Clinical behavior of bioactive glass ionomer (45S5) in moderate caries injuries: Study protocol of a clinical trial

Comportamento clínico de ionômero de vidro bioativo (45S5) em lesões de cárie moderada:
Protocolo de estudo de um ensaio clínico

Abstract
Bioactive materials that are capable of releasing remineralizing ions may be an interesting alternative treatment to prevent the development of carious lesions. The purpose of this study is to evaluate the effectiveness of the association of 45S5 with resin-modified glass ionomer cement (GIC-MR) for conservation and prevention of the progression of initial carious lesions in permanent molars. A total of 36 patients, aged 8 to 14 years, with at least two permanent molars homologous with ICDAS (International Caries Detection and Assessment System) 3 or 4 will be selected to participate in a clinical study of the split-mouth type. The DLF-T (Decayed, Lost and Filled Teeth index), ICDAS, BOP (Bleeding on probing), and VPI (Visible Plaque Index) will be analyzed. A complementary radiographic exam will be performed to assess the dentin underlying lesion. The selected teeth will be randomly assigned into two groups: GIC-MR and GIC-MR + 45S5. Parameters such as retention, the clinical performance of materials, and caries evolution will be evaluated into the two studied groups. Two calibrated evaluators will perform clinical, radiographic, and microscopic evaluations at one, six, 12, and 24 months of follow-up. The results obtained will be evaluated using a Chi-square test. The intention-to-treat protocol will be followed. The results of this study will help to assess the behavior of a bioactive glass ionomer in lesions with enamel microcavities. Innovative research projects, such as the one described here, are needed to determine whether new materials can be used as a treatment alternative.

Keywords: Pit and fissure sealants; Bioglass, Glass ionomer cements; Randomized controlled trial.

Resumo
Materiais bioativos, que são capazes de liberar íons, podem ser uma interessante alternativa de tratamento para prevenir o desenvolvimento de lesões cariosas. O objetivo deste estudo é avaliar a efetividade da associação do 45S5 ao cimento de ionômero de vidro modificado por resina (CIV-MR) na conservação e prevenção da progressão de lesões cariosas em molares permanentes. Um total de 36 pacientes, com idade de 8 até 14 anos, com pelo menos dois molares permanentes homólogos com ICDAS (International Caries Detection and Assessment System) 3 ou 4 serão selecionados para participar de um estudo clínico do tipo boca-dividida. O CPO-D (Índice de Dentes Cariados, Perdidos e Obturados), ICDAS, ISG (Índice de Sangramento Gengival) e IPV (Índice de Placa Visível) serão analisados. Um exame radiográfico complementar será realizado para avaliar a profundidade da lesão dentinária. Os dentes selecionados serão atribuídos aleatoriamente em dois grupos: CIV-MR e CIV-MR+45S5. Parâmetros tais como retenção, desempenho clínico dos materiais, e evolução da cárie serão avaliados nos dois grupos estudados. Dois
valiadores calibrados irão realizar avaliações clínica, radiográfica e microscópica com 1, 6, 12 e 24 meses de acompanhamento. Os resultados obtidos serão avaliados usando o teste Qui-quadrado. Será seguido o protocolo de intenção de tratar. Os resultados deste estudo irão ajudar a avaliar o comportamento de um ionômero de vidro bioativo em lesões com microcavidades em esmalte. Projetos de pesquisa inovadores, como o descrito aqui, são necessários para determinar se novos materiais podem ser usados como alternativa de tratamento.

**Palavras-chave:** Selantes de fossas e fissuras; Cimentos de ionômero de vidro; Ensaio clínico controlado aleatório.

### 1. Introduction

Dental caries has a great impact on oral health, significantly influencing people's quality of life (Barbosa & Gavião, 2008). The development of caries can cause irritation, masticatory limitation, and pain, in addition to impacting psychological and social aspects, especially in childhood (Barbosa et al., 2013). Even though the global rates of caries disease development have declined over the past few years (Peres et al., 2006), it is still necessary to find preventive and curative treatments that prevent their impact (Lima et al., 2018).

The evolution of caries concepts and updates in diagnoses, techniques, and biomaterials has generated a new perspective regarding the prevention and control of caries lesions. Allied to these factors, a change in the intervention approach validates the development of minimally invasive Dentistry (Innes et al., 2019). Contemporary dentistry recommends not removing all demineralized tissue, in cases where there is no visible exposure of dentin (Fontana et al., 2014). Therefore, it is necessary to follow-up on the development of caries lesions, categorizing them into inactive or active lesions (Innes et al., 2019). This clinical procedure is suggested to minimize intervention and preserve dental structure (Frenchen, 2017). Some validated instruments assist in the evaluation of caries lesion activity; this includes the International Caries Detection and Assessment System (ICDAS) (N Pitts, 2004).

Within this context, the use of sealants on non-cavitated lesions presents a lower chance of caries progression when compared to unsealed lesions (Griffin et al., 2008). Especially in cases of ICDAS-3 lesions (enamel breakdown without visible dentin exposure), the use of sealants is very relevant due to its effectiveness in controlling the evolution of carious lesions (Muñoz-Sandoval et al., 2019).

