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AVALIAÇÃO E COMPARAÇÃO DE INFILTRANTES CONTENDO SAL DE IODÔNIO E QUITOSANA E INFILTRANTES COMERCIAIS

EVALUATION AND COMPARISON OF INFILTRANTS CONTAINING IODONIUM SALT AND CHITOSAN AND COMMERCIAL INFILTRANTS

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Trabalho de Conclusão de Curso apresentado à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do título de Cirurgiã Dentista.

Udergraduate final work presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Dental Surgeon

Orientador: Prof.ª Dr.ª Giselle M. Marchi Baron

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RESUMO

O presente estudo teve como objetivo avaliar e comparar o grau de conversão (usando a espectroscopia de infravermelho com transformador de Fourier), resistência à flexão e módulo de elasticidade (por teste flexão de três pontos), sorção e solubilidade da água e análise antimicrobiana (Concentração Inibitória Mínima e Concentração Bactericida Mínima - CIM e CBM, respectivamente) de infiltrantes experimentais contendo sal de iodônio e quitosana e do infiltrante da marca comercial Icon®. O grupo de infiltrante na concentração de 1% de DFI e 0,25% de Quitosana, foi o que apresentou os melhores resultados nos testes realizados. Esse grupo foi semelhante ao infiltrante comercial Icon® em todos os quesitos, tendo o diferencial de apresentar atividade bactericida que a marca comercial não apresenta. Concluindo que a adição de sal de iodônio e Quitosana são boas alternativas para a melhoria das propriedades mecânicas e antibacterianas dos infiltrantes.

Palavras–chave: Resina composta. Infiltrante. Quitosana. Ônio compostos.

ABSTRACT

The present study aimed to evaluate and compare the degree of conversion (using infrared spectroscopy with Fourier transformer), flexural strength and modulus of elasticity (for three-point bending test), sorption and solubility of the water and microbial analysis (Minimum Inhibitory Concentration and Minimum Bactericidal Concentration - MIC and MBC, respectively) of experimental infiltrants containing iodonium salt and chitosan and infiltrate commercial - Icon®. The Group of infiltrant with the 1% iodonium salt concentration and 0.25% chitosan was presented the best results in the tests. This group was similar to commercial Icon® infiltrant on all counts, taking the present differential bactericidal activity that the trade mark does not present. Concluding that the addition of iodonium salt and chitosan are good alternatives to improve the mechanical properties and antibacterial properties of experimental infiltrants.

Key words: Composite resin. Infiltrants. Chitosan. Onium Compounds.

SUMÁRIO

1 INTRODUÇÃO

As lesões de cárie ainda são de grande prevalência na população (Stensson et al., 2010) sendo percebidas inicialmente como lesões de mancha branca e tendo a desmineralização do esmalte subsuperficial como primeira manifestação clínica (Alfaya et al., 2013; Lima, 2016). Os procedimentos relacionados ao tratamento de lesões de mancha branca são na sua maioria baseados no reequilíbrio entre os processos de desmineralização e remineralização (Buzalaf et al., 2011). Devido a boa acessibilidade às lesões nas superfícies vestibulares, a aplicação tópica de fluoreto e melhoria da higiene oral tem sucesso nas mesmas atingindo a remineralização do esmalte desmineralizado, porém lesões mais profundas tendem a remineralizar apenas superficialmente (Fejrskov et al., 2008). Sendo assim, tendo como base o conceito de odontologia minimamente invasiva, novas abordagens têm sido estudadas, buscando o tratamento para tais lesões sem que haja remoção de tecido sadio. Diante disso, ocorreu o desenvolvimento dos infiltrantes, que são materiais resinosos hidrofóbicos de baixa viscosidade (Paris et al., 2012; Meyer-Lueckel et al., 2016), e, assim, o tratamento microinvasivo das lesões cariosas tem se tornado uma alternativa para lesões não cavitadas (Paris et al., 2012). Os infiltrantes agem em profundidade nas lesões de cárie incipiente, preservando o tecido dental, e demonstraram apresentar altos coeficientes de penetração de lesão cariosa (Paris et al., 2006). Para melhorias desses infiltrantes, o sal de iodônio (DFI) (Gonçalves et al., 2013) e a quitosana (Elsaka, 2012) têm sido estudados por promover características benéficas aos materiais dentários. A quitosana como sendo um agente microbiano, quando adicionado aos materiais restauradores demonstra melhoria dos mesmos com relação às infiltrações bacterianas, o que pode aumentar seu desempenho clinico e diminuir o crescimento de biofilme (Inagaki, 2016). Já o sal de iodônio, por ter uma baixa energia de ligação entre carbono e iodo aumenta o potencial de polimerização dos materiais resinosos quando adicionados aos mesmos (Gonçalves et al., 2013). Sendo assim, são necessários mais estudos visando a melhoria na formulação dos infiltrantes. Diante do exposto, o presente estudo tem como objetivo avaliar e comparar o grau de conversão, resistência à flexão e módulo de elasticidade, sorção e solubilidade da água e análise antimicrobiana dos infiltrantes contendo sal de iodônio e quitosana e o infiltrante da marca comercial Icon®.

