética da área, ele apresenta algumas limitações em suas propriedades mecânicas, como resistência flexural, sorção e solubilidade, e na estabilidade de cor devido ao TEGDMA (Chen et. al, 2019; Pedreira et al., 2021). Por isso, o uso de BisEMA (etoxi bisfenol A glicidil dimetacrilato) é promissor, considerando que tem menor sorção de água proporcionando maior estabilidade de cor do que o TEGDMA (Sideridou et al., 2003; Fonseca et al., 2017), e foi incorporado em infiltrantes resinos experimentais mostrando bom potencial (Sfalcin et al., 2017; Mathias et al., 2019; Gaglianone et al., 2020; Pedreira et al., 2021).

Pesquisas têm sido desenvolvidas para formulações de infiltrantes experimentais com o objetivo de melhorar o desempenho do material (Paris et al., 2013; Araújo et al., 2013; Wang et al., 2020), principalmente visando uma melhor profundidade de penetração e/ou garantindo propriedades que ele não possua, como radiopacidade e adição de nanopartículas (Pedreira et al., 2021; Souza et al., 2023). Seria desejável um infiltrante que promova remineralização nas estruturas remanescentes, para garantir uma maior estabilidade de tratamento e um ganho mineral para o esmalte desmineralizado, e com esse objetivo têm surgido estudos sobre a incorporação de nanomateriais como hidroxiapatita (Andrade Neto, et al. 2016; Bonilla-Represa, et. al 2020; Souza et al., 2023).

Pensando no reforço de estrutura dental e na estabilidade de cor em tratamentos que visam a paralização da lesão de carie, o estudo de Ayad e colaboradores (2022) comparou a microdureza e a estabilidade de cor de lesões artificiais de mancha branca tratadas com infiltrante resinoso quando comparado ao grupo tratado com fosfato de cálcio amorfo, e foi concluído que o Icon aumentou a resistência do esmalte e diminuiu a susceptibilidade ao manchamento (Ayad et al., 2022)

A hidroxiapatita é um material composto de cálcio e fosfato presente abundantemente nos ossos e nos dentes, biocompatível e apresenta bioatividade por sua capacidade de adesão ao tecido mineralizado e indução da remineralização dos tecidos. Ao longo dos anos, estudos têm sido desenvolvidos com sucesso sobre sua incorporação em materiais odontológicos na forma de nano-hidroxiapatita (nano-HAP) e aplicações nos mais diversos segmentos, como implantodontia, engenharia tecidual, hipersensibilidade dentinária, clareamento e como agente remineralizante de lesões incipientes de cárie (Bordea, et. al 2020; Souza, et al., 2023; Andrade Neto et al., 2016).

Em um estudo prévio, Andrade et al (2016) observaram resultados positivos na incorporação de nano-HAP a um infiltrante na concentração de 10%wt; da mesma forma, a incorporação em selantes resinosos também se mostrou promissora (Andrade Neto et. al, 2016; Memarpour et. al, 2019; Haghgoo et. al, 2012). Mais recentemente, Elembaby e colaboradores (2022) incorporaram nanohidroxiapatita adquirida comercialmente ao Icon, e obtiveram resultados positivos nos grupos com concentrações de nano-HAP em 5 e 10%, melhorando as superfícies das lesões artificiais em esmalte quanto a sua lisura de superfície, densidade mineral e penetração do material. No estudo de Souza e colaboradores, em 2022, utilizando grupos com biossilicato e nanohidroxiapatita, o grupo nano-HAP apresentou bons resultados quanto ao grau de conversão e foi capaz de aumentar a densidade de conteúdo mineral nas lesões iniciais.

Outra questão metodológica para avaliação dos infiltrantes resinosos são os métodos para verificar sua penetração e reforço da estrutura. O teste mais comumente utilizado é a Microscopia Confocal, que permite obervar a infiltração dos monômeros nos microtúbulos de esmalte, porém é uma metodologia sensível e considerada qualitativa, além de que é possível avaliar a penetração, mas não identificar o conteúdo que foi capaz de infiltrar. Apesar das limitações, vários estudos demonstraram através desse método a penetração do infiltrante resinoso (Souza et al., 2023; Subraniam et al., 2014; Pedreira et al., 2021; Wang et al., 2020; Cerqueira et al., 2023; Ionta et al., 2016).

Ainda se tratando de metodologias, são escassos os trabalhos que conseguem verificar se a região infiltrada acometida pela lesão inicial de mancha branca realmente tem um reforço mecânico ou se a paralisação da progressão se dá apenas pela vedação superficial da estrutura dental. Um dos métodos bem estabelecidos para verificar a perda mineral é a microdureza transversal, permitindo o cálculo de ΔS onde se verifica o quanto de desmineralização a estrutura apresenta (Delbem, Cury, 2003; Argenta et al., 2003; Almeida et al., 2019), principalmente aliado com a microscopia de luz polarizada (Ayad et al., 2022), que nos permite visualizar o padrão da lesão de maneira qualitativa. Nesse aspecto, seria interessante avaliar através desse método a ação do infiltrante resinoso na superfície dental com lesão inicial de mancha branca.

Apesar de suas características desejáveis, ainda não há muitos estudos na literatura que avaliem outras propriedades da incorporação de nanohidroxiapatita em infiltrantes resinosos, como a estabilidade de cor. Como citado anteriormente, apesar do infiltrante estar penetrado nas microporosidades do esmalte, a região superficial está exposta ao meio ambiente oral, tendendo a incorporar pigmentos e sofrer mais manchamento do que uma resina convencional, devido à natureza de hidrofilicidade do TEGDMA (Chen et. al, 2019; Ceci et. al, 2017; Gelani et. al 2014).

