UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

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Antibacterial, biological, and physico-mechanical properties of a 1,3,5-triacryloylhexahydro-1,3,5-triazine containing luting agent

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Propriedades antibacterianas, biológicas e físico-mecânicas de um cimento resinoso contendo 1,3,5-triacriloyhexahidro-1,3,5-triazina

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Orientador: Profª. Drª. Ana Flávia Sanches Borges

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"Sem sonhos, a vida não tem brilho. Sem metas, os sonhos não tem alicerces. Sem prioridades, os sonhos não se tornam reais."

Augusto Cury

RESUMO

Propriedades antibacterianas, biológicas e físico-mecânicas de um cimento resinoso contendo 1,3,5-triacriloyhexahidro-1,3,5-triazina

Objetivos: O objetivo deste trabalho foi de formular e avaliar um cimento resinoso experimental com a adição de 1,3,5-triacryloylhexahidro-1,3,5-triazina (TAT).

Material e Métodos: O cimento resinoso experimental foi obtido pela mistura de 50% de Bisfenol A-Glicidil Metacrilato (BisGMA), 30% de Uretano Dimetacrilato (UDMA), 20% de Trietileno Glicol Dimetacrilato (TEGDMA) e iniciadores (% em peso). Vidro de silicato de bário foi utilizado como partícula de carga (45% em peso). A TAT foi utilizada como agente de carga em 15% de concentração, como monômero antibacteriano (C_{TAT}). Um grupo permaneceu sem a adição de triazine, sendo considerado o grupo controle ($C_{CONTROL}$). Os cimentos resinosos foram avaliados em relação ao grau de conversão, espessura de película, escoamento, resistência à flexão, dureza, citotoxicidade e atividade antibacteriana. O teste de micro cisalhamento (μSBS) foi avaliado em diferentes substratos após 7 e 30 dias. Os dados foram analisados pelo teste de Student e ANOVA à um critério. Para o teste de μSBS, foi utilizado ANOVA à três critérios seguido do teste de Tukey (α=0.05).

Resultados: C_{TAT} apresentou os maiores valores no grau de conversão (imediato e após 7 dias). A espessura de película estava de acordo com as recomendações da ISO 4049 em ambos os grupos. Baixa citotoxicidade e menor dureza foi observada no grupo C_{TAT} comparando-o ao $C_{CONTROL}$. Não houve diferença estatística entre os grupos nos testes de escoamento, resistência flexural, análise planctônica e atividade antibacteriana. No grupo C_{TAT} foi observado uma menor formação de biofilme. Em relação ao teste μSBS, os maiores valores foram obtidos para o substrato Y-TZP unido ao C_{TAT}.

Conclusão: O cimento resinoso experimental com triazina apresentou atividade antibacteriana, maior grau de conversão e reduzida citotoxicidade além de apresentar maior resistência de união ao substrato Y-TZP.

Palavras chaves: agente antibacteriano. Triazina. Agente de cimentação. Cárie. Cimentos dentais.

ABSTRACT

Antibacterial, biological, and physico-mechanical properties of a 1,3,5-triacryloylhexahydro-1,3,5-triazine containing luting agent

Objectives: The aim of this study was to formulate and evaluate an experimental luting agent with the addition of 1,3,5-triacryloylhexahydro-1,3,5-triazine (TAT).

Materials and Methods: Experimental luting agents were obtained by mixing 50wt% Bisphenol A-Glycidyl Methacrylate (BisGMA), 30wt% Urethane Dimethacrylate (UDMA), 20wt% Triethylene Glycol Dimethacrylate (TEGDMA) and initiators. Barium silicate glass was used as a filler (45wt%). The TAT was added to the filling agents at 15wt% concentration as an antibacterial monomer (C_{TAT}). One group remained without triazine as the control ($C_{CONTROL}$). The experimental luting agents were evaluated by their degree of conversion, film thickness, flow, flexural strength, softening solvent, cytotoxicity and antibacterial activity. The microshear bond strength test (μSBS) was evaluated in different substrates after 7 and 30 days. Data were analysed by the Student's t-test one-way ANOVA and for μSBS, three-way ANOVA with Tukey *post hoc* test (α=0.05).

Results: C_{TAT} showed a higher degree of conversion (immediately and after 7 days). The film thickness was in accordance with ISO 4049 in both groups. Lower cytotoxicity and lower softening solvent were observed for C_{TAT} when compared to the control. No statistical difference was shown between the groups for flow, flexural strength, in planktonic analysis and in antibacterial activity analysis. Reduced biofilm formation was observed in the C_{TAT} group. C_{TAT} resulted in higher μSBS values after 7 days of storage when applied on Y-TZP ceramics.

Conclusion: The experimental luting agent with TAT showed antibiofilm activity, increased degree of conversion and decreased the cytotoxicity. In addition, increased the bond strength in a Y-TZP substrate.

Keywords: Antibacterial agents. Triazine. Luting agent. Caries. Dental cements.

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1 INTRODUCTION

1 INTRODUCTION

The longevity of indirect restorations is affected by mechanical stress, host, bacterial biodegradation (STEWART & FINER, 2019), recurrent caries, fractures, marginal defects, wear (GOLSTEIN, 2010) and failure in the adhesion between the tooth and indirect restorations (MANSO et al, 2011). Recurrent caries is the cause of failure for 1% to 10% of indirect restorations (IOANNIDIS & BINDL, 2016; MORIMOTO et al, 2016), which may be induced by the presence of cariogenic bacteria close to the rehabilitated area. Biofilm accumulation at the cement-tooth interface induces demineralisation in regions where hygiene is difficult to perform (LEHMNENSIEK et al, 2018) contributing to the formation of carious lesions in restored teeth.

The reduction in bacteria colonisation rate in high-risk areas may be achieved by the development of antibacterial dental materials (NEDELJKOVIC et al, 2015; GARCIA et al, 2020). Antibacterial compounds are added to dental materials (GARCIA et al, 2020; ALTMANN et al, 2015; ALTMANN et al, 2017; SCHIROKY et al, 2017; GARCIA et al, 2019) to impair the colonisation of bacteria that remain viable during the treatment. The strategy aims to avoid the colonisation of bacteria that may gain access to the cavity through gaps in the restorative procedures. Different antibacterial agents are studied in dentistry and quaternary ammonium compounds have been used due to their ability to reduce the viability of cariogenic bacteria (GARCIA et al, 2020; ALTMANN et al, 2015; ALTMANN et al, 2017; SCHIROKY et al, 2017; GARCIA et al, 2019; COCCO et al, 2015) and to copolymerise with the methacrylate resin matrix leading to a post-curing antibacterial effect and reliable material stability (SCHIROKY et al, 2017).

Copolimerisable methacrylate-based antibacterial agents, such as the 1,3,5-triacryloylhexahydro-1,3,5-triazine (TAT), were previously added to dental materials (ALTMANN et al, 2015; ALTMANN et al, 2017; SCHIROKY et al, 2017). The three aliphatic double bonds (C=C) (SCHIROKY et al, 2017) are responsible for its copolymerisation capacity and when TAT was added to different methacrylate-based materials, higher resistance to softening, increased bond strength and significantly reduced *Streptococcus mutans* growth were observed (ALTMANN et al, 2015; ALTMANN et al, 2017). Thus, the addition of TAT in a luting agent may reduce the colonisation of cariogenic bacteria near indirect restorations. The

aim of this study was to formulate and evaluate an experimental luting agent with the addition of 1,3,5-triacryloylhexahydro-1,3,5-triazine (TAT).

2 ARTICLES

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2.1 ARTICLE 1

Antibacterial, biological, and physico-mechanical properties of a 1,3,5-triacryloylhexahydro-1,3,5-triazine containing luting agent

This article was submitted to *Journal of Dentistry* and was in accordance with this journal.

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ABSTRACT

Objectives: The aim of this study was to formulate and evaluate an experimental luting agent with the addition of 1,3,5-triacryloylhexahydro-1,3,5-triazine (TAT).

Materials and Methods: Experimental luting agents were obtained by mixing 50% Bisphenol A-Glycidyl Methacrylate (BisGMA), 30% Urethane Dimethacrylate (UDMA), 20% Triethylene Glycol Dimethacrylate (TEGDMA) and initiators. Barium silicate glass was used as a filler (45wt%). The TAT was added in 15 wt % as an antibacterial monomer (C_{TAT}) and one group remained without triazine, as the control group ($C_{CONTROL}$). The experimental luting agents were evaluated by their degree of conversion, film thickness, flow, flexural strength, softening solvent, cytotoxicity and antibacterial activity. Data were analysed by the Student's t-test and one-way ANOVA with Tukey *post hoc* test (α=0.05).

Results: C_{TAT} showed a higher degree of conversion (immediately and after 7 days). The film thickness was in accordance with ISO 4049 in both groups. Lower cytotoxicity and lower softening solvent were observed for C_{TAT} when compared to the control. No statistical difference was shown between the groups for flow and flexural strength and neither in the planktonic analysis, while a reduced biofilm formation was observed in the C_{TAT} group.

Conclusion: The addition of TAT showed antibiofilm activity, increased degree of conversion and decreased the cytotoxicity for an experimental luting agent.

Clinical significance: The luting agent developed in this study with antibacterial activity could be a reliable alternative due to the copolymerisation with the resin matrix, reducing the colonisation of cariogenic bacteria near indirect restorations and increased the degree of conversion.

Keywords: Antibacterial agents. Triazine. Luting agent. Caries. Dental cements.

Introduction

The longevity of indirect restorations is affected by mechanical stress, host, bacterial biodegradation [1], recurrent caries, fractures, marginal defects, wear [2] and failure in the adhesion between the tooth and indirect restorations [3]. Recurrent caries is the cause of failure for 1% to 10% of indirect restorations [4,5] which may be induced by the presence of cariogenic bacteria close to the rehabilitated area. Biofilm accumulation at the cement-tooth interface induces demineralisation in regions where hygiene is difficult to perform [6] contributing to the formation of carious lesions in restored teeth [7].

Antibacterial compounds are added to dental materials [8-12] to prevent bacterial-derived enzymes that degrade composites and collagen components of the hybrid layer and to decrease bacterial load around restoration [1]. Consequently, impair the colonisation of bacteria that remain viable during the treatment and avoiding the colonisation of bacteria that may gain access to the cavity through gaps in the restorative procedures [7-12]. Different antibacterial agents are studied in dentistry and quaternary ammonium compounds have been used due to their ability to reduce the viability of cariogenic bacteria [8-13] and to copolymerise with the methacrylate resin matrix leading to a post-curing antibacterial effect and reliable material stability [11].

Copolimerisable methacrylate-based antibacterial agents, such as the 1,3,5-triacryloylhexahydro-1,3,5-triazine (TAT), were previously added to dental materials [9-11]. The three aliphatic double bonds (C=C) (Fig. 1) [11] are responsible for its copolymerisation capacity and when TAT was added to different methacrylate-based materials, higher resistance to softening, increased bond strength and significantly reduced *Streptococcus mutans* growth was observed [9,10]. Thus, the addition of TAT in a luting agent may reduce the colonisation of cariogenic bacteria near indirect restorations. The aim of this study was to formulate and evaluate an experimental luting agent with the addition of 1,3,5-triacryloylhexahydro-1,3,5-triazine (TAT).