Resin-modified glass ionomer cement (RMGIC) is a material that presents mechanical resistance and abrasion (Di Nicolo et al., 2007) besides high retention rates (Pereira et al., 2003). However, RMGIC may have a limited ability to release fluoride ions when compared to conventional GIC (Kucukyilmaz et al., 2017).

Bioactive materials have been studied due to their effects on antimicrobial capacity (Martins et al., 2011; Waltimo et al., 2007), mineral release, and influence on the pH of the medium (Bauer et al., 2019; Yli-Urpo et al., 2005). 45S5 bioglass is one of the most widespread bioactive materials, with the ability to release calcium and phosphate ions (Bauer et al., 2019).
45S5 (45% SiO$_2$, 24.5% Na$_2$O, CaO, 6% P$_2$O$_5$) reacts in aqueous medium and results in the formation of hydroxyapatite and/or hydroxy carbonate apatite layer (Hench, 2006). An interactive layer is formed and it is resistant to abrasion (Bakry et al., 2014). One characteristic of 45S5 glass is that it is transformed into hydroxyapatite crystals when in the presence of a remineralizing solution (Bakry et al., 2014). The incorporation of bioactive particles to RMGIC could promote improvements that allow this material to act effectively in demineralized structures.

This study is motivated by the lack of randomized clinical studies reporting the clinical behavior of these bioactive materials to prevent the evolution of moderate carious lesions. Due to the need for clinical information to confirm the in vitro studies, this pragmatic clinical trial aimed to propose a clinical evaluation of the effectiveness of 45S5 associated with RMGIC in preventing the progression of moderate carious lesions in permanent molars. Additionally, this study proposes the evaluation of the retention and clinical quality criteria of material remaining in different evaluation periods, as well as checking the patients’ oral health condition. The null hypotheses to be tested are that there will be no difference in: (1) the retention of the different materials evaluated; (2) the quality of the material remaining in each group; (3) changing the ICDAS of treated teeth.

2. Methodology

This protocol followed the recommendations of the Standard Protocol Items: Recommendations for Intervventional Trials (SPIRIT).

2.1 Ethical aspects and registration

This clinical trial was approved by the Research Ethics Committee of the Federal University of Maranhão, under the number 2.284.768, and registered in the database for clinical studies: Brazilian Registry for Clinical Studies (ReBEC) with registration RBR-389y6y.

The study will be carried out in São Luís city, in the state of Maranhão, Brazil. The authorization for volunteers to participate in the research will be through the signing of a written informed consent by the parents/guardians of the patients, and an assent form signed by the child.

All the patients will be coded by a number to ensure the confidentiality and anonymity of the information. The study-related information will be stored securely and reports, data collection, process, and administrative forms will be identified by a coded ID [identification] number only to maintain participant confidentiality.

2.2 Study design

The design will be a randomized, triple-blind, split-mouth clinical study. The patients, operators, and evaluators will not know which treatment is being applied on each side (teeth). The flowchart of the study steps according to CONSORT (interventions, and evaluation diagram) is shown in Figure 1. The primary outcome of the study will be a reduction in the risk of caries progression, and the secondary outcome will be an evaluation of the evolution of caries disease, shape, contour and fracture of the material.
Figure 1. Clinical trials flowchart.

The flow diagram shows the steps that will be followed throughout the study.

2.3 Sample size calculation

The primary outcome of the study should be a reduction in the risk of caries progression. The sample size calculation, carried out by the www.sealedenvelope.com system, is based on the rate related to the reduction in the risk of developing caries with resin-modified glass ionomer sealant, which is reported to be approximately 56% (Wright et al., 2016). Thus, 64 patients have to have an 80% chance of detecting a significant reduction in the measurement of the primary outcome, from 56% in the control group to 86% in the experimental group, at the 5% level. Given the research will be of the mouth-divided type, the number of patients will be 32. Considering a possible rate of patient loss, the sample number has been increased by 10%, thus the number of patients involved will be 36.
2.4 Eligibility

This study will include volunteers aged between eight and 14 years, with good oral health in general, who have at least two molars with an ICDAS carious lesion between scores 3 and 4, verified through clinical and radiographic examination. Additionally, these teeth should have pulp vitality; be free of cavitated carious lesions and restorations on any of the sides of the treated tooth, and all of these items will be recorded in a clinical anamnensis form.

Volunteers with periodontitis, those undergoing orthodontic treatment, systemic changes, or who report previous allergies to the material will be excluded.

2.5 Recruitment and setting

The recruitment will be carried out between September and December 2021, at the clinics of postgraduate courses in Pediatric Dentistry (São Luís, MA, Brazil). Each patient will be involved in the study for about 25 months: 1 month for treatment and 24 months for follow-up.

2.6 Randomization and Groups Allocation (Study Protocol)

The randomization will be carried out through a specialized system, through the website www.sealedenvelope.com. Allocation details will be recorded on cards stored in opaque envelopes, numbered sequentially, and sealed by a person blind to the conditions and who will not participate in other stages of the study.