Por haver apenas um infiltrante comercial (Icon®), que por conta de sua hidrofilicidade, oriunda do TEGDMA, há uma redução de suas propriedades como a resistência à degradação e favorecendo o machamento, justifica-se a necessidade de desenvolver outras formulações que apresentem características mecânicas e desempenho clínico mais promissores (Paris, Meyer-Lueckel, 2010; Araújo et. al, 2013), e também torna-se atrativa a possibilidade de incorporação de nanohidroxiapatita visando aumentar a resistência da estrutura dental (Elembaby, 2022).

Considerando as possibilidades de novas formulações, julga-se interessante a incorporação de nano hidroxiapatita na concentração de 10%wt e avaliações sobre sua estabilidade de cor frente a um manchamento artificial, e seu reforço estrutural por meio da microdureza seccional, em comparação ao controle comercial Icon® e ao controle experimental. As hipóteses nulas a serem testadas foram: 1) não haverá diferença entre os grupos infiltrados quanto à perda mineral em relação ao controle mancha branca e 2) não há diferença entre os grupos na estabilidade de cor das superfícies.

2 ARTIGO: INCORPORATION OF NANO-HYDROXYAPATITE INTO EXPERIMENTAL RESIN INFILTRANT AND ITS PERFORMANCE ON COLOR STABILITY AND REINFORCEMENT IN DEMINERALIZED ENAMEL: IN VITRO STUDY

Submetido no periódico Clinical Oral Investigations

Incorporation of nano-hydroxyapatite into experimental resin infiltrant and its performance on color stability and reinforcement in demineralized enamel: *in vitro* study

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ABSTRACT

Objective: The aim of the study was to evaluate the influence of the incorporation of 10% nano-hydroxyapatite into an experimental resin infiltranton color stability and cross-sectional microhardness.

Material and methods: 135 blocks were divided into five groups: H: healthy; MB: white spot; I: Icon®; E: experimental; EH: experimental containing 10% nano-hydroxyapatite. For color evaluation (n=15), CIEL*a*b* values were obtained at the following time points: T0 (before immersion), T1 (14 days after immersion), and T2 (28 days after). Data were applied to CIEDE2000 formula. Cross-sectional microhardness (n=12) data from the MB, I, E, and EH groups were applied to the mineral loss formula (ΔS). Polarized Light Optical Microscopy images were obtained (n=5) at 40x magnification. Shapiro-Wilk test was used to assess data normality for color stability and mineral loss. One-way ANOVA analysis was performed, followed by Bonferroni's post hoc test (color stability) and Tukey's test (mineral loss).

Results: In color stability results, regardless of time, there was no significant difference between H and MB groups; at 14 days and at 28 days, MB differed from all infiltrated groups, and H differed from E and EH. For ΔS, MB group showed a significant difference compared to I and EH groups but did not differ from E.

Conclusion: E and EH showcased similar performance to I regarding color variation. In terms of ΔS, I and EH had less mineral loss, suggesting a reinforcement of the dental structure.

Clinical Relevance: Predict color stability and structural reinforcement of resinous infiltrants applied to white spot lesions.

Key words: Dental caries. Demineralization. Resin infiltrants. Color stability.

INTRODUCTION

Non-invasive methods are very important for the treatment of early-stage carious lesions, such as the white spot lesion, ideally being able to reinforce the structure weakened by demineralization caused by cariogenic biofilms (1). Aiming to promote enamel remineralization, the most widely used and safe therapies are the topical application of fluoride and mouth rinses or tooth pastes containing stannous fluoride (2,3,4).

Besides the potential progression to cavitation when left untreated, early white spot lesions have a different refractive index from healthy enamel, hence being characterized by its whitish appearance that can cause aesthetic damage to the patient (5).

Considering the need to halt the lesion at an early stage and mask its coloration, resin infiltrants have been recommended as treatment (6, 7, 8). Their mechanism of action starts with the activity of 15% hydrochloric acid, which dilates the enamel microtubules to allow the material to penetrate (9). As the commercially available infiltrant Icon is still patented by the manufacturer DMG its exact formulation is proprietary, but it is known to contain triethylene glycol dimethacrylate (TEGDMA) and additives.

These monomers are responsible for ensuring that the material has low viscosity and can penetrate the enamel microtubules. Through this penetration, the microtubules are occluded, leading to the interruption of the interaction between acids from the biofilm and the dental structure, thereby halting the lesion. Also, by filling the tubules, it masks the whitish coloration, providing an aesthetic result (6; 8; 10).

Icon presents a behavior similar to dental adhesives due to its composition, for this reason, Icon does not offer good color stability over time, as well as its flexural strength and physicochemical properties are inferior when compared to sealants and resins (7; 11). However, several studies aim to improve these properties, such as with the incorporation of particles and alternative use of monomers (12; 13; 14; 15; 16).

Hydroxyapatite, abundantly present in the enamel and highly biocompatible, has been employed in various materials to improve their performance, as seen in sealants, resins, and adhesives (17; 18; 19). The incorporation of hydroxyapatite into resin infiltrants has shown good results in terms of mineral density and a high degree of conversion, resulting in the good physical and mechanical performance of the material (11;20).

However, since the infiltrant is a material that is intended to act within the dental structure, the methodology for evaluating it becomes challenging. Studies assessing their structural reinforcement are restricted to surface microhardness, confocal microscopy to verify its penetration, and quantification methods of chemical elements (EDS) (11; 20). Frequently used in cariology studies, cross-sectional microhardness is employed to measure the internal hardness of the dental structure and therefore can be useful in evaluating the action of resin infiltrants, as demonstrated (21; 22).