Material and methods

Formulation of experimental luting agents

Dual-cure luting agents were prepared by mixing 50% Bisphenol A-Glycidyl Methacrylate (BisGMA), 30% Urethane Dimethacrylate (UDMA) and 20% Triethylene Glycol Dimethacrylate (TEGDMA). As dual-cure activation was used, two pastes were produced.

Paste A had an addition of 1 mol% of Camphorquinone (CQ), 1 mol% of Ethyl 4-dimethylamino-benzoate (EDAB), 1 mol% of Dihydroxyethyl-para-toluidine (DHEPT) and 0.01 wt% Butylated hydroxytoluene (BHT) as a photo-initiator system. Paste B had an addition of 1wt% of benzoyl peroxide (monomers and photo-initiators were from Sigma Aldrich - St Louis, MO, USA). Barium Silicate glass 45wt% (Esstech, Essington, PA, USA) was used as a filler in both pastes.

Two experimental groups were formulated:

- Cement with Triazine (C_{TAT}) contain: 15wt% of Triazine (TAT - 1,3,5-Triacryloylhexahydro-1,3,5-triazine) (Sigma Aldrich - St Louis, MO, USA) was added as an antibacterial monomer and 45wt% of barium silicate glass filler. The concentration of TAT was determined in a previous study [9].
- The control group ($C_{CONTROL}$) contain: 45wt% barium silicate glass filler, without antibacterial monomers.

The particles were hand mixed and sonicated for 8 minutes to ensure adequate dispersion.

To prepare the specimens, equal parts of paste A and B were weighed and mixed for 10 seconds. Luting agents were photo-activated for 60 seconds with a light emitting diode unit (Radii Cal, SDI, Bayswater, VIC, Australia) at 1200 mW/cm^2.

Degree of conversion

The degree of conversion of the groups was accessed by Fourier transform infrared spectroscopy (FTIR- Vertex 70, Bruker Optics, Ettlingen, Baden-Württemberg Germany) with attenuated total reflectance (Platinum ATR-QL; Bruker Optics). The pastes were mixed and placed in a polyvinylsiloxane mould in a horizontal diamond crystal with a 45° mirror angle (n = 3). A spectrum was then obtained in a range of 4000 to 400 cm^{-1} and 64 scans were obtained with a 4 cm^{-1} resolution. The luting agent was photo-activated for 60 seconds and a new spectrum was generated. The specimens were stored and after 7 days a new spectrum was obtained. The degree of conversion was calculated by the absorbance of aliphatic carbon bonds at 1640 cm^{-1} and the aromatic carbon bonds at 1610 cm^{-1}, as previously described [14].

Film thickness

Film thickness was evaluated according to International Standard Organization (ISO) 4049 [15]. Two glass plates (200 mm^2 and 5 mm thickness) had their thickness measured (T1) with a digital calliper. Then, the materials were mixed and dispensed in the centre of one glass plate while another glass plate was placed above the agent. A constant load of 150N was applied centrally to the superior plate during 180 ± 10 seconds. The load was removed, and the luting agents were photo-activated for 60 seconds. The plates were measured again (T2) and the difference between T1 and T2 was used as the film thickness of each specimen. Three specimens were used for each group (n = 3).

Flow

The luting agent flow was measured according to ISO 6876 [16]. The agents were placed between two glass plates (200 mm^2 and 5 mm thickness). A load of 100 grams was applied into the plates and after 180 ± 10 seconds luting agents were photo-activated. The largest and the smallest diameter of the luting agents were measured with a digital calliper. The mean value between the diameters was recorded. Three samples from each group were measured (n = 3).

Flexural strength

Flexural strength was tested according to ISO 4049 [15] except by the size of the rectangular specimens (20 mm x 2 mm x 2 mm) since what it is important is the span. The luting agents were placed in stainless steel moulds on the top of polyester strips and photo-activated in two irradiations of 30 seconds each at the top and the bottom of the specimen. The flexural strength was determined with a three-point test device with a span of 8 mm, at a cross-head speed of 0.5 mm/min in a mechanical testing machine (Shimadzu EZ-SX, Shimadzu Corp., Kyoto, Kyoto, Japan). Five specimens were used for each group (n = 5).

Softening solvent

Disc-shaped measuring 4 mm in diameter and 1 mm in height were produced and embedded in acrylic resin. The samples were polished with silicon carbide abrasive papers numbers: #1200 and 2000 and felt disks with alumina suspension (alumina 0.5μm; Arotec, Cotia, SP, Brazil) prior to a Knoop microhardness measurement (KHN). Specimens were submitted to an initial KHN analysis (KHN1), where three indentations were performed in each specimen, with a load of 10 grams for 5 seconds in a microhardness tester (HMV 2, Shimadzu). Specimens were then immersed in 70% ethanol solution for 2 hours and washed with distilled

water. Then new microhardness measurements were performed (KHN2). The difference between KHN1 and KHN2 was used to calculate the %KHN for the softening solvent analysis. Three specimens were used for each group (n = 3).

Cytotoxicity

Cytotoxicity was tested against the primary pulp cells. This research was approved by the Ethics Committee of Federal University of Rio Grande do Sul under protocol number CAAE: 10241519.3.3001.5347. Cells were obtained from an extracted third molar from a healthy patient that agreed to donate and signed an informed consent form. Cells were cultivated in supplemented Dubellco's modified essential medium (DMEM) (Thermo Fischer Scientific, Waltham, MA, EUA) at 37ºC and 5% CO_2. Specimens (n = 3; 4 mm diameter x 1 mm height) were sterilised in ethylene oxide and immersed in 1 mL of DMEM 24 hours prior to cell treatment and kept at 37ºC to produce a conditioned medium. Cells were treated with a conditioned medium and were stored for 72 hours. Wells with a medium without conditioning were cultivated as well. After treatment, cells were fixed and stained with 0.4% Sulforhodamine B (SRB - Sigma-Aldrich). Quantification was performed in a microplate spectrophotometer (Multiskan GO, Thermo Fisher Scientific) at an absorbance of 560 nm. The number of cells in the wells without treatment were used to normalise the number of cells in the tested groups. The results were expressed by the percentage of viable cells.

Antibacterial activity

The analyses of biofilm and planktonic bacteria viability were performed to evaluate the antibacterial property of the formulated materials. Six specimens (4 mm diameter x 1 mm thickness) were immersed in brain heart infusion (BHI) broth (Sigma-Aldrich) with a suspension of Streptococcus mutans (NCTC 10449) in 48-well plates and incubated at 37°C for 24 hours. For biofilm quantification, samples (n = 3) per group were placed inside a micro-tube containing 900 µL of saline and vortexed for 1 minute to remove the adhered biofilm. The dilutions were made up to 10^{-6} for the biofilm quantification. Two 25 µL-drops of each dilution were platted in BHI agar Petri dishes and incubated for 48 hours at 37°C. For planktonic analysis, the medium on the wells was collected and dilutions were made up to 10^{-6}. Dilutions were platted in BHI agar Petri dishes and incubated for 48 hours at 37ºC. As a negative control, Teflon matrices were used. The number of colony forming units (CFU) was visually counted by optical microscopy and transformed to log CFU/mL.

The flowchart illustrates the experimental steps (Fig. 2).

Statistical analysis

The normality of the data was assessed with the Shapiro-Wilk test. A normal distribution was found for all analyses. A t-test was used for film thickness, flow, flexural strength, softening in solvent, cytotoxicity and antibacterial activity analysis. The degree of conversion analysis was performed by two-way repeated measures ANOVA and Tukey for different groups and time comparison. For the softening in solvent, the differences between KHN1 and KHN2 were analysed by a paired t-test. Planktonic analysis, in the antibacterial analysis, was performed with a one-way ANOVA and the Tukey test. All tests were performed with $\alpha = 0.05$.

Results

The degree of conversion of the experimental luting agents is shown in Table 1. The C_{TAT} showed a higher degree of conversion when compared to the $C_{CONTROL}$ in the immediate and 7 days measurements ($p = 0.001$). After 7 days, the degree of conversion values reached 73.91% (± 3.09) and 66.72% (± 3.47) for the C_{TAT} and $C_{CONTROL}$, respectively, with a statistical difference between the groups ($p = 0.003$).

Film thickness was higher for materials with the addition of TAT ($p = 0.013$) and all values were in accordance with ISO 4049 standards (> 50 μm). For flow results, no difference was found between the groups ($p = 0.133$). The C_{TAT} mean flow value was 16.47mm (± 1.88). The flexural strength results showed no difference between the groups ($p = 0.820$), with a mean value of 41.77 MPa for the C_{TAT} and 42.85 MPa for the $C_{CONTROL}$ (Table 1).

For the softening solvent, both luting agents had similar initial Knoop hardness values ($p = 0.265$). $C_{CONTROL}$ showed a higher degradation between KHN1 and KHN2, with a significant reduction after ethanol immersion. Comparing the %KHN, no statistical difference was found between the groups (Table 2).

Table 3 shows the cytotoxicity and antibacterial activity results. Higher cell viability was observed for C_{TAT} when compared to $C_{CONTROL}$. C_{TAT} showed 103.82% (± 2.59) of viable cells while $C_{CONTROL}$ presented 93.35% (± 6.26) ($p = 0.028$). The analysis of biofilm formation in the samples showed reduced bacterial growth in the C_{TAT} specimens when compared to the $C_{CONTROL}$, with a statistical difference between the groups. For planktonic analysis, no statistical difference was found between the groups and the negative control.

Discussion

Antibacterial agents are used to reduce the viability of bacteria [1,13] in risk areas, such as the cementation line in indirect restorations. In this study, a dual-cure luting agent was formulated with 1,3,5-triacryloylhexahydro-1,3,5-triazine as an antibacterial monomer for controlling the biofilm formation at indirect restorations that may fail due to recurrent caries [4,5]. The TAT-containing luting agent showed higher degree of conversion and higher cell viability with a reduced adhesion of bacteria at the material's surface.

The 15% TAT concentration selected to be used in this study was previously tested by Altmann et al. [9] with orthodontic adhesives (10%, 15% and 20% TAT), and they obtained promising antibacterial materials, especially those with 15% and 20% TAT, and because of this, in this study the TAT 15% was choose. TAT is a well-known quaternary ammonium compound that has been studied in different applications as an antibacterial agent [8-11,13]. Besides, the presence of three C=C functional groups in TAT structure (Figure 1), it is able to copolymerize with methacrylate monomers which have been shown to increase the degree of conversion of different dental restorative materials [9-11]. The copolymerization characteristics shown for triazine compounds influence the formation of the polymeric structure and the antibacterial property [13], and in this study a higher degree of conversion was observed for luting agents with TAT addition both immediately and after 7 days. As a dual-cure system was used, an increased degree of conversion was found after 7 days for the C_{TAT} and $C_{CONTROL}$. While a higher degree of conversion is related to an increased to higher mechanical properties as shown for different dental composites [17,18] the flexural strength values of developed luting agents did not present statistically significant differences between groups.