2 ARTIGO: EVALUATION AND COMPARISON OF INFILTRANTS CONTAINING IODONIUM SALT AND CHITOSAN AND COMMERCIAL INFILTRANTS

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Title: Evaluation and comparison of infiltrants containing iodonium salt and chitosan and commercial infiltrants.
Short title: Evaluation and comparison of infiltrants.

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Key words: Composite Resin; Infiltrants; Chitosan; Onium Compounds.

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FULL TITLE: EVALUATION AND COMPARISON OF INFILTRANTS CONTAINING IODONIUM SALT AND CHITOSAN AND COMMERCIAL INFILTRANTS

SUMMARY TITLE: EVALUATION AND COMPARISON OF INFILTRANTS

ABSTRACT: **Purpose:** The current study had as an objective the evaluation and comparison of infiltrants containing iodonium salt and chitosan and commercial infiltrants. **Methods:** Comparing the degree of conversion (using infrared spectroscopy with Fourier transformer), flexural strength and modulus of elasticity (for three-point bending test), sorption and solubility of the water and microbial analysis (Minimum Inhibitory Concentration and Minimum Bactericidal Concentration - MIC and MBC, respectively) of experimental infiltrants containing iodonium salt and chitosan and commercial infiltrant - Icon®. **Results:** The Group of infiltrant with the 1% iodonium salt concentration and 0.25% chitosan presented the best results in the tests. This group was similar to commercial Icon® infiltrant on all counts, taking the present differential bactericidal activity that the trade mark does not present.

CLINICAL SIGNIFICANCE: the addition of iodonium salt and chitosan are good alternatives to improve the mechanical properties and antibacterial properties of experimental

Key words: Composite Resin; Infiltrants; Chitosan; Onium Compounds.

INTRODUCTION

Caries lesions are still highly prevalent in the population [1]. Being a chronic disease, its etiology is multifactorial: results of the interaction between microbial factors, diet, host conditions and time [2]. As a first clinical manifestation, there is demineralization of the subsurface enamel, clinically perceived as a white spot lesion due to the increase of porosity within the body of the lesion, resulting in this characteristic whitish appearance [3].

Treatment procedures of white spot lesions are mostly based on the rebalancing between demineralization and remineralization, minimizing the continuity of demineralization [4]. Topical application of fluoride and oral hygiene improvement are the common treatment strategy to achieve the remineralization of the demineralized enamel [5]. Due to the good accessibility of the lesions on the vestibular surface, these approaches have success in them, however, deeper lesions tend to remineralize only superficially [5].

Therefore, new approaches have been studied, based on the concept of minimally invasive dentistry, seeking treatment for such lesions without removing healthy tissue. In this way, the development of low-viscosity hydrophobic resin materials known as infiltrants occurred [6, 7]; and thus the microinvasive treatment of carious lesions has become an alternative for non-cavitated lesions [6].