Regarding color stability, there are studies that evaluate staining in several solutions and demonstrate that the material tends to become highly pigmented (23; 24). Clinically, this would lead to the need for intervention due to its non-aesthetic appearance, as the resin would have a different coloring from the dental substrate.

Reflecting on new formulations for resin infiltrants and their proposed action, the present study aimed to evaluate the incorporation of nano-hydroxyapatite into experimental infiltrant, comparing it with a commercial infiltrant, an experimental infiltrant, and untreated surfaces. The study verified color stability against staining by an extrinsic solution, and internal reinforcement of the dental structure through cross-sectional microhardness and mineral loss calculation. The null hypotheses are: 1) the infiltrated groups will not differ in color stability from untreated surfaces, and 2) there will be no difference in mineral loss among the infiltrated groups compared to the white spot control.

MATERIALS AND METHODS

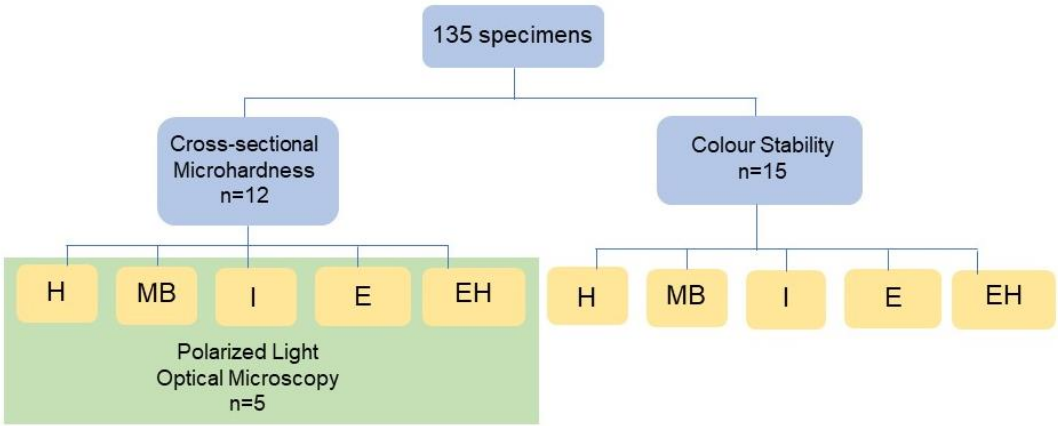
A total of 135 bovine incisors were obtained, cleaned using scalpel blades and pumice stone, and stored in a 0,1% thymol solution. Specimens following the 4x4 standard were prepared from these incisors using cutting disks in a metallographic cutter (Buehler LTD., Lake Bluff, IL, USA). The back surface was flattened using 600-grit sandpaper, and the buccal surfaces were polished with 600, 1200, and 2000-grit water sandpaper on a polisher (Arotec S/A Indústria e Comércio, Cotia-SP, Brazil) for 30 seconds each, with ultrasonic baths in between for 5 minutes. They were then manually polished with diamond paste on felt for 2 minutes. Finally, the varnish (Colorama, São Paulo-SP, Brazil) was applied to all lateral and back walls, leaving the buccal surface exposed. The groups were divided into healthy enamel, initial white spot lesion, Icon, Experimental, and Experimental containing 10% nano-hydroxyapatite (Table 1). They were allocated to the tests according to the flowchart 1.

Table 1. Groups Division and their composition.

Group	Composition
Healthy (H)	No treatment
White spot (MB)	Superficial white spot lesion
Icon (I)	Resin infiltrant TEGDMA based, iniciators and additives*
Experimental (E)	Experimental resin infiltrant: 75% TEGDMA; 25% Bis-EMA; 1% EDAB; 0,5% CQ
Experimental containing 10% nano-hydroxyapatite (EH)	Experimental resin infiltrant : 75% TEGDMA; 25% Bis-EMA; 1% EDAB; 0,5% CQ; 10% nano-hydroxyapatite

*According to manufactor DMG, Hamburg, Germany.
Experimental resin infiltrants: ethoxy bisphenol A glycidyl dimethacrylate (Bis-Ema - Sigma Aldrich, St. Louis, EUA); triethylene glycol dimethacrylate (TEGDMA - Sigma Aldrich, St. Louis, EUA); camphorquinone (CQ - Sigma Aldrich, St. Louis, EUA); tertiary amine dimethylaminoethyl benzoate (EDAB - Sigma Aldrich, St. Louis, EUA); nano-hydroxyapatite (Sigma Aldrich, Steinheim, German)

Flowchart 1. Experimental design.



1. Induction of initial superficial white spot lesion

After obtaining the specimens, three surface microhardness readings were performed using a 50 g load for 10 seconds on the exposed surface of each specimen. The specimens were randomized into the healthy enamel group, and for the other groups, randomization occurred after a new surface microhardness reading following the demineralization-remineralization (DES-RE) protocol. The solutions was composed as described: DES 0.1 M acetate buffer containing 1.28 mM Ca, 0.74 mM Pi and 0.03 µg F/mL (3 mL/mm2) with pH= 5; RE composed of 1.5mM Ca, 0.9 mM Pi, 150 mM KCL, 0.05 µg F/mL, 0.1M Tris buffer, pH 7.0 (25). To simulate the demineralization cycle, the specimens were individually immersed in the DES solution for 4 hours at 37°C in an incubator, in an amount of solution in mililiters based on their surface area, then rinsed with deionized water and immersed in the RE solution for 20 hours. Seven cycles of DES-RE were performed, with each cycle corresponding to 24 hours, and on the eighth day the specimens remained in RE solution for 24 hours. The solutions were changed on the fourth day (25).