The addition of antibacterial monomers into composites may affect the stability of the polymeric network [19,20] in the developed luting agents. The softening of the polymeric matrix was assessed after ethanol immersion and no statistically significant difference was found between C_{TAT} samples before and after immersion in the solvent, which may indicate that the copolymerization ability leads to a polymeric structure less prone to degradation due to the increased stability of the formed polymeric chains [19]. The reduced polymer softening along with the increased in the degree of conversion might be the reason why an increased cell viability was observed for C_{TAT} materials when compared to the control group. The unreacted methacrylate monomers, especially TEGDMA, are known to induce a reduction in cell viability in different cell types [1–3] which was not observed in the present study. The presence of an antibacterial agent into the luting agent showed increased cell viability when compared to $C_{CONTROL}$ which may also be related to the absence of TAT leaching compounds from the matrix due to the copolymerization. This was in accordance with the findings of the antibacterial

analysis, where no increased planktonic activity was observed indicating that TAT was not released in the culture medium. Although the formation of the polymer was adequate, the addition of TAT increased the film thickness. Despite the statistical significance, TAT-containing luting agents and the control group presented values within the ISO 4049 requirements [15]. The higher film thickness may impact the biofilm formation in the cementation line and in this case, the antibiofilm properties that were found for TAT-containing luting agents may reduce the risk of caries development.

The copolymerization ability of antibacterial agents could reduce the bacterial viability with higher stability and possible long-term activity [1,9,13,21]. TAT has antibacterial activity against Gram-positive and Gram-negative bacteria as well as an antibiofilm activity against *S. aureus* [22,23] and *S mutans* [9-11]. And further, 1,3,5-triazine can decrease bacterial growth beacause it is a small compound that mimics the hydrophobic and charge standards founded in the pharmacophore of short cationic antimicrobial peptides, resulting in the lysis of bacteria's membrane leading to its death [9,24,25]. This effect may be observed in the present study after the contact of bacteria with the luting agents during the antibacterial analysis for biofilm formation. The reduction in the biofilm indicates the potential of TAT to decrease *S. mutans* viability when in contact with materials, which may be important to reduce the number of viable bacteria in cementation line. This behavior was not found in the planktonic analysis, as reported, due to the ability of TAT to bond to the polymeric chains preventing its leaching [9,10]. The fact that the antibacterial effect is driven for the contact between the materials and bacteria and not by the leaching, and consequently by the degradation of the developed luting agents, may prevent the increase in the porosity of materials, the reduction in Physico-chemical properties and the loss of marginal seal [26].

The antibacterial activity of luting agents assumes their clinical relevance as the biofilm formation near the material and in this case, close to the cementation line may be related to the incidence of recurrent caries in restored teeth [6,7]. In addition to the development of a luting agent with antibacterial activity, the results of this study suggest that the proposed material could be more stable and consequently, an alternative for establishing an adequate marginal seal, preserving the tooth/indirect restoration interface.

Conclusion

The experimental luting agent formulated in this study with the addition of 1,3,5-triacryloylhexahydro-1,3,5-triazine are a promising luting agent with antibacterial activity,

because it exhibited anti-biofilm activity, increased degree of conversion and decreased the cytotoxicity.

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References

1. C.A. Stewart, Y. Finer Y, Biostable, antidegradative and antimicrobial restorative systems based on host-biomaterials and microbial interactions, Dent Mater. 35 (2019) 36-52. https://doi: 10.1016/j.dental.2018.09.013

2. G.R. Golstein, The longevity of direct and indirect posterior restorations is uncertain and may be affected by a number of dentist-, patient-, and material-related factors, J Evid Based Dent Pract. 10 (2010)30-31. https:// doi: 10.1016/j.jebdp.2009.11.015.

3. A.P. Manso, N.R.F.A. Silva, E.A. Bonfante, T.A. Pegoraro, R.A. Dias, R.M. Carvalho, Cements and adhesives for all-ceramic restorations, Dent Clin North Am. 55 (2011) 311-332. https:// doi: 10.1016/j.cden.2011.01.011

4. A. Ioannidis, A. Bindl A, Clinical prospective evaluation of zirconia-based three-unit posterior fixed dental prostheses: Up-to ten-year results, J Dent. 47 (2016) 80–85. https:// doi: 10.1016/j.jdent.2016.01.014

5. S. Morimoto, F.B. Rebelo de Sampaio, M.M. Braga, N. Sesma, M. Özcan, Survival rate of resin and ceramic inlays, onlays, and overlays: a systematic review and meta-analysis, J Dent Res. 95 (2016) 985–994. https:// doi: 10.1177/0022034516652848

6. M. Lehmensiek, H. Askar, F. Brouwer, U. Blunck, S. Paris, F. Schwendicke, Restoration integrity, but not material or cementation strategy determined secondary caries lesions next to indirect restorations in vitro, Dent Mater. 34 (2018) e317-323. https:// doi: 10.1016/j.dental.2018.09.004

7. I. Nedeljkovic, W. Teughels, J. De Munck, B. Van Meerbeek, K.L. Van Landuty, Is secondary caries with composites a material-based problem? Dent Mater. 31 (2015) e247-277. https:// doi: 10.1016/j.dental.2015.09.001

8. I.M. Garcia, S.B. Rodrigues, G.S. Balbinot, F. Visioli, V.C.B. Leitune, F.M. Collares, Quaternary ammonium compound as antimicrobial agent in resin-based sealants, Clin Oral Investig. (2020). https://doi: 10.1007/s00784-019-02971-4

9. A.S. Altmann, F.M Collares, F.A. Ogliari, S.M.W. Samuel, Effect of methacrylated-based antibacterial monomer on orthodontic adhesive system properties, Am J Orthod Dentofacial Orthop. 147 (2015) S82-87. https:// doi: 10.1016/j.ajodo.2015.01.015

10. A.S. Altmann, F.M. Collares, V.C.B. Leitune, R.A. Arthur, A.S. Takimi, S.M.W. Samuel, In vitro antibacterial and remineralizing effect of adhesive containing triazine and niobium pentoxide phosphate inverted glass, Clin Oral Investig. 21 (2017) 93-103. https:// DOI 10.1007/s00784-016-1754-y

11. P.R. Schiroky, V.C.B. Leitune, I.M. Garcia, F.A. Ogliari, S.M.W. Samuel, F.M. Collares, Triazine compound as copolymerized antibacterial agent in adhesive resins, Braz Dent J. 28 (2017) 196-200. https:// doi: 10.1590/0103-6440201701346

12. L.S.G. Garcia, A.C.B. Delbem, J.P. Pessan, M. Dos Passos Silva, F.N.S. Neto, L.F. Gorup, E.R. de Camargo, M. Danelon, Anticaries effect of toothpaste with nano-sized sodium hexametaphosphate, Clin Oral Investig. 23 (2019) 3535-3542. https://doi.org/10.1007/s00784-018-2773-7

13. A.R. Cocco, W.L. Rosa, A.F. Silva, R.G. Lund, E. Piva, A systematic review about antibacterial monomers used in dental adhesive systems: current status and further prospects, Dent Mater. 31 (2015) 1345-1362. https:// doi: 10.1016/j.dental.2015.08.155

14. F.M. Collares, F.F. Portella, V.C.B. Leitune, S.M.W. Samuel, Discrepancies in degree of conversion measurements by FTIR, Braz Oral Res. 28 (2014)9–15. https:// doi: 10.1590/S1806-83242013000600002

15. ISO 4049:2009 - Dentistry – Polymer-based restorative materials.

16. ISO 6876:2012 - Dentistry – Root canal sealing materials.

17. J.L Ferracane, E.H. Greener, The effect of resin formulation on the degree of conversion and mechanical properties of dental restorative resins, J Biomed Mater Res. 20 (1986) 121-131. https://doi:10.1002/jbm.820200111

18. J.P. Santerre, L. Shajii, B.W. Leung, Relation of dental composite formulations to their degradation and the release of hydrolyzed polymeric-resin-derived products, Crit Rev Oral Biol Med. 12 (2001) 136–151. https://doi:10.1177/10454411010120020401

19. V.B. Michelsen, G. Moe, M.B. Strøm, E. Jensem, H. Lygre, Quantitative analysis of TEGDMA and HEMA eluted into saliva from two dental composites by use of GC/MS and tailor-made internal standards, Dent Mater. 24 (2008) 724-731. https://doi:10.1016/j.dental.2007.08.002

20. P. Pongprueksa, J. de Munck, R.C. Duca, K. Poels, A. Covaci, P. Hoet, L. Godderis, B. Van Meerbeek, K.L. Van Landuyt, Monomer elution in relation to degree of conversion for different types of composite, J Dent. 43 (2015) 1448-1455. https://doi: 10.1016/j.jdent.2015.10.013

21. L. Cheng, M.D. Weir, K. Zhang, D.D. Arola, X. Zhou, H.H. Xu, Dental primer and adhesive containing a new antibacterial quaternary ammonium monomer dimethylaminododecyl methacrylate, J Dent. 41 (2013) 345–355. https://doi: 10.1016/j.jdent.2013.01.004

22. K.M. Al-Zaydi, H.H. Khalil, A. El-Faham, S.N. Khattab, Synthesis, characterization and evaluation of 1,3,5-triazine aminobenzoic acid derivates for their antimicrobial activity, Chem Cent J. 11 (2017) 39. https://doi.org/10.1186/s13065-017-0267-3.

23. S. Katugampala, I.C. Perera, C. Nanayakkara, T. Perera, Synthesis, characterization, and antimicrobial activity of novel sulfonated copper-triazine complexes, Bioinorg Chem Appl. 29 (2018). https://doi.org/10.1155/2018/2530851

24. C. Zhou, J. Min, Z. Liu, A. Young, H. Deshazer, T. Gao, Y.T. Chang, N.R. Kallenbach, Synthesis and biological evaluation of novel 1,3,5-triazine derivatives as antimicrobial agents, Bioorg Med Chem Lett. 18 (2008) 1308-1311. https://doi: 10.1016/j.bmcl.2008.01.031

25. S.N. Lewis, Z. Garcia, R. Hontecillas, J. Bassaganya-Riera, D.R. Bevan, Pharmacophore modeling improves virtual screening for novel peroxisome proliferator-activated receptor-gamma ligands, J Comput Aided Mol Des. 29 (2015) 421-439. https://doi: 10.1007/s10822-015-9831-x

26. C.C. Centenaro, F.V. Rostirolla, V.C.B. Leitune, C.F. Parolo, F.A. Ogliari, S.M.W. Samuel, F.M. Collares, Influence of addition of 2-[3-(2H-benzotriazol-2-YL) -4-hydroxyphenyl] ethyl methacrylate to an experimental adhesive system, Acta Odontol Latinoam. 28 (2015) 72-78. https://doi: 10.1590/S1852-48342015000100010

Table 1. The degree of conversion (immediate and 7 days), film thickness, flow and flexural strength results for the C_{TAT} and $C_{CONTROL}$.