The infiltrants act deeply on incipient caries lesions, preserving the dental tissue, and have shown high coefficients of penetration in carious lesion [8]. For improvement of these infiltrants, the Iodonium Salt (Diphenyliodonium Hexafluorophosphate, DPI) [9] and Chitosan [10] have been studied for promoting beneficial characteristics of dental materials. Chitosan, as a microbial agent, when added to restorative materials reduces bacterial infiltration, which can increase its clinical performance and decrease biofilm growth [11]. The iodonium salt, because of its low energy of carbon-iodine bonding, increases the polymerization potential of the resinous materials when added to them [9]. Therefore, further studies are needed to improve the formulation of infiltrating agents.

In view of the above, the present study aims to evaluate and compare the degree of conversion, flexural strength and modulus of elasticity, water sorption and solubility, and antimicrobial analysis of the infiltrants containing Iodonium Salt and Chitosan and also the trademark infiltrant Icon®.

MATERIALS AND METHODS

2.1 Experimental infiltrants formulation

Based on a previous study [11, 12], three groups of experimental infiltrants besides the Icon® infiltrant were tested. The experimental infiltrants were composed essentially of BisEma (25%), TEGDMA (75%), HEMA (10%), CQ (0.5%) and EDAB (1%). What varied between them was the concentration of Iodonium Salt in 0%; 0.5% or 1% and the Chitosan concentration at 0%; 0.12% or 0.25%.

The materials were weighed in a high precision analytical balance (Chyo JEX-200, YMC Co Ltda, Tokyo, Japan) and handled in a yellow light environment with controlled humidity and temperature.

Table 1. Infiltrants groups to be evaluated.

Group 1	Iodonium Salt 0%; Chitosan 0%
Group 2	Iodonium Salt 0,5%; Chitosan 0,12%
Group 3	Iodonium Salt 1%; Chitosan 0,25%
Group 4	Icon®

Basic composition of experimental infiltrants: BisEma (25%), TEGDMA (75%), HEMA (10%), CQ (0.5%), EDAB (1%).

2.2 Modulus of elasticity and flexural strength

Ten bar shaped specimens (7mm x 2mm x 1mm, ISO 178: 2001) from each group were made and the three-point bending test was performed with the universal test machine (Instron, model 4111, Instron Corp., Canton, MA, USA), with velocity of 1.0mm/min and load of 50N, until the moment of the fracture. Before the test, the dimensions of each species were obtained with the aid of a digital caliper (Mitutoyo, Tokyo, Japan). The dimensions were recorded and transferred to the Bluehill 2 software (Instron Corp., Canton, MA, USA) to calculate the modulus of elasticity in GPa and the Flexural Strength in MPa according to the dimensions and sustained tension.

2.3 Water Sorption and Solubility

The sorption and solubility test was carried out according to the ISO 4049 specification. Disks (5mm x 1mm thickness, n=3) were prepared, and soon after the polymerization they were placed in a desiccator and stored in an oven at 37º C. The specimens were weighed repeatedly, at 24-h intervals, until a constant initial mass (m1) with a variation of less than 0.2 mg was obtained. The thickness and diameter of the samples were measured using a digital caliper and these measurements were used to calculate the volume (V) of each specimen (in mm3). Subsequently, they were individually stored at 37° C in ependorfs containing 1.5 distilled water. After seven days of storage, the containers were removed from the oven and left at room temperature for 30 minutes. The specimens were washed in running water, dried with absorbent paper and weighed in analytical balance (m2). After this period of storage in water, the samples were dried in a desiccator containing silica gel and, once again, weighed daily until obtaining a new constant mass (m3). The values of sorption (SO) and solubility (SL) were calculated using specific formulas (SO = m2 - m3 / V and SL = m1 - m3 / V).

2.4 Degree of conversion

Degree of conversion measurements (DC, in %) were performed as follows: discs were made (5mm x 1mm thickness, n=6, ISO 4049: 2009) between glass slides and analyzed with infrared spectroscopy (Nexus 6700 IR Spectrometer, Thermo Scientific, Waltham, MA, USA) in transmission mode.