Specimens designated for color evaluation were not subjected to surface microhardness testing and were randomized before the DES-RE protocol based on the Lightness (L*) value obtained from three readings on each specimen using a light hanger with a spectrophotometer (Konica Minolta Sensing Americas, Inc, USA).

2. Experimental infiltrants formulation

The experimental infiltrant are composed of Bis-EMA (25%) and TEGDMA (75%) as the monomeric base, using Edab (1%), and camphorquinone as photoinitiator at 0.5% concentration (14, 26, 27). All components were calculated for the manipulation of 5ml of the material and weighed on an analytical scale under a yellow light environment. They were then stored in sealed vials and taken to the high-frequency mixer SpeedMixer (FlackTeck, Inc, Landrum-SC, USA) for 5 minutes at 3000 rotations to ensure homogeneity.

For the manipulation of the material containing nano-hydroxyapatite, the same monomeric base described for the experimental group was used, the only difference being the addition of commercially acquired hydroxyapatite nanoparticles (Sigma-Aldrich, Steinheim, Germany) at a concentration of 10% (15,17) so it could later be taken to the SpeedMixer. Prior to use, both materials were stirred in a magnetic stirrer (Model M089, Piracicaba-SP, Brazil) for 5 minutes.

3. Resin infiltrants application

To apply the material onto the specimen surfaces the protocol suggested by the manufacturer DMG (Hamburg, Germany) was followed. Etching was performed with 15% hydrochloric acid for 2 minutes, followed by thorough rinsing, Icon dry application for 30 seconds, infiltrant application for 3 minutes, photopolymerization (Valo corded, 395-480nm, irradiance 1000mW/cm², Ultradent, South Jordan-UT, USA), followed by a new 1-minute application and polymerization. Then finally polishing the surface using low-speed abrasive rubbers (TDV Dental, Pomerode-SC, Brazil) for 20 seconds.

4. Colour stability

The specimens were fixed in acrylic plates with sticky wax on the back wall and sides, exposing only the buccal surface, and were immersed in a container with 1.5 liters of coffee solution (Mellita do Brasil Indústria e Comércio, Avaré-SP, Brazil) for a period of 28 days in an incubator at 37º C, with daily solution changes (23,28). For each color evaluation, the samples were removed from the acrylic plate, rinsed thoroughly with distilled water, color assessed, and then reattached to the plates.

The color evaluation was performed using a spectrophotometer (Konica Minolta Sensing Americas, Inc, USA) at three distinct time periods: (T0) before immersion, (T1) 14 days after immersion, and (T2) 28 days after immersion.

The data obtained from the CIEL*a*b* system, which correspond to L* for lightness, a* for chromaticity (red-green), and b* (blue-yellow), were used in the CIEDE2000 color variation formula (∆E00) between the T0-T1, T0-T2, and T1-T2 intervals.

5. Cross-sectional microhardness

The specimens for this analysis were sectioned in the buccolingual direction right in the center of the sample using a cutting disk on a metallographic cutter (Buehler LTD., Lake Bluff, IL, USA). Half of each sample was randomly selected and all were placed in an embedding device (Arotec S/A Indústria e Comércio, Cotia-SP, Brasil) with the surface to be analyzed facing the metal platform to be embedded in clear acrylic resin (VIPICril Plus, Pirassununga-SP, Brazil). Once the stubs with the samples were obtained, they were taken to the polisher (Arotec S/A Indústria e Comércio, Cotia-SP, Brasil) where they were polished for 10 minutes at low speed with 600-grit sandpaper, for 13 minutes at high speed on 1200-grit sandpaper, and finally for 15 minutes on felt with a polishing solution. At each interval they were placed in an ultrasonic bath for 5 minutes.

Next, the samples were taken to the microdurometer (Future Tech FM Hardness Tester, Future Tech Corporation, Kawasaki, Japan) for analysis. After a pilot test, the distances were established as follows: 3 columns spaced 200 μm apart, each containing 10 rows spaced 30 μm apart, with the first reading also taken 30 μm from the surface. A load of 25 g was used for 5 seconds

The data were allocated into a spreadsheet where the calculation of ∆S was performed. This value is attained from the areas calculated by numerical integration of the hardness values versus depth (kg/mm2 × μm), using the trapezoidal rule (29).

6. Polarized Light Optical Microscopy

From the halves not used for microhardness testing, 5 samples from each group were selected to be taken to the Polarized Light Microscope (Leica DMLP, Optika Microscopes, Ponteranica-BG, Italy). To prepare these samples, they were polished in a polisher with 1200-grit sandpaper until they reached a thickness of 0.6mm. Then, they were manually polished with 600-grit sandpaper until they reached a thickness of 0.3mm, followed by 1200-grit sandpaper until they reached a thickness of 0.2mm, and finally, 2000-grit sandpaper until they had a thickness between 110 to 170 μm. At last, they were polished for 1 minute on felt with diamond paste. They were stored in a humid environment under refrigeration and taken to the microscope, where they were analyzed between a glass slide and coverslip, containing distilled water at the interface. The images were obtained with a 40x objective.

7. Statistical analysis

For color stability, R program version 4.2.2 was used. The normality assumption of the data was accepted through the Shapiro-Wilk test, and a one-way ANOVA test was applied for each $\Delta E00$ interval, followed by Bonferroni post hoc test. For internal hardness (ΔS), SigmaStat 4.0 program was used. The data were square root transformed, and the normality assumption was accepted using the Shapiro-Wilk test, followed by one-way ANOVA and Tukey's post hoc. In both statistics, the significance level was set at 5%.

The images obtained through polarized light microscopy were analyzed qualitatively.