	Immediate DC (%)	7-dayDC (%)	Film thickness[1]	Flow[2]	Flexural strength[1]
C_{TAT}	60.88 (± 2.44)^{Ab}	73.91 (± 3.09)^{Aa}	43.0 µm (± 9.0)^{B}	16.47 mm (± 1.88)^{A}	41.77 MPa (± 8.90)^{A}
$C_{CONTROL}$	49.19 (± 3.50)^{Bb}	66.7 (± 3.47)^{Ba}	30.0 µm (± 10.0)^{A}	17.97 mm (± 0.69)^{A}	42.85 MPa (± 5.14)^{A}

Different uppercase letters indicate a statistical difference between the different groups. Different lowercase letters indicate a statistical difference between the different times in the DC. (1) analysis performed according to ISO 4049 (2) according to ISO 6876:2012.

Table 2. Softening solvent results. KHN1 and KHN 2 were used to calculate the %KHN.

	KHN1	KHN2	%KHN
C_{TAT}	31.53 (± 1.79)^{Aa}	27.72 (± 1.59)^{a}	11.83 (± 6.70)^{A}
$C_{CONTROL}$	32.70 (± 2.35)^{Aa}	24.27 (± 2.11)^{b}	25.66 (± 5.60)^{A}

Different uppercase letters indicate a statistical difference between the lines. Different lowercase letters indicate a statistical difference between the columns. KHN 1: Initial Knoop microhardness measurements. KHN 2: Knoop microhardness analysis after immersion in ethanol. %ΔKHN: percentage reduction in Knoop microhardness after immersion in ethanol.

Table 3. Percentage of viable cells quantified by SRB. Antibacterial activity results in log CFU/ml for the C_{TAT} and $C_{CONTROL}$.

	Cell viability	Biofilm (Log CFU/ml)	Planktonic (Log CFU/ml)
C_{TAT}	103.82% (± 2.59)^{A}	4.75 (± 0.069)^{A}	7.85 (± 0.12)^{A}
$C_{CONTROL}$	93.35% (± 6.26)^{B}	5.47 (±0.015)^{B}	8.05 (± 0.23)^{A}
Negative Control	-	-	8.25 (± 0.06)^{A}

SRB (Sulforhodamine B). CFU (Colony Forming Unit). Different uppercase letters indicate a statistical difference between the different groups.

Fig. 1. The structural formula of 1,3,5-triacryloylhexahydro-1,3,5-triazine (TAT) used in the present study.

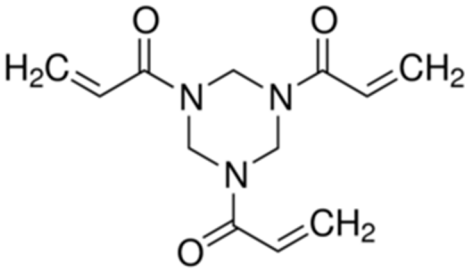
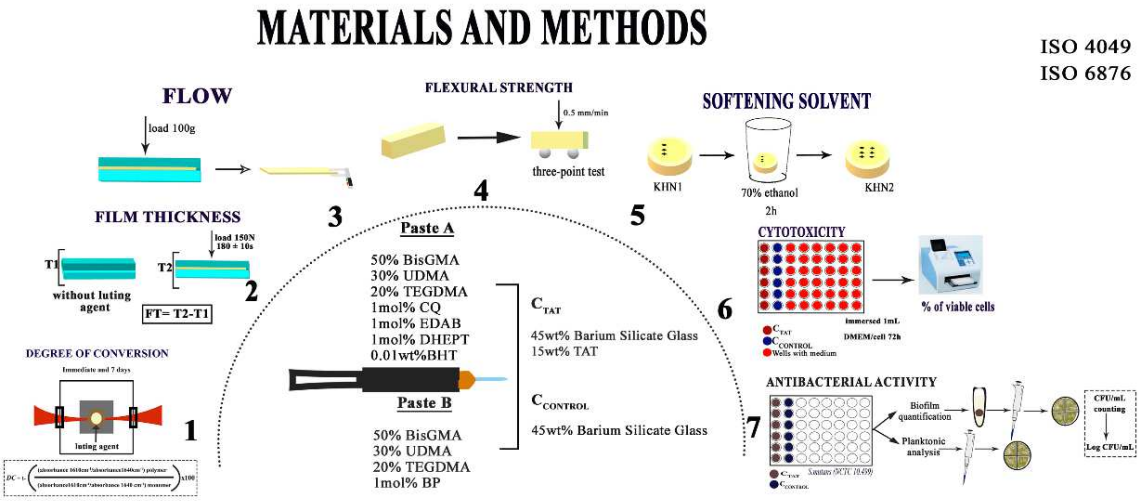


Fig. 2. Experimental flowchart steps.



2.2 ARTICLE 2

Can triazine resin luting cement increase bond strength to ceramics and dental structure?

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ABSTRACT

Objectives: The use of 1,3,5-triacryloylhexahydro-1,3,5-triazine (TAT), an antibacterial agent, has been shown to significantly improve the physico-mechanical properties of adhesives. The aim of this study was to formulate and evaluate an experimental resin luting cement with the addition of TAT, and evaluate the bond strength to different ceramics and to dental structure, at different storage times.

Material and Methods: Experimental resin luting cements were obtained by mixing 50% BisGMA, 30% UDMA, 20% TEGDMA and initiators. Barium silicate glass was used as a filler. The triazine compound (TAT) was added in 15 wt % as an antibacterial monomer and one group remained without triazine as the control group. The microshear bond strength test (μSBS) and failure pattern of the resin luting cements bonded to lithium disilicate (LD), celtra duo (CD), yttrium-stabilized tetragonal polycrystalline zirconia (ZirCAD and inCoris), enamel and dentin was evaluated after 7 and 30 days. Data was analysed by 3-way ANOVA, followed by Tukey test and Chi-square for the failure pattern ($\alpha = 0.5$).

Results: The μSBS was improved for the resin luting cement with TAT for ZirCAD even over time (9.96MPa to 14.23MPa for 7 and 30 days, respectively). The higher μSBS was obtained for LD and CD when cemented with the control resin luting cement. The predominantly failure pattern was mixed, except for ZirCAD and InCoris.

Conclusion: The use of an antibacterial resin luting cement with TAT provided better μSBS just for ZirCAD even over the period analysed, for the other substrates evaluated it was not possible to improve the μSBS.

Key words: resin luting cement, lithium disilicate, zirconia, dental structure, triazine

INTRODUCTION

Different types of all ceramics have been developed during the last few decades to assist patients and dentists seeking esthetic [1,2]. However, multiple factors could impact on the success of indirect restorations such as preparation design, oral hygiene, plaque retention, occlusion, restorative materials, compatibility with oral tissues and especially long-term survival in the oral cavity [3-5]. Dental luting agents establish a connection between indirect restoration and the dental structure [3], consequently, the proper resin luting cement selection should be based on knowledge of physical and biological properties and other characteristics of both restorative materials and resin luting cement [6].

The resin luting cement use became prevalent instead of the conventional cements due to the lower hydrolytic degradation, higher mechanical properties [7], reduced risk of microleakage and staining [8] and higher bond strength, allowing minimally invasive procedures [6], and after resin luting cementation (the adhesive protocol), dental-cement-restoration, behaves as a single body [7]. Therefore, resin luting cements must bond to different substrates such as enamel and dentin, ceramics, gold, metal alloys and indirect composite [9]. When using resin luting cements, the internal surface of the indirect restoration must be treated differently from the tooth surface, and further, the surface treatment of the indirect restoration depends on the type of used material [10].

Glass ceramics indirect restorations obtained higher fractures strength after being etching with hydrofluoric acid and the application of a silane-coupling agent [11], when bonded with a resin luting cement compared with the conventional cements [12]. Considering the significant amount of vitreous phase in its composition, thus being acid etched and silane treated [13]. However, zirconia such as yttrium-stabilized tetragonal zirconia polycrystal (Y-TZP) ceramics are almost unaffected by the current bond protocols, it is highly chemically inert, not etchable [13,14], taking into account the higher hardness and crystallinity [15]. When submitted to other bonding strategies such as: sandblasting, the use of primers and/or resin luting cements with phosphate ester 10-metacriloiloxidecil dihydrogen phosphate (MDP) [14] resin luting cements provide stronger bonding for Y-TZP restorations showing better mechanical properties than conventional cements [13,14,15].

In other to establish proper retention, marginal seal and durability of indirect restorations, a proper choice of luting agents and the cementation procedure is crucial [3, 6,10,16]. Thus, the use of resin luting cements with antibacterial agents [17-19], could reduce the risk of recurrent carries that may develop from gaps through the margins of the restoration

by decreasing the viability of bacteria [20,21]. 1,3,5-triacryloylhexahydro-1,3,5-triazine (TAT) is an antibacterial methacrylate, with three aliphatic double bonds (C=C) [19] which are responsible for its copolymerization capacity, higher resistance to softening, increased bond strength and significantly reduced *Streptococcus mutans* growth [18,19]. The objective of this study was to formulate and evaluate an experimental resin luting cement with the addition of 1,3,5-triacryloylhexahydro-1,3,5-triazine (TAT), and evaluate the bond strength to different ceramics and to dental structure, at different storage times. The hypothesis was that the bond strength would be significantly improved by the use of a resin luting cement containing TAT.

MATERIAL AND METHODS

Resin luting cement formulation

The experimental dual cure resin luting cement used in this study were formulated by mixing Bisphenol A-Glycidyl Methacrylate (BisGMA), Urethane Dimethacrylate (UDMA) and Triethylene Glycol Dimethacrylate (TEGDMA) in a 50:30:20 mass ratio, and divided into two pastes. The paste A had the addition of 1mol% of Camphorquinone (CQ), 1mol% of Ethyl 4-(dimethylamino) benzoate (EDAB), 1mol% of Dihydroxyethyl-para-toluidine (DHEPT) and 0.01 mol% Butylated hydroxytoluene (BHT) as photoinitiator system. The paste B had the addition of 1mol% of benzoyl peroxide (BP). Barium Silicate glass 45vol% (Esstech, Essington, Pennsylvania, USA) was used as a filler in both pastes. All monomers and photoinitiators were from Sigma Aldrich (St Louis, MO, USA).

After that, two experimental resin luting cements were formulated: Triazine (TAT - 1,3,5-Triacryloylhexahydro-1,3,5-triazine) was added as an antibacterial monomer. For TAT-containing resin luting cement (C_{TAT}), 15wt% of TAT and 45vol% of filler were added. A resin luting cement formulated without TAT and with 45Vol% of barium glass served as a control resin luting cement ($C_{CONTROL}$). The particles were hand mixed to the paste and sonicated for 8 minutes to adequate dispersion.

Specimen preparation

The ceramic materials used in this study were: lithium disilicate ceramic (LD) (IPS e.max CAD, Ivoclar Vivadent, Liechtenstein), celtra duo ceramic (CD) (CELTRA Duo, Dentsply-Sirona, Bensheim, Germany), yttrium-stabilized tetragonal polycrystalline zirconia: ZirCAD (IPS e.max ZirCAD LT; Ivoclar Vivadent, Schaan, Liechtenstein) and inCoris (inCoris

TZI mono L, Dentsply-Sirona, Bensheim, Germany). These materials bond strengths to enamel and dentin were evaluated.