Measurements of the degree conversion of the materials tested for each experimental group were carried out in a Fourier transform infrared spectrometer - FTIR (Spectrum 100 Optica; PerkinElmer, MA, USA).

The assay was conducted from the initial reading of non-photoactivated infiltrants from each experimental group. Afterwards, the specimens were placed on the crystal, and then infrared spectra were obtained with the aid of the Spectrum program (PerkinElmer) present in a microcomputer connected to the spectrometer. The program was used in the scan monitoring mode, Happ-Genzel apodization, with resolution of 4 cm-1 and the spectra being collected in the range of 1608 to 1755 cm-1 for those based of TEGDMA.

The baseline technique [13], traced by the Spectrum program itself, was used for the calculation using specific formulas.

2.5. Antibacterial activity

2.5.1 Microorganisms and microbial sensitivity tests

The microorganisms selected and used were Streptococcus mutans UA159 from the Laboratory of Microbiology and Immunology of the School of Dentistry of Piracicaba (State University of Campinas, Piracicaba, São Paulo, Brazil), for being indicators of presence of caries and generally associated with white spot lesions [14].

A microdilution method was used following the recommendations of the modified M7-A619 protocol for the preparation of S. mutans strains. They were kept in BHI medium (Brain Heart Infusion - Difco Laboratories) with 20% glycerol at -20° C. Each species was reactivated on BHI plates and incubated under the conditions of 10% CO_2 /37° C.

After the growth period, the absorbance was adjusted in a spectrophotometer (10mV Genesys, Thermo Electron Corporation, USA) to give an inoculum concentration equivalent to 1.5×10^8 cells/mL.

Serial dilution was then made so as to reach a concentration of 1.0×10^6 cells/ml in BHI broth (Disco Laboratories, U.S.A.). Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays were performed, respectively, to evaluate the inhibitory and bactericidal activities of experimental infiltrants.

2.5.2 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Substances with antibacterial effects are generally divided into two groups: primarily bacteriostatic and primarily bactericidal [15].

In the spectrophotometer (Genesys 10mV, Thermo Electron Corporation, USA), the strains of both microorganisms were adjusted to the absorbance of 0.05 and 625 nm, to determine the MIC of the groups.

According to the recommendations of the Clinical and Laboratory Standards Institute (CLSI - Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard - Sixth Edition), Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) determinations were performed.

Bacterial cultures of S. mutans were grown in BHI medium for 24 h. From these cultures, were then selected from 3 to 5 colonies and placed in 5 mL of BHI medium. This new broth culture was incubated at 35° C until an optical turbidity comparable to the McFarland standard solution of 0.5 (absorbance reading in spectrophotometer, λ = 625 nm, absorbance of 0.05) was obtained, resulting in an inoculum containing approximately 1 to 2 x 10^8 CFU/mL (Colony Forming Units/mL). Into 96-well plates, 50 μL of each infiltrating group to be tested were dispersed. The sample size of the infiltrant was divided in half for each subsequent well, so that the first well had 50 μL, the second 25 μL and so on until the infiltrating concentration was equal to 0 (zero) μL.

Afterwards, the samples were polymerized and the wells were filled with 50 μL of bacterial inoculum, which upon reaching the desired turbidity, was diluted to contain $5x10^5$ CFU/mL; and from this new bacterial suspension 50 μL was added to each well of the plate, resulting in a final concentration of 5 x 10^4 CFU/well.

The plates were analyzed visually, after incubation for 24 h in aerobiosis, to verify the presence or absence of turbidity in the medium. The MIC was considered for the lowest concentration of the compound that did not show turbidity. For the determination of MBC, a 10 μL aliquot of each well where there was no bacterial growth was transferred to a Petri dish containing BHI-agar medium and incubated for 24 h. The MBC was considered in the plaque referring to the lowest concentration where there was no bacterial growth. Growth of microorganisms was examined after 24 and 48 hours.