RESULTS

1. Color stability

One-way ANOVA revealed a significant difference in ∆E00 T0-T1 between the treatment groups. Multiple comparisons (post hoc) exposed significant differences in averages between the H and MB groups compared to the infiltrated groups. H and MB did not differ from each Other (Table 2).

For ∆E00 at T0-T2, one-way ANOVA also showed a statistically significant difference between the groups. Multiple comparisons showed differences between the H, E, and EH groups, but H did not differ significantly from I and MB groups. MB, on the other hand, differed from all three infiltrated groups (Table 2).

Regarding ∆E00 between T1-T2 time intervals, the one-way ANOVA revealed no significant difference between the groups.

Table 2. Color stability through CIEDE2000 (∆E00).

	Groups					
Time	**H**, N = 15[1]	**MB**, N = 15[1]	**I**, N = 15[1]	**E**, N = 15[1]	**EH**, N = 15[1]	**p-value**[2]
T0 -T1	9.6 ± 2.1 **A**	9.7 ± 3.3 **A**	15.0 ± 3.9 **B**	17.6 ± 4.0 **B**	18.4 ± 3.2 **B**	<0.001
T0 – T2	16.9 ± 2.1 **AB**	15.0 ± 3.0 **A**	20.7 ± 5.0 **BC**	23.7 ± 4.1 **C**	24.8 ± 3.9 **C**	<0.001
T1 – T2	9.30 ± 1.85 **A**	7.19 ± 1.29 **A**	8.93 ± 4.89 **A**	8.43 ± 1.49 **A**	9.19 ± 1.65 **A**	0.2

[1]Mean ± SD

[2]One-way ANOVA

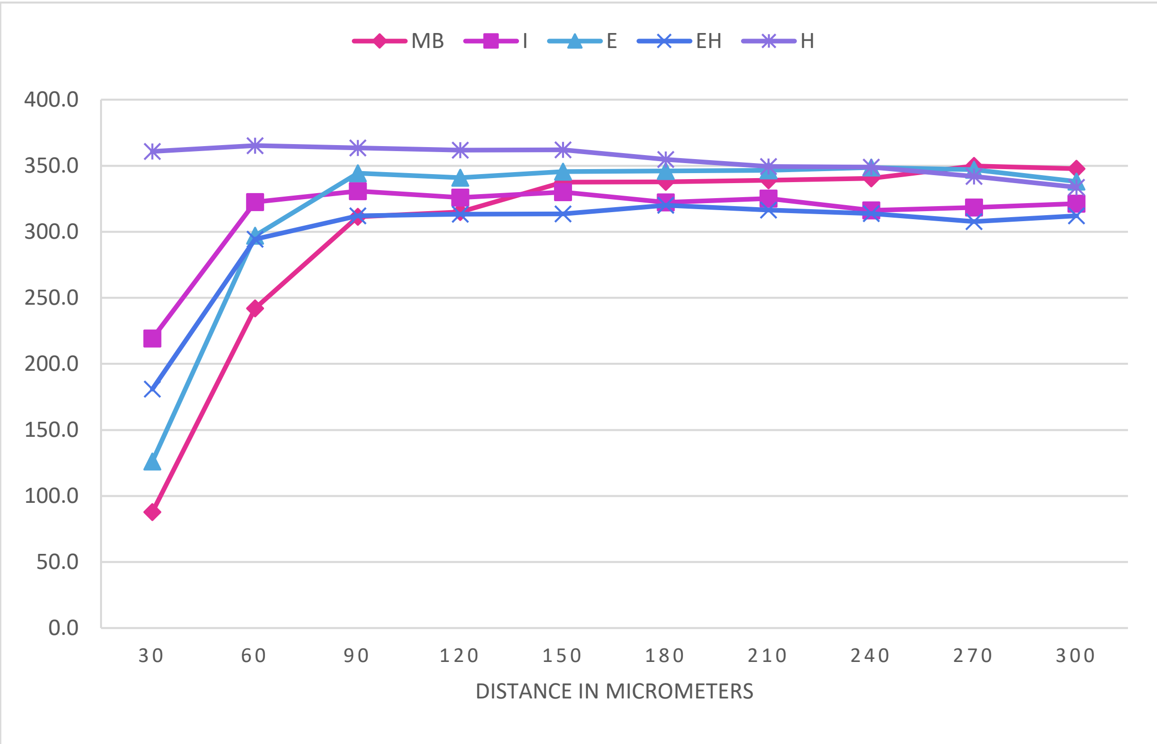
Different letters on the lines means statistical difference between groups.

2. Cross-sectional microhardness

The distribution of ∆S data is shown in Graphic 1. As described, microhardness was performed in all groups; however, due to the healthy group not presenting demineralization, its ∆S formula calculation resulted in 0 for all samples since it presented a mineral plateau, making the statistical analysis of this group impossible. Nonetheless, data were obtained to verify the absence of demineralization and it was possible to draw a graph to visually compare with the other groups (Graphic 1).

The MB and E groups did not show a statistically significant difference between them, as did the I and EH groups. However, MB and E differed statistically from the I and EH groups (Table 3).

Graphic 1. Microhardness in function of distance.



H: Healthy; MB: White spot; I: resin infiltrant Icon; E: Experimental infiltrant; EH: Experimental infiltrant containing 10% nano-hydroxyapatite.

Table 3. ∆S means and standard deviantion.

	∆S
Group	**Mean (SD)**
MB	107,75 (26,63) **A**
I	55,58 (15,5) **B**
E	88,36 (20,37) **A**
EH	65,48 (18,55) **B**

Distintic letters represents significant statistical difference (p<0,05).