Ceramic preparations

Slices were obtained from the ceramic's blocks, by sectioning under water irrigation with a double-faced diamond disk (Wafer blade, 5 inch × 0.15 inch × 0.15 inch; Extec) in a cutting machine (IsoMet 1000; Buehler) to obtain specimens (14mm long, 12mm wide, 1.5mm-thick). Materials were processed according to the manufacturer's instructions.

Afterward, the slices were fixed in (10-mm-high and 19 mm-width) polyvinyl chloride tubes with acrylic resin (Jet; Dental Articles Classic). Then, they were finished with #800 and #1200 silicon carbide abrasive papers (Carbimet; Buehler) (Politriz APL-4 AROTEC, Cotia, São Paulo, Brazil) and polished with 1-μm diamond solution (MetaDi water-based suspension; Buehler) on fine-grained felt disks followed by ultrasound cleaning with deionized water (USC 750; Unique Group) for 10 minutes. The Y-TZP groups were sandblasted with 30 μm silica-coated aluminum oxide particles (CoJet™ Sand S30, 3M ESPE, USA) for 15 s (at a distance of 5mm and 2.5 bar of pressure) and ultrasonically cleaned for 10 min.

Teeth preparations

Twenty caries-free extracted human third molars were used in this study. In the same teeth the enamel and dentin surface were used for analysis, independent, like as described below. This research was approved by the Ethics Committee of Bauru School of Dentistry and Federal University of Rio Grande do Sul under Protocol Number CAAE.10241519.3.0000.5417. The extracted teeth were cleaned and stored in a 0.2% thymol solution up to one month. The roots were sectioned 3 mm below to the cementoenamel junction. The occlusal third of each tooth crown was perpendicular cut to the longitudinal axis of the tooth using a water-cooled diamond disc (Wafer blade, 5 inch × 0.15 inch × 0.15 inch; Extec) to expose a flat mid-coronal dentin surface with a 4 mm at a distance of 2.5/2.0 mm of the pulp. The dentin surface was ground flat, and a smear layer was standardized by means a #600 grift silicon carbide paper under cooling water for 30 seconds and ultrasonically cleaned for 15s.

The occlusal third of each tooth was used to evaluate the enamel surface. The oclusal enamel surface was polished using #600 grift silicon carbide paper under cooling water for 60 seconds and ultrasonically cleaned for 15s, to obtain a flat surface. Subsequently, the specimens of dentin and the enamel were fixed in (10-mm-high and 19 mm-width) polyvinyl chloride tubes with acrylic resin (Jet; Dental Articles Classic) with the analysed area direction upwards.

Microshear bond strength (μSBS) test

For microshear bond strength, 3 factors were analysed: substrate (at 6 levels), resin luting cement (at 2 levels C_{TAT} and $C_{CONTROL}$) and time (7 days and 30 days) with the amount of 24 groups (n=16) as follow: [Lithium Disilicate + C_{TAT}, Lithium Disilicate + $C_{CONTROL}$, Celtra Duo + C_{TAT}, Celtra Duo + $C_{CONTROL}$, ZirCAD + C_{TAT}, ZirCAD + $C_{CONTROL}$, InCoris + C_{TAT}, InCoris + $C_{CONTROL}$, Enamel + C_{TAT}, Enamel + $C_{CONTROL}$, Dentin + C_{TAT} and Dentin + $C_{CONTROL}$]^2 (7 days and 30 days – independent samples). The specimen surface receives the treatment recommended according material, as followed in Table 1.

Table 1. Specimen treatment.

Material	Surface Treatment
Lithium Disilicate	a) Etched with 10% hydrofluoric acid (Condac porcelain, FGM Dental Products, Brazil) for 20 s, cleaned with water and oil-free compressed air for 30 s b) silanated (Silano, Angelus, Londrina, Brazil) c) a layer of Adhesive (Scotchbond Multi-Purpose, 3M ESPE, St. Paul, MN, USA) and photopolymerized
Celtra Duo	a) Etched with 10% hydrofluoric acid (Condac porcelain, FGM Dental Products, Brazil) for 30 s, cleaned with water and oil-free compressed air for 30 s b) silanated (Silano, Angelus, Londrina, Brazil) c) a layer of Adhesive (Scotchbond Multi-Purpose, 3M ESPE, St. Paul, MN, USA) and photopolymerized
YTZP	a) Clean surface with alcohol and dry with compressed air; b) Dispense Signum Zirconia (Heraeus Kulzer, Hanau, Germany) bond I and apply with suitable brush to entire surface and air dry for 5 s; c) Apply Signum Zirconia bond II (Heraeus Kulzer, Hanau, Germany) and photopolymerized for 40 s
Enamel	a) Etched with 35% phosphoric acid 30s, cleaned with water 30s, dried b) Layer of Adhesive (Scotchbond Multi-Purpose, 3M ESPE, St. Paul, MN, USA) and photopolymerized
Dentin	a) Etched with 35% phosphoric acid 15s, cleaned with water 30s, wet-dried b) Layer of Primer (Scotchbond Multi-Purpose, 3M ESPE, St. Paul, MN, USA) c) layer of Adhesive (Scotchbond Multi-Purpose, 3M ESPE, St. Paul, MN, USA) and photopolymerized

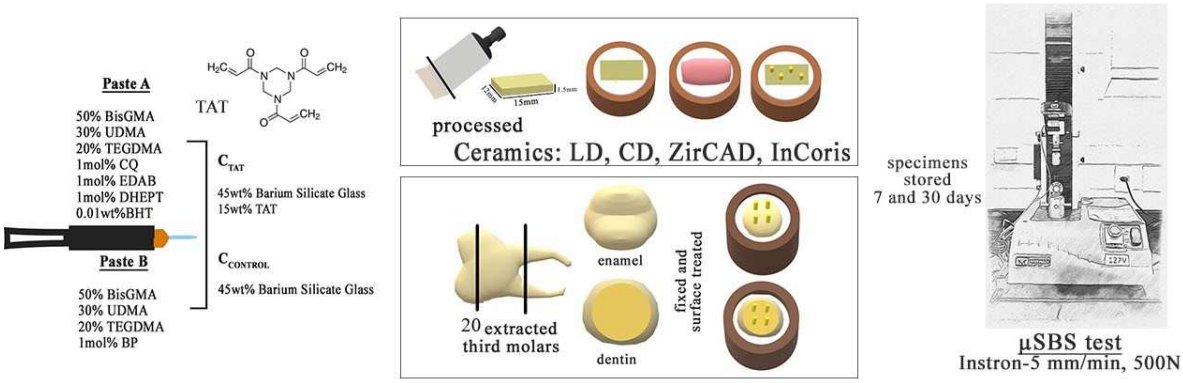
Surgical catheters with an inner diameter of 1.40 mm and a height of 1 mm were used to produce resin cement cylinders. Then, to use the resin luting cement, equal parts of paste A and B were weighted and mixed for 10s. The resin luting cements were applied inside specimens according to the groups. Resin luting cements were photoactivated for 60s with a light emitting diode unit (Radii Cal, SDI, Victoria, Australia) at 1200mW/cm^2. Four cylinders were made from each specimen. After, the surgical catheters were removed with #12 scalpel blades to expose the resin luting cement cylinders. Specimens were stored in deionized water at a temperature of 37°C for 7 days or 30 days (independent samples). Specimens were tested in a universal testing machine (Instron 3342; Illinois Tool Works), at a crosshead speed of 0.5

mm/min, 500N-load cell using a 0,2 mm metal wire. Data were obtained in MPa by dividing the force in Newton (N) by the bonding surface area (A) (A=π*r2, where *r* is the cement cylinder radius=0.7 mm). The mean fracture strength (MPa) in each specimen was calculated.

The fractured surfaces were evaluated by stereomicroscope (Modular Stereomicroscope Leica MZ6; Leica Microsystems) and the failure patterns classified as adhesive, cohesive or mixed.

The flowchart illustrates the experimental steps [Figure 1].

Figure 1. Flowchart



Statistical analysis

Statistical analysis was performed by using the software Statistica10 (Stat Soft Inc). Microshear bond strength data were analysed using 3-way ANOVA, following by Tukey test (α = 0.5). Chi-square test was used to assess the failure pattern (α = 0.5).

RESULTS

ANOVA indicated a significant interaction between substrate vs. resin luting cement (p = 0.0000) and between substrate vs. time (p = 0.0013). The interactions substrate vs. resin luting cement vs. time (p = 0.3259) and resin luting cement vs time (p = 0.1278) revealed no statistically significant differences. Table 2 displays the mean values, standard deviations, and statistical differences of the microshear bond strength (μSBS).

At 7 days, ZirCAD exhibited the highest C_{TAT} resistance value compared to $C_{CONTROL}$ (9.96 MPa and 3.26 MPa for $C_{CONTROL}$). All $C_{CONTROL}$ of the other substrates had better μSBS.

In the comparison substrate vs resin luting cement, LD and CD $C_{CONTROL}$, at both evaluation times (7 and 30 days), had the highest μSBS. In the comparison substrate vs time, overtime, ZirCAD C_{TAT} had the highest μSBS at 30 days (14.23MPa) than at 7 days (9.96MPa). Enamel and Dentin showed no statistically significant differences in relation to both the resin luting cement type and time. InCoris exhibited no statistically significant differences in relation to the resin luting cement and time.

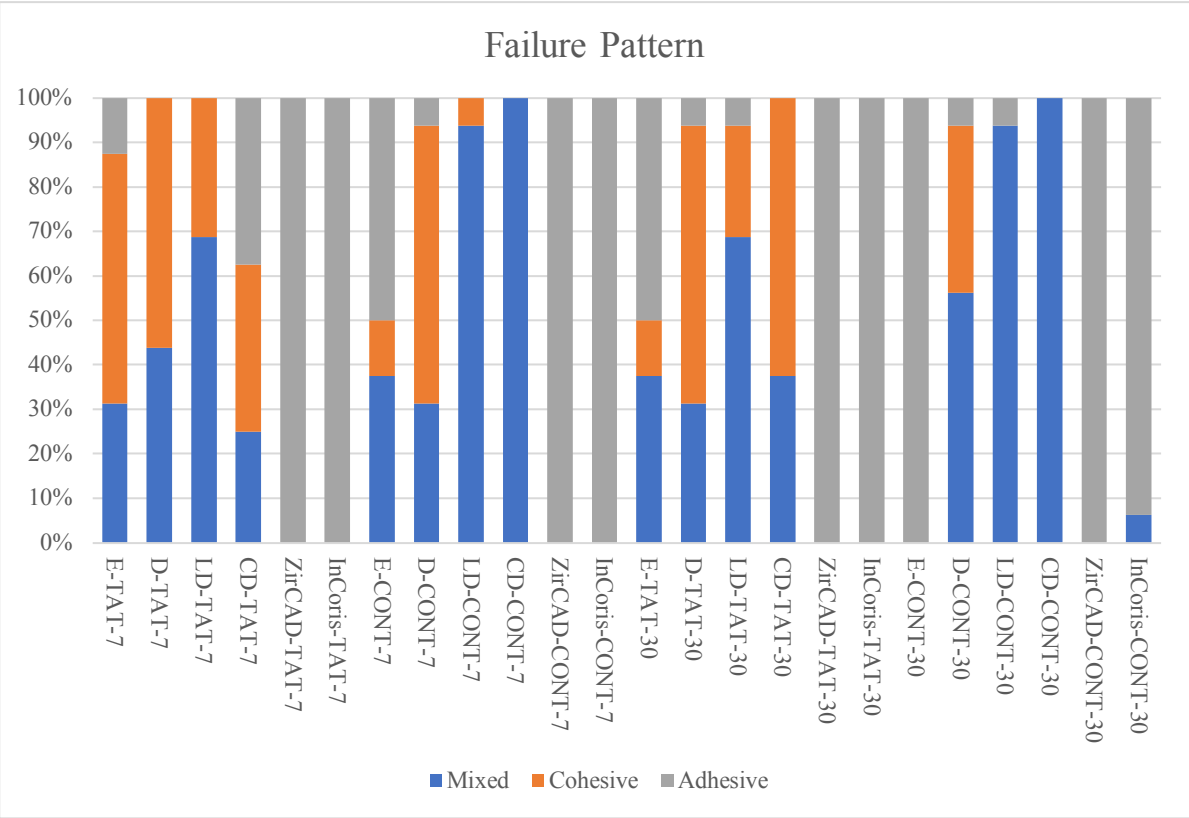
Table 2. Mean μSBS and standard deviation for different times.