2.6. Data analysis

The data obtained were analyzed for the normality and homogeneity of the variables by the Biostat program, with the exception of bacterial tests.

The results for the conversion degree, flexural strength, modulus of elasticity, sorption and solubility were analyzed by analysis of variance (ANOVA one-way), considering the factors "DPI concentration" and "Chitosan concentration", and Tukey's test.

RESULTS

In relation to the modulus of elasticity, we can observe in Table 2 that the group 1 (DPI 0%, Chitosan 0%) and 3 (DPI 1%, Chitosan 0.25%) presented the highest values of modulus of elasticity, being equivalent in values to the infiltrating Icon®. Only group 2 (DPI 0.5%, Chitosan 0.12%) had the lowest values of modulus of elasticity. The same result can be noted for the flexural strength. We can verify that, with the exception of group 2 (DPI 0.12%, Chitosan 0.5%), the other groups obtained high values of modulus of elasticity and flexural strength.

Table 2. Mean values of modulus of elasticity (MPa) and flexural strength (GPa) (standard deviation) of infiltrants groups.

	Modulus of elasticity (MPa)	Flexural strength (GPa)
Group 1	1,14 (0,17) **a**	92,84 (10,70) **a**
Group 2	0,55 (0,18) **b**	54,33 (11,01) **b**
Group 3	1,24 (0,16) **a**	99,90 (12,80) **a**
Group 4	1,13 (0,21) **a**	84,36 (17,58) **a**

Different letters indicate statistical difference ($p \leq 0.05$).

As for water sorption and solubility values (Table 3), we can observe that there was no difference between the groups in relation to water sorption. In relation to solubility, only group 2 (DPI 0.5%, Chitosan 0.12%) presented high values, being the most soluble among all.

Table 3. Mean values of Water Sorption and Solubility (μg/mm^3) (standard deviation) of infiltrants groups.

	Water Sorption (μg/mm^3)	Solubility (μg/mm^3)
Group 1	50,57 (11,70) **a**	11,262 (4,78) **b**
Group 2	44,02 (4,42) **a**	35,986 (7,77) **a**
Group 3	45,08 (8,27) **a**	11,816 (4,35) **b**
Group 4	32,29 (13,64) **a**	5,816 (19,77) **b**

Different letters indicate statistical difference ($p \leq 0.05$).

In relation to the degree of conversion (Table 4), group 1 (DPI 0%, Chitosan 0%) was the one that obtained the best result being statistically similar to group 3 (DPI 1%, Chitosan 0.25%). Icon® was the one that obtained the lowest values of degree of conversion, being similar to group 2 (DPI 0.5%, Chitosan 0.12%).

Table 4. Mean values of Degree of conversion (%) (standard deviation) of infiltrants groups.

	Degree of conversion (%)
Group1	84,30 (10,64) **a**
Group 2	61,23 (15,28) **bc**
Group 3	76,39 (1,40) **ab**
Group 4	49,42 **c**

In relation to the antimicrobial activity (Table 5), Group 1 (DPI 0%, Chitosan 0%) and Group 4 (Icon®) did not present antibacterial activity, having presented microbial growth since the lowest concentration tested. However, Group 2 and Group 3 presented antibacterial activity for S. mutans, being bactericidal.

Table 5. Antibacterial activity of the infiltrants groups (MIC / MBC).

		S. mutans	
	MIC (µg/mL)	MBC (µg/mL)	MBC:MIC
Group 1	-	-	-
Group 2	1.56*	3.13	2:1
Group 3	1.56*	3.13	2:1
Group 4	-	-	-

*maximum for the concentrations tested.

DISCUSSION

This study aimed to evaluate the mechanical and antibacterial properties of experimental infiltrants containing different concentrations of DPI and chitosan.

As the first criterion of mechanical property, we evaluated the modulus of elasticity and flexural strength. These properties are interconnected and match the stiffness of the polymer formed, governed by the extent of polymerization [16].