H: Healthy; MB: White spot; I: resin infiltrant Icon; E: Experimental infiltrant; EH: Experimental infiltrant containing 10% nano-hydroxyapatite.

1. Polarized Light Optical Microscopy

The images were obtained using a 40 objective, as can be seen in the following figures. In the image captured from the H (Figure 1) group the uniformity of the enamel structure can be observed, while in the MB (Figure 2) group, an initial subsurface lesion is visible. In the infiltrated groups (Figures 3, 4 and 5) there is a superficial deposition of resin material, and near the edges of the specimen a decline in the mineral surface can be noticed (Figure 6). No initial white spot lesion was detected in the infiltrated groups.

Figure 1. Healthy group, 40x magnification.

Figure 2. White spot (MB), 40x magnification.

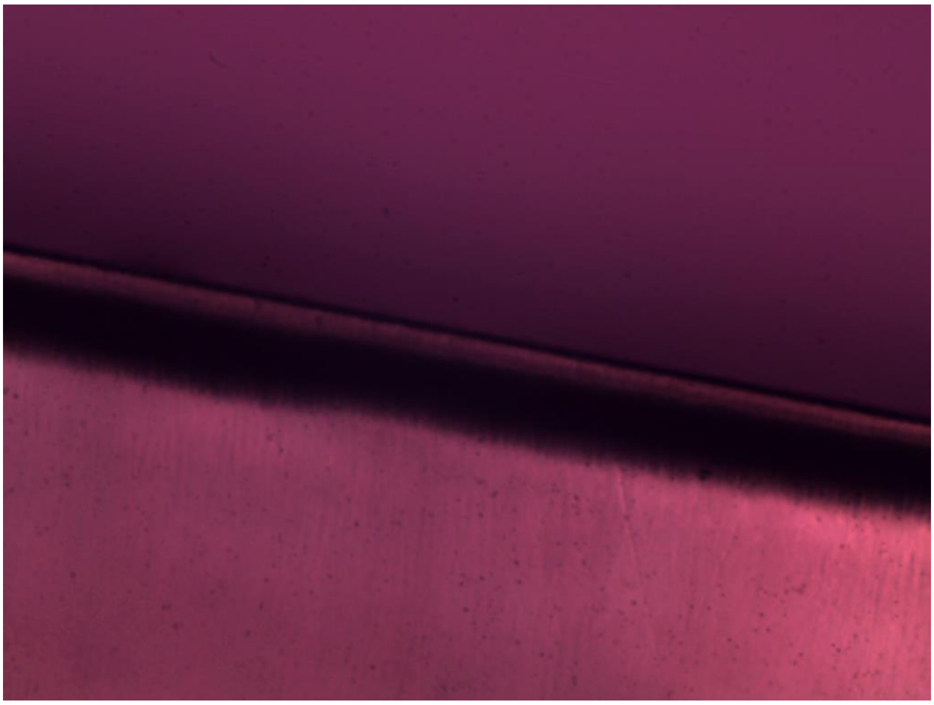


Figure 3. Resin infiltrant Icon (I), 40x magnification.

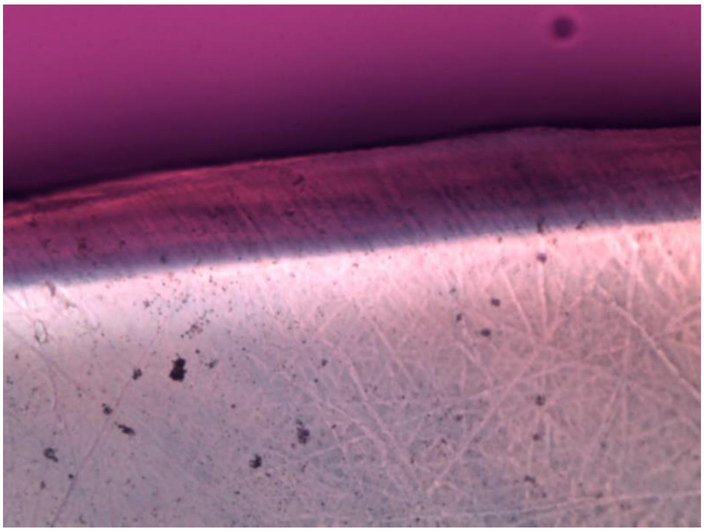


Figure 4. Experimental resin infiltrant (E), 40x magnification.

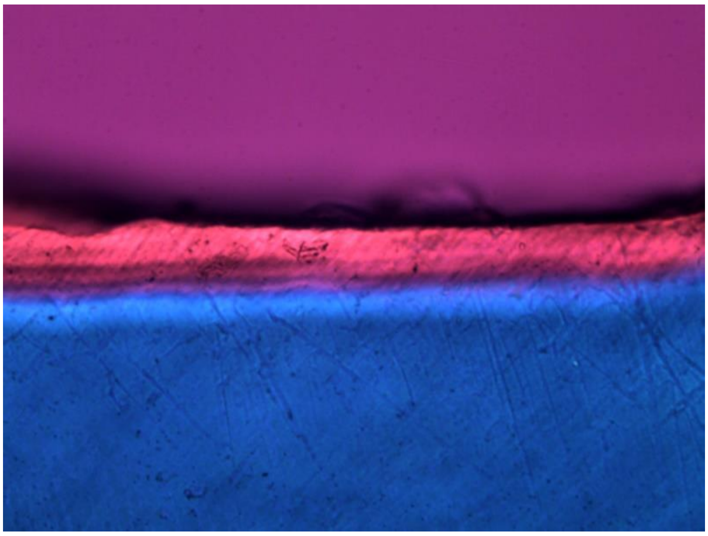


Figure 5. Experimental resin infiltrant containing 10% nano-hydroxyapatite (EH), 40x magnification.