Substrate	Cement	7 days	30 days
E	CONTROL	10.93 (3.69) ^{D}	11.68 (3.95) ^{D}
D		9.80 (4.52) ^{D}	8.08 (3.54) ^{D}
LD		22.41 (3.26) ^{A}	21.82 (7.80) ^{A}
CD		22.84 (5.47) ^{A}	22.76 (5.37) ^{A}
ZirCAD		3.26 (5.25) ^{D}	9.02 (4.69) ^{D}
InCoris		9.64 (3.51) ^{D}	9.57 (6.03) ^{D}
E	TAT	8.86 (2.21) ^{D}	10.85 (3.08) ^{D}
D		8.68 (3.02) ^{D}	8.47 (3.06) ^{D}
LD		16.67 (2.67) ^{D}	15.18 (4.19) ^{D}
CD		13.08 (2.22) ^{D}	16.04 (3.17) ^{D}
ZirCAD		9.96 (5.73) ^{C}	14.23 (6.22) ^{B}
InCoris		6.02 (5.50) ^{D}	10.96 (4.40) ^{D}

Different superscript letters indicate statistically significant differences between groups ($p<.05$).

The failure patterns after μSBS test varying according substrate, resin luting cement and time (p= 0.0000) (Graph 1).

Graphic 1. Resin luting cement x substrate failure pattern.



DISCUSSION

The results of this study demonstrate that 1,3,5-triacryloylhexahydro-1,3,5-triazine (TAT) resin luting cement improved only ZirCAD bond strength overtime. Therefore, the null hypothesis was partially accepted. TAT cement did not improve the bond strength for all the other substrates tested. Notwithstanding, the greatest microshear bond strength (μSBS) was obtained with Lithium Disilicate and Celtra Duo ceramics when cemented with $C_{CONTROL}$ even over time.

Compared with conventional cements such as polycarboxylate or glass ionomer cement, resin luting cements were introduced to promote all-ceramic restoration retention [22,23]. They can be classified as light-cured, self-cured, or dual-cured based in the polymerization mode [4]. The dual-curing resin luting cements are most appropriate for luting procedures in which light

transmission is limited [7] and increase the degree of conversion by means of a chemical activation of the monomeric system [24].

Resin luting cements not only provide stronger and more durable bonding between ceramics and tooth, but they also obtain better aesthetic performance and maintain higher ceramic strength [4,25]. Many factors related to the cement (monomer composition, filler content, curing mode and curing capability [26,27]) as well as to the surface type (enamel, dentine, alloys, glass ceramics, polycrystalline ceramics and composites [28,29]) influence on the bonding ability of resin luting cements. Thus, the clinician's choice may depend on several clinical factors, such as the need for additional retention, improving the strength of the crown, and even whether an adequate isolation can be obtained [23].

Each ceramic is unique in terms of composition. Therefore, to reach a successful outcome, it is necessary to understand that the ceramic surface treatment before cementation varies according to the type of ceramic used, to choose the proper resin luting cement, and to execute the cementation procedure correctly [3,16].

Enhancing the bond strength of resin luting cements to Y-TZP indirect restorations is an important issue for clinicians [13] because the surface cannot be etched by hydrofluoric acid to obtain micro-mechanical adhesion [4]. The inert nature of this substrate reduces the chemical interaction with resin luting cements [14,15]. Different substrate strategies for improving Y-TZP bonding has been analyzed such as: sandblasting, to form a surface roughness and irregularities [30]; treating the restorations with a combination of tribochemical silica and 10-metacriloiloxidecil dihydrogen phosphate (MDP); or using different primers based on phosphate and carboxylate functional monomers or a combination of MDP and a metal primer [16]. Others tried to use titanium dioxide (TiO_2) nanotubes applied to the surface of zirconia associated with MDP primers to obtained better bond strength [31]. In this research, the use of C_{TAT} with ZirCAD associated with sandblasting and an MDP primer improved μSBS overtime. The rationale behind this finding would probably be due to some interaction between Y-TZP surface (ZirCAD tested) and the cement. Nonetheless, the fracture pattern observed was adhesive. On the other hand, the same surface treatment with InCoris did not improve μSBS even overtime, probably because different zirconia materials have different surface features and internal structure, grain size, shape, composition, and hardness [15,32]. Therefore, the effect of any surface treatment and the bond strength to different materials may vary [15,32].

The bonding interface of glass ceramics and resin luting cements is an important factor for the long-term durability of ceramic restorations [4]. Hydrofluoric acid etching increases ceramic surface roughness, creating a micromechanical interlock between the ceramic and resin

luting cement, and followed by silanization has become the most established surface treatment for glass ceramics [4] ensuring chemical bonding to the resin luting cements [13,33] and providing better fracture resistance [34]. The glass ceramics evaluated in this study has the same μSBS performance for both cements, with better results for the control resin luting cement, supporting the well kwon acceptable performance of the surface treatment of this substrate type. They presented the highest μSBS values of all tested substrates.

The adhesive characteristic of the cement affects the retention and consequently the longevity of minimally prepared indirect restorations [35]. Resin luting cements providing higher bond strength to restorative materials and hard dental tissues, minimal solubility, stability, and biocompatibility has contributed to the development of a strong and durable tooth-restoration interface, with minimal removal of sound tooth tissues [29]. The enamel structure adhesion to resin luting cements is durable and reliable because of its homogeneity [36]. The dentin adhesion to resin luting cement depends on the morphology and the extent of demineralization, infiltration, and polymerization that determines the quality of tissue hybridization [35,37].

Although μSBS and tensile bond strength methods measure regional mapping, they are very conducive to characterize different substrates. μSBS test is still useful and necessary for the screening of new products and studying experimental variables [38]. μSBS test causes the predominance of tensile bond strength in the interface which could cause irregularities leading to the failure initiation [13]. The load applied might trigger an amount of stress concentration into the resin luting cement, resulting in the fracture within the structure bulk [39]. Therefore, the shear bond strength may be lower when the debonding occurs in the interface due to a weak link between the resin luting cement and the ceramic [11]. This was observed in YTZP ceramics, which showed adhesive failures. Lithium Disilicate and Celtra Duo presented better bonding to either TAT and Control cements, in which the cohesive or mixed failures predominantly occurs. In relation to the dental structure, enamel mostly had mixed and cohesive failures. However, C_{TAT} at 30 days exhibited just adhesive failures, demonstrating a weak interaction. Dentin had either mixed or cohesive failures.

The interface ceramic/resin luting cement is subjected to a complex environment in the oral cavity influenced by extrinsic factors such as temperature change, saliva, daily food and drinks, chewing, and other habits, so laboratory testing is important to enable the development of superior materials and surface preparation methods that provide long-term durability [4]. Consequently, the adhesion to tooth structure should provide retentive strength, marginal seal, be relatively simple to achieve, and have clinical durability [40].

Another important aspect for dental materials is the development of materials with antibacterial properties [20,41]. Antibacterial agents that copolymerizes with the monomers should be the first choice for antibacterial materials [17]. TAT is an antibacterial monomer that copolymerizes with methacrylate resin monomers [17,18]. This copolymerization provides an immobilization of the antibacterial component, preventing its leaching that could result in a porous material, with low physical and chemical properties [20], and loss of marginal sealing [42]. Therefore, antibacterial resin luting cements are clinically relevant as the biofilm formation near the material and close to the cementation line may be related to the incidence of recurrent caries in restored teeth [21,43]. Consequently, antibacterial resin luting cements may prevent the colonization of remnant viable bacteria through gaps in the indirect restoration/cement/tooth interface [20,21].

This study did not evaluate the antibacterial activity of TAT resin luting cement, but the literature reports the antibacterial activity of in bonding materials through reduced bacterial growth against *S. mutans* after direct contact test [17,18].

CONCLUSION

The experimental resin luting cement formulated in this study with 1,3,5-triacryloyhexahydro-1,3,5-triazine, an antibacterial component, provided increased μSBS to one zirconia type even over time. For the other substrates evaluated it was not possible to improve the μSBS.

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REFERENCES

[1] Silva NRFA, Thompson VP, Valverde GB, Coelho PG, Powers JM, Farah JW, Esquivel-Upshaw J. Comparative reliability analyses of zirconium oxide and lithium disilicate restorations in vitro and in vivo. J Am Dent Ass 2011; 142:4s-9s.

[2] Sailer I, Makarov NA, Thoma DS, Zwahlen M, Pjetursson BE. All ceramic or metal-ceramic tooh-supported fixed dental prostheses (FDS)? A systematic review of the survival and complication rates. Part I: Single crowns (SCs). Dent Mater 2015; 31:603-23.

[3] Ladha K, Verma M. Conventional and contemporary luting cements: an overview. J Ind Prosth Soc 2010; 10:79-88.

[4] Tian T, Tsoi JKH, Matinlinna JP, Burrow MF. Aspects of bonding between resin luting cements and glass ceramics materials. Dent Mater 2014; 30:e147-e162.

[5] Malament KA, Natto ZS, Thompson V, Rekow D, Eckert S, Weber HP. Ten-year survival of pressed, acid-etched e.max lithium disilicate monolithic and bilayered complete-coverage restorations: performance and outcomes as a function of tooth position and age. J Prosthet Dent 2019; 121:782-90.