The results were compared to a previous study [17] which reported higher modulus of elasticity values for DPI-containing adhesives, demonstrating improvements in the mechanical properties of adhesives with salt incorporation. Chitosan may improve the mechanical resistance of the infiltrant, since it serves as a reinforcing structure for methacrylates [18].

As for water sorption and solubility values (Table 3), which correspond to the processes capable of causing hydrolytic degradation of the restorative materials and consequently negatively influence the mechanical properties of these, we can observe that there was no difference between the groups in relation to water sorption. In relation to solubility, only group 2 (DPI 0.5%, Chitosan 0.12%) presented high values, being the most soluble among all, being then beneficial the addition of DPI and Chitosan in the

concentrations of 1% and 0.25% respectively, against the previous study results [19], where DPI adversely affected the solubility of experimental adhesives at 1% concentration.

In relation to the degree of conversion (Table 4), the results were against of the study done by Gonçalves et al. [9], where the addition of 0.5% DPI in experimental cements has been shown to increase the degree of conversion of these. Also going against Elsaka [10] study, where the presence of chitosan at a concentration of 0.25% was related to a lower degree of conversion into an adhesive, but going to meet the same study Elsaka [10], where the concentration of 0.12% addition of Chitosan, had a lower degree of conversion of the polymer evaluated.

In relation to the antimicrobial activity (Table 5), Group 2 and Group 3 presented antibacterial activity for S. mutans, being bactericidal since, according to Levison and Levison [15], a substance is considered bactericidal when MIC value coincides with MBC value, or when MIC ratio by MBC is at most 4 times, which is in agreement with previous studies that indicated good results in the addition of Chitosan to dental materials [10], and the bactericidal and bacteriostatic action of the same [20], in addition to the broad-spectrum inhibitory action of DPI against bacteria [21]. It was concluded that the addition of the Iodonium Salt and Chitosan was beneficial for the antibacterial activity of the infiltrating agent.

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3 CONCLUSÃO

O grupo de infiltrante que obteve os melhores resultados nos testes realizados foi o de concentração de 1% de DFI e 0,25% de Quitosana, sendo semelhante em todos os quesitos ao infiltrante comercial Icon®, tendo o diferencial de apresentar atividade bactericida que a marca comercial não apresenta.

Concluimos que a adição de sal de iodônio e Quitosana são boas alternativas para a melhoria das propriedades mecânicas e antibacterianas dos infiltrantes.