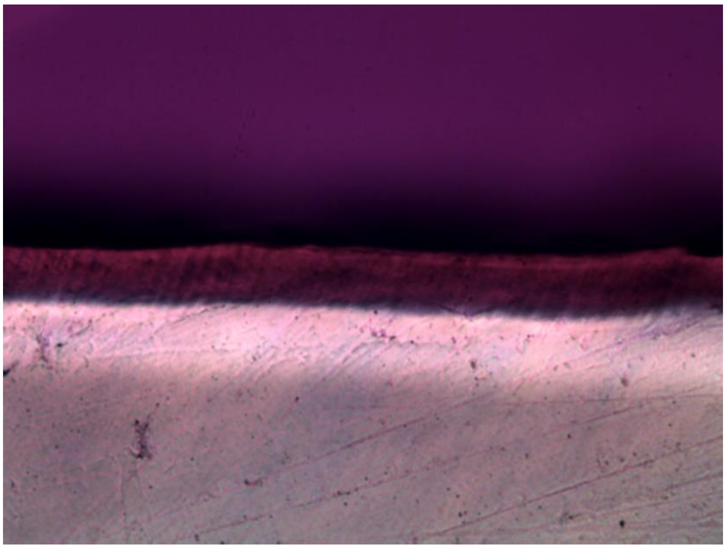
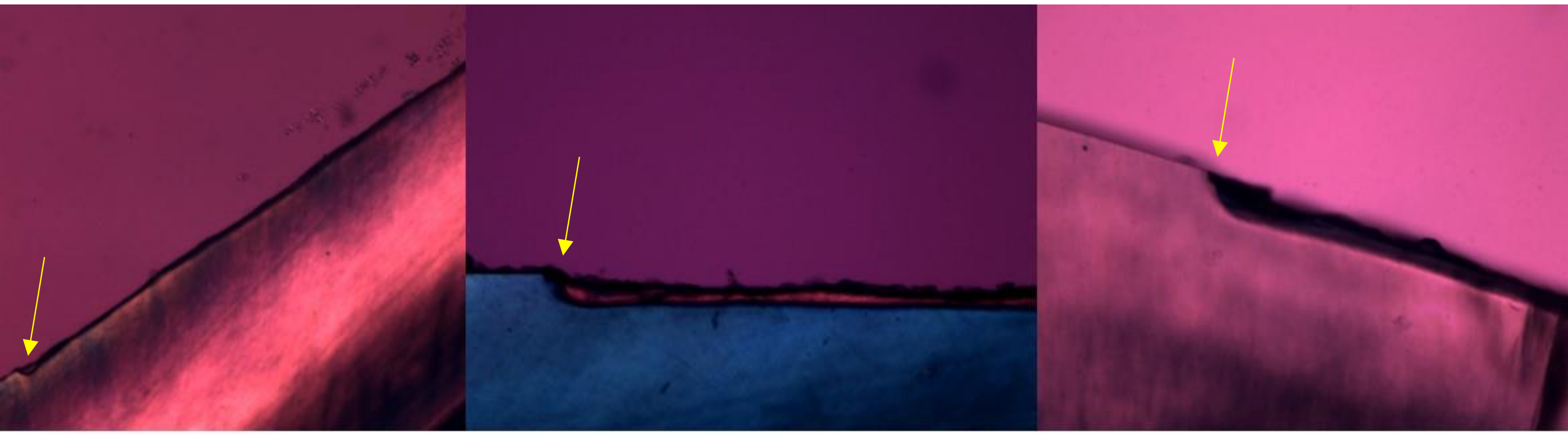


Figure 6. Declines on surface, Icon (I) in 5x magnification (left), Experimental (E) in 10x magnification (center), Experimental containing 10% nano-hydroxyapatite (EH) in 10x magnification.



DISCUSSION

The first hypothesis that there would be no difference between the infiltrated groups and the untreated surface groups was rejected. Due to the material's high sorption properties (13,30), the infiltrant tends to incorporate pigments, represented by the ∆E00 evaluation. It was noticed that the three infiltrated groups (I, E, EH) showed higher staining compared to the healthy surface and initial white spot lesion groups during the 14-day period. This conclusion is in line with the study by Leland et al. from 2016, where they found a higher ∆E in surfaces treated with Icon compared to the healthy surface.

The choice of coffee as a pigmentation agent was based on studies that also evaluated the color stability of resin infiltrants, such as the studies by Leland et al. from 2016, Ayad et al. from 2022, and Ceci et al. from 2017 (23, 31, 28). Coffee was chosen because it is commonly consumed by patients in their daily lives and has a high pigmentation capacity. Most studies use the CIEL*a*b* calculation to obtain ∆E for color variation between periods. However, this study used the CIEDE2000 calculation, which is more sensitive in identifying color changes and better mimics human vision (32).

In the 28-day period (T0-T2), the I group did not differ statistically from the H group, suggesting that its long-term pigment incorporation was similar to that of the dental structure. Nevertheless, considering the perceptibility and acceptability thresholds for ∆E00, which are 0.8 (perception) and 1.8 (acceptance), it can be assumed that a value higher than 0.8 makes the color difference perceptible, and when higher than 1.8, it can be considered as clinically acceptable or not by the observer (33). Thus, although there is no significant difference between these two groups, it can be inferred that under clinical conditions this difference may be perceptible and potentially aesthetically unfavorable, which could result in the need for clinical intervention to ensure aesthetic outcomes for the patient.