[6] Pameijer CH. A review of luting agents. Int J Dent 2012;752861. doi:10.1155/2012/752861

[7] Albuquerque PPAC, Duarte MFB, Moreno MBP, Schneider LF, Moraes RR, Cesar PF, Rodrigues Filho LE. Physicochemical properties and microshear bond strength of experimental self-adhesive resin cements to dentin or Yttria-stabilized Tetragonal Zirconia Polycrystal. J Adhes Dent 2019; 21:133-41. doi: 10.3290/j.jad.a42363

[8] Kermanshahi S, Santerre JP, Cvitkovich DG, Finer Y. Biodegradation of resin-dentin interfaces increase bacterial microleakage. J Dent Res 2010; 89:996 - 1001. doi: 10.1177/0022034510372885

[9] Ferracane JL, Stansbury JW, Burke JT. Self-adhesive resin cements - chemistry, properties and clinical considerations. J Oral Rehab 2011; 38:295-314. doi:10.1111/j.1365-2842.2010.02148.x

[10] Stamatacos C, Simon JF. Cementation of indirect restorations: an overview of resin cements. Clin Comp 2013; 34:42-4.

[11] Chen JH, Matsumura H, Atsuta M. Effect of different etching periods on the bond strength of a composite resin to a machinable porcelain. J Dent 1998; 28:53-58.

[12] Heintze SD, Cavalleri A, Büchler A, Zappini G. Fracture frequency of all-ceramic crowns during dynamic loading in a chewing simulator using different loading and luting protocols. Dent Mater 2008; 24:1352-61.

[13] Bacchi A, Spazzin AO, de Oliveira GR, Pfeifer C, Cesar PF. Resin cements formulated with thio-urethanes can strengthen porcelain and increase bond strength to ceramics. J Dent 2018; 73:50-6.

[14] Paes PNG, Bastian FL, Jardim PM. The influence of Y-TZP surface treatment on topography and ceramic/resin cement interfacial fracture toughness. Dent Mat 2017; 33:976-89.

[15] Tzanakakis EGC, Tzoutas IG, Koidis PT. Is there a potential for a durable adhesion to zirconia restorations? A systematic review. J Prosthet Dent 2016; 115:9–19.

[16] Vargas MA, Bergeron C, Diaz-Arnold A. Cementing all-ceramic restorations: recommendations for success. J Dent Assoc 2011; 142:s20-4. doi: 10.14219/jada.archive.2011.0339

[17] Altmann AS, Collares FM, Ogliari FA, Samuel SM. Effect of methacrylated-based antibacterial monomer on orthodontic adhesive system properties. Am J Orthod Dentofacial Orthop 2015;147: S82-7.

[18] Altmann AS, Collares FM, Leitune VC, Arthur RA, Takimi AS, Samuel SM. In vitro antibacterial and remineralizing effect of adhesive containing triazine and niobium pentoxide phosphate inverted glass. Clin Oral Investig 2017; 21:93-103.

[19] Schiroky PR, Leitune VCB, Garcia IM, Ogliari FA, Samuel SMW, Collares FM. Triazine compound as copolymerized antibacterial agent in adhesive resins. Braz Dent J 2017; 28:196-200.

[20] Cocco AR, Rosa WL, Silva AF, Lund RG, Piva E. A systematic review about antibacterial monomers used in dental adhesive systems: current status and further prospects. Dent Mater 2015; 31:1345-62.

[21] Nedeljkovic I, Teughels W, De Munck J, Van Meerbeek B, Van Landuty KL. Is secondary caries with composites a material-based problem? Dent Mat 2015; 31:e247-77.

[22] el-Mowafy O. The use of resin cements in restorative dentistry to overcome retention problems. J Can Dent Assoc 2001; 67:97–102.

[23] Lawson NC, Litaker MS, Ferracane JL, Gordan VV, Atlas AM, Rios T, Gilbert GH, McCracken MS. Choice of cement for single unit crowns. JADA 2019; 50:522-30.

[24] De Souza G, Braga RR, Cesar PC, Lopes GC. Correlation between clinical performance and degree of conversion of resin cements: a literature review. J Appl Oral Sci 2015; 23:358-68. doi: 10.1590/1678-775720140524

[25] Addison O, Marquis PM, Fleming GJ. Quantifying the strength of a resin-coated dental ceramic. J Dent Res 2008; 87:542–7.

[26] Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Peumans M, Lambrechts P, et al. Adhesives and cements to promote preservation dentistry. Oper Dent 2001; 6:119–44.

[27] Yang B, Ludwing k, Adelung R, Kern M Micro-tensile bond strength of three luting resins to human regional dentin. Dent Mater 2006; 22:45–56.

[28] Hikita K, Van Meerbeek B, De Munck J, Ikeda T, Van Landuyt K, Maida T, et al. Bonding effectiveness of adhesive luting agents to enamel and dentin. Dent Mater 2007; 23:71–80.

[29] Al-Assaf K, Chakmakchi M, Palaghias G, Karanika-Kouma A, Eliades G. Interfacial characteristic of adhesive luting resins and composites with dentine. Dent Mater 2007; 23: 829-39.

[30] Abd El-Ghany OS, Sherief AH. Zirconia based ceramics, some clinical and biological aspects: Review. Fut Dent J 2016; 2:55-64.

[31] Dos Santos AF, de Lucena FS, Borges AFS, Lisboa-Filho PN, Furuse AY. Incorporation of TiO_2 nanotubes in a polycrystalline zirconia: synthesis of nanotubes, surface characterization, and bond strength. J Prosthet Dent 2018; 120:589-95. doi: 10.1016/j.prosdent.2017.10.027

[32] Calvacanti A, Foxton R, Watson T, Oliveira M, Giannini M, Marchi G. YTZP ceramics: key concepts for clinical application. Oper Dent 2009; 34:344-51.

[33] Bayne SC, Ferracane JL, Marshall GW, Marshall SJ, Van Noort R. The evolution of dental materials over the past century: silver and gold to tooth color and beyond. J Dent Res 2019; 98: 257-65.

[34] Pagniano RP, Seghi RR, Rosenstiel SF, Wang R, Katsube N. The effect of a layer of resin luting agent on the biaxial flexure strength of two all-ceramic systems. J Prosthet Dent 2005; 93:459–66.

[35] Aung SSMP, Takagaki T, Ko AK, Halabi S, Sato T, Ikeda M, Nikaido T, Burrow MF, Tagami J. Adhesion durability of dual-cure resin cements and acid-base resistant zone formation on human dentin. Dent Mater 2019; 35:945-52.

[36] Cardoso MV, de Almeida Neves A, Mine A, Coutinho E, Van Landuyt K, De Munck J, et al. Current aspects on bonding effectiveness and stability in adhesive dentistry. Aust Dent J 2011; 56:31–44.

[37] Cerqueira LAC, Costa AR, Spohr AM, Miyashita E, Miranzi BAS, Filho SC, Correr-Sobrinho L, Borges GA. Effect of dentin preparation mode on the bond strength between human dentin and different resin cements. Braz Dent J 2018; 29:268-74.

[38] Armstrong S, Geraldeli S, Maia R, Raposo LHA, Soares CJ, Yamagawa J. Adhesion to tooth structure: a critical review of "micro" bond strength test methods. Dent Mater 2010; 26: e50-62.

[39] Braga RR, Meira JB, Boaro LC, Xavier TA. Adhesion to tooth structures: a critical review of macro test methods. Dent. Mater 2010; 26:e38‑49.

[40] Armstrong S, Breschi L, Özcan M, Pfefferkorn F, Ferrari M, Van Meerbeek B. Academy of dental materials guidance on in vitro testing of dental composite bonding effectiveness to dentin/enamel using micro-tensile bond strength (μTSB) approach. Dent Mater 2017; 33:133-43.

[41] Leung D, Spratt DA, Pratten J, Gulabivala K, Mordan NJ, Young AM. Chlorhexidine-releasing methacrylate dental composite materials. Biomaterials 2005; 26:7145–53.

[42] Centenaro CC, Rostirolla FV, Leitune VC, Parolo CF, Ogliari FA, Samuel SM, Collares FM. Influence of addition of 2-[3-(2H-benzotriazol-2-YL)-4-hydroxyphenyl] ethyl methacrylate to an experimental adhesive system. Acta Odontol Latinoam 2015; 28:72-8.

[43] Lehmensiek M, Askar H, Brouwer F, Blunck U, Paris S, Schwendicke F. Restoration integrity, but not material or cementation strategy determined secondary caries lesions next to indirect restorations in vitro. Dent Mater 2018; 34:e317-23.

3 FUNDAMENTED DISCUSSION

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Antibacterial agents are used to reduce the viability of bacteria (STEWART & FINER, 2019; COCCO et al, 2015) in risk areas, such as the cementation line in indirect restorations. In this study, a dual-cure luting agent was formulated with 1,3,5-triacryloylhexahydro-1,3,5-triazine as an antibacterial monomer for controlling the biofilm formation at indirect restorations that may fail due to recurrent caries (IOANNIDIS & BINDL, 2016; MORIMOTO et al, 2016). The TAT-containing luting agent showed higher degree of conversion and higher cell viability and a reduced adhesion of bacteria at the material's surface while increased the bond strength to one zirconia substrate after 7 and 30 days.

TAT is a well-known quaternary ammonium compound that has been studied in different applications as an antibacterial agent (MONTEIRO et al, 2020; GARCIA et al, 2020; ALTMANN et al, 2015; ALTMANN et al, 2017; SCHIROCKY et al, 2017; COCCO et al, 2015). Besides, the presence of three C=C functional groups in TAT structure, it is able to copolymerize with methacrylate monomers which have been shown to increase the degree of conversion of different dental restorative materials (ALTMANN et al, 2015; ALTMANN et al, 2017; SCHIROCKY et al, 2017; GARCIA et al, 2019). The copolymerization characteristics shown for triazine compounds influence the formation of the polymeric structure and the releasing of antibacterial products (COCCO et al, 2015). In this study a higher degree of conversion was observed for TAT luting agents both immediately and after 7 days. As a dual-cure system was used, it is expected that the chemical activated polymerization continues after photoactivation and, for this reason, the degree of conversion was assessed immediately and after 7 days, when increased values were found for C_{TAT} and $C_{CONTROL}$. While a higher degree of conversion is related to higher mechanical properties as shown for different dental composites (FERRACANE & GREENER, 1986; SANTERRE et al, 2001), the flexural strength values of developed luting agents did not present statistically significant differences between groups. Besides, formation of the polymeric network in these materials may be related to their ability to adhere to substrates in a clinical application. In this study, C_{TAT} presented higher μSBS results for one substrate (YTPZ- ZirCAD 9.96 MPa at 7 days and 14.23MPa at 30 days) only with adhesive failure, which may be related to the quality of the formed polymer for the developed luting agents (NOVAIS et al, 2017).

The addition of antibacterial monomers into composites may affect the stability of the polymeric network (MICHELSEN et al, 2008; PONGPRUEKSA et al, 2015) in the developed luting agents. The polymeric matrix softening was assessed after ethanol immersion and no statistically significant differences was found between C_{TAT} samples before and after immersion in the solvent, which may indicate that the copolymerization ability leads to a polymeric structure less prone to degradation due to the increased stability of the formed polymeric chains (MICHELSEN et al, 2008). The reduced polymer softening along with the increased degree of conversion might be the reason why increased cell viability was observed for C_{TAT} materials compared to the control group. The unreacted methacrylate monomers, especially TEGDMA, are known to induce a reduction in cell viability in different cell types (STEWART & FINER, 2019; GOLSTEIN, 2010; MANSO et al, 2011) which was not observed in the present study. The presence of an antibacterial agent into the luting cement showed increased cell viability when compared to $C_{CONTROL}$ which may also be related to the lack of TAT leaching from the matrix due to copolymerization. This agrees with the findings of the antibacterial analysis, in which no increased planktonic activity was observed indicating that TAT was not released in the culture medium. Although the formation of the polymer was adequate, the addition of TAT increased the film thickness. Despite the statistical significance, values of both TAT-containing and control group luting agents meets ISO 4049 requirements. The higher film thickness may impact on the biofilm formation in the cementation line and, in this case, the antibiofilm properties of TAT-containing luting agents may reduce the risk of caries development.