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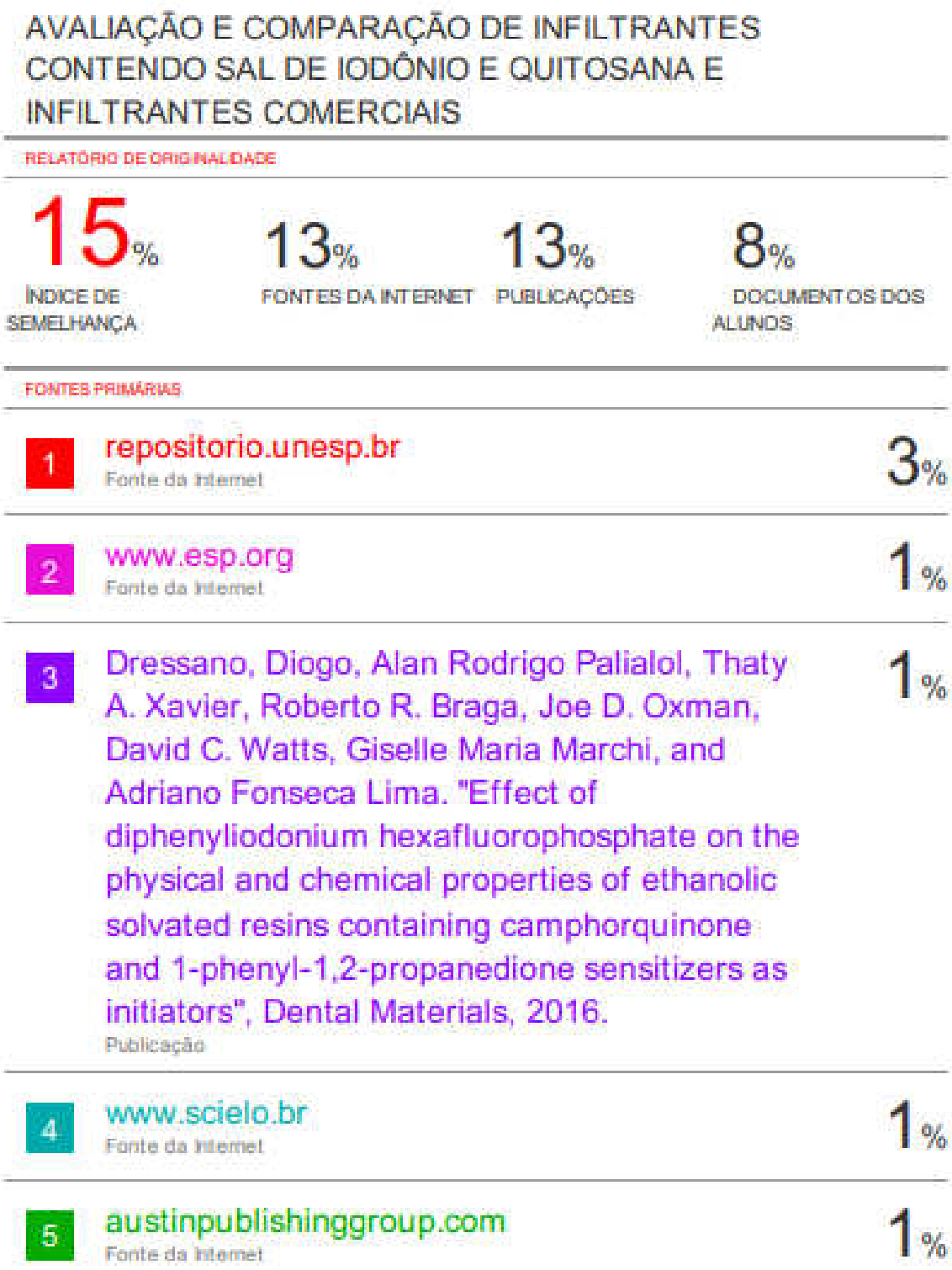
ANEXOS

Anexo 1 – Verificação de Originalidade e Prevenção de Plágio



AVALIAÇÃO E COMPARAÇÃO DE INFILTRANTES CONTENDO SAL DE IODÔNIO E QUITOSANA E INFILTRANTES COMERCIAIS

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Anexo 2 – Iniciação Científica

Relatório Final

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Pela análise do relatório final de atividades, percebe-se que a aluna finalizou a parte experimental e escreveu a discussão dos resultados e conclusão, além da atualização da bibliografia. Houve um problema com um dos ensaios, mas isso não impediu que a aluna vivenciasse a rotina de laboratório esperada em uma bolsa de iniciação científica. Os resultados são interessantes e serão submetidos para a publicação em uma revista de circulação internacional. O coeficiente de rendimento da aluna continua muito bom, inclusive melhorando sua posição em relação aos demais alunos de sua turma. Assim, considero muito boa as atividades apresentadas pela bolsista e recomendo a aprovação do relatório final de atividades.

Aprovado

Anexo 3 - Submissão do Artigo

From: **Franklin Garcia-Godoy** <godoy@amjdent.com>
Date: ter, 18 de set de 2018 às 14:18
Subject: Re: Manuscript Submission
To: Mariana Dias Flor Ribeiro <marianadiasflor@gmail.com>

Dr. Ribeiro:

I received the paper. I will submit it to at least two reviewers for their comments and will contact you immediately after I hear from all of them.

Thank you for considering the **American Journal of Dentistry** for possible publication of your work.

Sincerely,

Franklin Garcia-Godoy, DDS, MS, PhD, PhD

Editor

_____

Franklin Garcia-Godoy, DDS, MS, PhD, PhD

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