In terms of color stability, the experimental groups behaved similarly to the commercial group, with no statistical difference between them. This can be explained by their composition, which includes a higher constitution in the monomeric base such as TEGDMA that incorporates pigments, similar to Icon, as described by Alqahtani and Ceci (24,28). The formulation of the experimental infiltrants without the addition of nanoparticles is also supported by other studies, such as Cerqueira and Pedreira (34,14), where the depth of penetration, sorption and solubility results, cohesive strength, and degree of conversion were found to be similar or superior to Icon.

There was no statistical difference between the groups in the T1-T2 period, suggesting that the resin infiltrants reach color saturation during this time, i.e. having sufficient pigment impregnation. But as described previously, even if there is no statistically significant difference, these values may indicate a clinical perception, leading to aesthetic dissatisfaction.

Although not evaluated in this study, other research has reported that polishing can provide some reversibility of staining (23,35). Therefore, it is important to consider that patients should be instructed to undergo regular check-ups for control and guidance regarding the material (36,37).

The calculation of mineral loss (ΔS) is widely used in the fields of biochemistry and cariology to assess the amount of superficial enamel demineralization (29,38,39). The calculation is based on data obtained from cross-sectional microhardness, which allows the evaluation of the internal enamel surface. As the premise of the resin infiltrants is to penetrate and reinforce the dental structure, this methodology was applied to determine if there was indeed a difference in mineral loss compared to the untreated initial lesions

As observed in the table 2, the MB and E groups did not show a statistical difference, and we can infer that the E group was not able to reinforce the mineral structure. This may be due to its composition, consisting only of TEGDMA, BisEMA, EDAB, and camphorquinone, which did not provide it with mechanical strength (14, 27, 40).

On the other hand, the I and EH groups exhibited lower mineral loss, suggesting that they were able to reinforce the demineralized enamel. Therefore, the second hypothesis was also rejected. EH showed this behavior most likely because of its hydroxyapatite nanoparticles, which, as reported by Elambaby et al.(20), were able to improve the penetration of the Icon material and the mineral density. Another important characteristic for new materials is the degree of conversion, and Souza et al. reported that the experimental infiltrant containing 10% nano-hydroxyapatite achieved a high degree of conversion when compared to other infiltrants, including Icon(15).

As described by Meyer-Lueckel and Paris (1), the penetration depth of the infiltrant composed of TEGDMA is approximately 200 μm in natural lesions in human enamel. Therefore, it is important that the methodology employed for the internal surface evaluation be established accordingly. In this study, the distance was designated at 30 μm, extending up to 300 μm to ensure we were evaluating this infiltrated area.

The study by Ayad et al. (22) demonstrated that the initial white spot lesion group treated with Icon was able to reinforce the dental structure. However, despite corroborating the results presented in the table, some data differ, such as the distance used in the test. They employed an initial distance of 100 μm and did not calculate ∆S but rather the average at each distance to determine hardness.

Despite the results showing the lowest mineral loss, a finding of the present study was the presence of a decline in the majority of samples analyzed under polarized light microscopy. This decline can be attributed to the acid etching carried out with 15% hydrochloric acid for 2 minutes, as established by the manufacturer DMG, which is capable of removing the superficial micrometric layer (40 μm) and allowing the resin material to settle, as the surface was subtly removed, exposing the fully demineralized area (41).

Esteves-Oliveira (21) employed the same ∆S calculation methodology to evaluate the internal surface of specimens treated with resin infiltrant and different polishing protocols, differing from this study only in the distance employed. However, the values assumed for the first indentation were similar to the I and EH groups.

Considering the color and ∆S analyses, the EH group presented great promise by presenting results similar to the commercial product and, combined with its well-established biocompatibility potential in the literature, should be investigated with other methodologies capable of accurately mimicking or predicting the performance of resin infiltrants. As expected, the MB group had the highest mineral loss and also the greatest variability, yet it did not differ from the E group, demonstrating that this group was not able to provide reinforcement for the demineralized enamel region.

The premise of using resin infiltrants is highly beneficial for non-invasive dentistry and for halting the progression of early lesions. However, attempts to improve the properties of the material and, most importantly, maintain the integrity of demineralized enamel are valid. Hence, it is of utmost importance that this line of research be maintained and new methodologies be developed that more precisely access the mechanism of action of these materials and find further ways to enhance their viability.

CONCLUSION

- The infiltrated groups I, E, and EH exhibited similar behavior, with greater staining compared to the untreated H and MB groups at the 14-day evaluation, while at 28 days, group I showed results similar to H;
- The infiltrated groups I and EH showed lower mineral loss than the E and MB groups, suggesting a reinforcement of the demineralized dental structure;
- The EH experimental group exhibited a behavior similar to the commercial Icon in terms of structural reinforcement and staining, proving to be promising.

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3. CONCLUSÃO

De acordo com os dados obtidos no estudo foi possível concluir que a variação de cor foi maior nos grupos que receberam tratamento com infiltrantes resinosos no período de 14 dias, mas em 28 dias o grupo comercial Icon apresentou resultado semelhante à superfície hígida. Em relação à perda mineral, o grupo contendo nanohidroxiapatita teve desempenho semelhante ao Icon, e ambos apresentaram menor perda mineral em comparação com os demais. Considerando os resultados, o material experimental contendo nanohidroxiapatita se mostrou promissor.

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ANEXOS

Anexo 1 – Verificação de originalidade e prevenção de plágio

INCORPORAÇÃO DE NANOHIDROXIAPATITA À INFILTRANTE RESINOSO EXPERIMENTAL E SEU DESEMPENHO NA ESTABILIDADE DE COR E REFORÇO NO ESMALTE DESMINERALIZADO: ESTUDO IN VITRO

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