The literature reports the copolymerization ability of antibacterial agents to reduce the bacterial viability with higher stability and possible long-term activity (STEWART & FINER, 2019; ALTMANN et al, 2015; COCCO et al, 2015; CHENG et al, 2013). TAT has antibacterial activity against Gram-positive and Gram-negative bacteria as well as an antibiofilm activity against *S. aureus* (AL-ZAYDY et al, 2017; KATUGAMPALA et al, 2018) and *S mutans* (MONTEIRO et al, 2020; ALTMANN et al, 2015; ALTMANN et al, 2017; SCHIROKY et al, 2017). The disruption of the bacteria's membrane integrity is the main responsible for TAT antibacterial activity. Due to its positive charge and lipophilic structure, TAT is easily detected by bacteria's membrane and TAT has the ability of mimicking antimicrobial peptides (AMPs) in the bacteria metabolism. The AMP's are released by the immune system when bacterial infection is detected and results in the lysis of bacteria's membrane leading to its death. This effect may be observed in the present study after the contact of bacteria with the luting agents during the antibacterial analysis for biofilm formation (ZHOU et al, 2018; LEWIS et al, 2015).

The reduction in the biofilm indicates the potential of TAT to decrease *S. mutans* viability when in contact with materials, which may be important to reduce the number of viable bacteria in the cementation line. This behavior was not found in the planktonic analysis, as reported, due to the ability of TAT to bond to the polymeric chains preventing its leaching (ALTMANN et al, 2015; ALTMANN et al, 2017). The fact that the antibacterial effect is driven by the contact between the materials and bacteria but not by the leaching (and consequently not by the degradation of the developed luting agents), may prevent the increase in the materials' porosity, the reduction in physicochemical properties, and the loss of marginal seal (CENTENARO et al, 2015).

The μSBS of developed luting agents were tested after 7 and 30 days of storage and different substrates were used as a screening for the *in vitro* bonding ability of C_{TAT} and $C_{CONTROL.}$ No statistically significant differences were observed in both enamel and dentin after 30 days, suggesting no effect of the TAT addition on adhesion to tooth tissue. The control luting agent presented higher values for the glass ceramic materials (lithium disilicate and celtra duo) at both time periods.

The adhesion to zirconia substrates is known to be a challenge due to the inert surface of these ceramics (QUIGLEY et al, 2020; PASSOS et al, 2015). Several attempts have been made to promote increased mechanical interlocking between the zirconia surface and the luting agents, which may increase the bond strength results and the stability of bonding over time (WEIGL et al, 2019; ABOUSHELIB et al, 2018). In this study, the use of TAT with ZirCAD associated with sandblasting and MDP primer has optimized the μSBS over time because some interaction with the surface of the Y-TZP (ZirCAD tested) and maybe with the luting agent could have occurred. Nonetheless, the fracture pattern observed was adhesive. On the other hand, the same surface treatment with InCoris did not improve μSBS even overtime, probably because different zirconia materials have different surface features and internal structure, grain size, shape, composition, and hardness (TZANAKAKIS et al, 2016; CAVALCANTI et al, 2009). Therefore, the effect of any surface treatment and the bond strength to different materials may vary (TZANAKAKIS et al, 2016; CAVALCANTI et al, 2009).

Therefore, antibacterial resin luting cements are clinically relevant as the biofilm formation near the material and close to the cementation line may be related to the incidence of recurrent caries in restored teeth (LEHMENSIEK et al, 2018; NEDELJKOVIC et al, 2015). In addition to the development of a luting agent with antibacterial activity, the results of this study

suggest that the proposed material could be more stable and consequently, an alternative for establishing an adequate marginal seal, preserving the tooth/indirect restoration interface.

4 CONCLUSIONS

4 CONCLUSIONS

The experimental luting agent formulated in this study with the addition of 1,3,5-triacryloylhexahydro-1,3,5-triazine showed anti-biofilm activity, increased degree of conversion and decreased the cytotoxicity. The luting agent with TAT increased the bond strength for one Y-TZP type even over the time.

REFERENCES

REFERENCES

ABOUSHELIB, M.N. et al. Ultrastructural analysis and long-term evaluation of composite-zirconia bond strength. *The Journal of Adhesive Dentistry*, v. 20, n. 1, p.33-39, mar. 2018.

ALTMANN, A.S. et al. Effect of methacrylated-based antibacterial monomer on orthodontic adhesive system properties. *American Journal of Orthodontics and Dentofacial Orthopedics*, v. 147, n. supl.4, p. S82-87, abr. 2015.

ALTMANN, A.S. et al. In vitro antibacterial and remineralizing effect of adhesive containing triazine and niobium pentoxide phosphate inverted glass. *Clinical Oral Investigation*, v. 21, n. 1, p. 93-103, jan. 2017.

AL-ZAYDI, K.M. et al. Synthesis, characterization and evaluation of 1,3,5-triazine aminobenzoic acid derivates for their antimicrobial activity. *Chemistry Central Journal*, v. 11, n. 1, p. 39, maio, 2017.

CENTENARO, C.C. et al. Influence of addition of 2-[3-(2H-benzotriazol-2-YL) -4-hydroxyphenyl] ethyl methacrylate to an experimental adhesive system. *Acta Odontológica Latinoamerica*, v. 28, n. 1, p. 72-78, abr. 2015.

CHENG, L. et al. Dental primer and adhesive containing a new antibacterial quaternary ammonium monomer dimethylaminododecyl methacrylate. *Journal of Dentistry*, v. 41, n. 4, p. 345–355 abr. 2013.

COCCO, A.R. et al. A systematic review about antibacterial monomers used in dental adhesive systems: current status and further prospects. *Dental Materials*, v. 31, n. 11, p. 1345-1362, nov. 2015.

FERRACANE, J.L. & GREENER, E.H. The effect of resin formulation on the degree of conversion and mechanical properties of dental restorative resins. *Journal of Biomed Materials Research*, v. 20, n. 1, p. 121-131, jan. 1986.

GARCIA, I.M. et al. Quaternary ammonium compound as antimicrobial agent in resin-based sealants. *Clinical Oral Investigation*, v. 22, n. 4, p. 777-784, fev. 2020.

GARCIA, L.S.G et al. Anticaries effect of toothpaste with nano-sized sodium hexametaphosphate. *Clinical Oral Investigation*, v. 23, n. 9, p. 3535-3542, set. 2019.

GOLSTEIN, G.R. The longevity of direct and indirect posterior restorations is uncertain and may be affected by a number of dentist-, patient-, and material-related factors. *The Journal of Evidence-Based Dental Practice*, v. 10, n.1, p. 30-31, mar. 2010.

IOANNIDIS, A. & BINDL, A. Clinical prospective evaluation of zirconia-based three-unit posterior fixed dental prostheses: Up-to ten-year results. *Journal of Dentistry*, v. 47, p. 80–85, abr. 2016.

KATUGAMPALA, S. et al. Synthesis, characterization, and antimicrobial activity of novel sulfonated copper-triazine complexes. *Bioinorganic Chemistry and Application*, v. 29. 2018

LEHMENSIEK, M. et al. Restoration integrity, but not material or cementation strategy determined secondary caries lesions next to indirect restorations in vitro. *Dental Materials,* v. 34, n. 12, p. e317-323, dez. 2018.

LEWIS, S.N. et al. Pharmacophore modeling improves virtual screening for novel peroxisome proliferator-activated receptor-gamma ligands. *Journal of Computer-Aided Molecular Design*, v. 29, n: 5, p. 421-439, maio. 2015.

MANSO, A.P. et al. Cements and adhesives for all-ceramic restorations. *Dental Clinics of North America*, v. 55, n. 2, p. 311-332, abr. 2011.

MICHELSEN, V.B. et al. Quantitative analysis of TEGDMA and HEMA eluted into saliva from two dental composites by use of GC/MS and tailor-made internal standards. *Dental Materials*, v. 24, n. 6, p. 724-731, jun. 2008.

MONTEIRO, J.C. et al. Dental Sealant Empowered by 1,3,5-Tri Acryloyl Hexahydro1,3,5-Triazine and α-Tricalcium Phosphate for Anti-Caries Application. *Polymers (Basel)*, v. 12, n. 4, p.e895, abr. 2020.

MORIMOTO, S. et al. Survival rate of resin and ceramic inlays, onlays, and overlays: a systematic review and meta-analysis. *Journal of Dental Research*, v. 95, n. 9, p. 985–994, ago. 2016.

NEDELJKOVIC, I. et al. Is secondary caries with composites a material-based problem? *Dental Materials*, v. 31, n. 11, p. e247-277, nov. 2015.

NOVAIS, V.R. et al. Degree of conversion and bond strength of resin-cements to feldspathic ceramic using different curing modes. *Journal of Applied Oral Science*, v. 25, n. 1, p. 61-68, jan-fev. 2017.

PASSOS, S.P. at al. The effect of air-abrasion and heat treatment on the fracture behaviour of Y-TZP. *Dental Materials*, v. 31, n. 9, p.1011-1021, set. 2015.

PONGPRUEKSA, P. et al. Monomer elution in relation to degree of conversion for different types of composite. *Journal of Dentistry*, v. 43, n. 12, p. 1448-1455, dez. 2015.

QUIGLEY, N.P. et al. Clinical efficacy of methods for bonding to zirconia: A systematic review. Journal of Prosthetic Dentistry, fev. 2020.

SANTERRE, J.P. et al. Relation of dental composite formulations to their degradation and the release of hydrolyzed polymeric-resin-derived products. *Critical Reviews in Oral Biology Medicine*, v. 12, n. 2, p. 136–151, 2001.

SCHIROKY, P.R. et al. Triazine compound as copolymerized antibacterial agent in adhesive resins. *Brazilian Dental Journal*, v. 28, n. 2, p. 196-200, mar-abr. 2017.

STEWART, C.A. & FINER, Y. Biostable, antidegradative and antimicrobial restorative systems based on host-biomaterials and microbial interactions. *Dental Materials*, v. 35, n. 1, p. 36-52, jan. 2019.

WEIGL, P. et al. Screw-retained monolithic zirconia vs. cemented porcelain-fused-to-metal implant crowns: a prospective randomized clinical trial in Split-Mouth Design. *Clinical Oral Investigation*, v. 23, n. 3, p.1067-1075, mar. 2019.

ZHOU, C. et al. Synthesis and biological evaluation of novel 1,3,5-triazine derivatives as antimicrobial agents. *Bioorganic & Medicinal Chemistry Letter*, v. 18, n. 4, p. 1308-1311, fev. 2008.