Once the patient is eligible for the procedure, the allocation assignment will be revealed by this envelope to be opened at the time of the application of the treatments by an assistant, who will make the selected material available to the operator.

The treatments will be with resin-modified glass ionomer cement (RMGIC) and RMGIC associated with 45S5 (CIV-MR + 45S5). The treatment labels will be sealed, being identified only as A and B so that neither the operator nor the patients can identify which treatment is being applied on each side.

2.7 Interventions

The procedures will be performed at the Dentistry Clinics of the Federal University of Maranhão (São Luís, MA, Brazil). Before carrying out the treatments, patients will undergo screening in order to select those that meet the inclusion criteria. The parents/guardians of the patients selected for the research will be guided and invited to sign a written informed consent form, and the child will sign an assent form.

For the clinical examination, a mouth mirror, an exploratory probe, and a millimeter probe will be used to register the visible plaque index (VPI), Bleeding on probing (BOP), decayed, lost, and filled teeth index (DLF-T), and oral health conditions. Interproximal radiographs will be taken, and all the data will be recorded on the patients' clinical files.

After the clinical examination, a prophylaxis of the teeth with pumice and water will be performed. The tooth, with ICDAS lesion, will be completely isolated, first on the right side and an envelope containing the randomized treatment will be opened. The material drawn to the right side will be applied to the tooth, while the left side tooth will receive the treatment mentioned second.

The treatment will be performed applying the material on the occlusal surface of the tooth, sealing the lesions of ICDAS 3 or 4. The application of the materials will be in accordance with the manufacturer's instructions for the resin-modified glass ionomer cement (Vitro Fill Nova DFL, Rio de Janeiro, RJ, Brazil) (Table 1). After the treatments are performed, the occlusion will be checked to remove possible premature contacts. Finally, a layer of glaze will be applied and light-cured for 20s using a LED Bluephase N (Ivoclar Vivadent, Schaan, Liechtenstein).
Table 1. Specifications of the materials to be used, composition, and manipulation.

| GROUPS | MATERIAL/MANUFACTURER | COMPOSITION | MANIPULATION |
|--------|-----------------------|-------------|--------------|
| CIV- MR | Vitro Fill LC A3/ DFL, Rio de Janeiro, RJ, BR | Powder: Strontium-Aluminum Silicate, Load, Activators and Iron Oxide. Liquid: 2-Hydroxyethyl Methacrylate, Aqueous Solution of Polyacrylic and Tartaric Acids, Benzoyl Peroxide and Camphorquinone. Primer: Modified Metacrylated Polyacids, Stabilizer, Catalyst and Ethyl Alcohol | 1. Conditioning the enamel surface with polyacrylic acid for 30s and then washing it for 30s. 2. After the acid conditioning, the primer will be applied and light-cured for 20s. The material will be applied, in the region of grooves, pits, and fissures on the occlusal surface, with the aid of a blunt-ended explorer probe, and light-cured for 20 seconds. |
| 45S5 | Bioactive glass SYLC/ Osspray Ltd, London, UK | 45% SiO2; 24.5% Na2O; 24.5% CaO; 6% P2O5 | 1. Incorporation of 10% of the SYLC to the powder of the CIV-MR with the aid of a high-precision scale (Ohaus, New Jersey, USA); 2. The treatment will be performed according to the protocol for application of the resin-modified glass ionomer cement (Vitro Fill Nova DFL, Rio de Janeiro, RJ, Brazil). |

Source: Authors.

In Table 1, it is possible to verify the materials used, their composition and way of manipulation them.

2.8 Post-treatment guidelines

Patients will be instructed to brush their teeth regularly using fluoride toothpaste and floss. Brochures with oral hygiene guidelines will also be provided.

All patients will be instructed to return to follow-up after one month, six months, one year, and two years. A single calibrated researcher will perform all the evaluations.

Direct and indirect assessments of the biological and functional properties of the material will be carried out. The evaluators will be calibrated and masked as to the type of the evaluated treatment.

The operator and evaluator will be calibrated in order to reduce possible study failures. The calibration for the use of the ICDAS index and the quality criteria of the United States Public Health Service (USPHS), technical application of the material and evaluation of the treatments will be carried out by means of expository classes given by a specialist professor. Training will also be carried out on the teeth of dental mannequins, standardizing the form of application of ionomeric sealants. The microscopic analysis evaluator will participate in four classes followed by four calibration tests.

2.9 Assessment

2.9.1 Direct assessment of the functional and biological properties of the material

Regarding the functional properties of the material, the following aspects will be evaluated: anatomical shape and contour, marginal discoloration, fracture, and retention of the material. The aspects of shape, anatomical contour, and marginal discoloration will be assessed according to the modified USPHS criterion (Perdigão et al., 2012). The retention of the material will be measured according to the criteria of Simonsen et al., 1991: 1- total retention of the sealant, 2- partial loss of the sealant, and 3- total loss (Simonsen, 1991).

Regarding the biological aspects, caries' regression or progression will be evaluated. This control will be performed
clinically and with radiographic examination, using the bite-wing technique. In cases of material loss, the presence of superficial opacity and color change (white/darkened) will be checked visually, and the roughness change will be verified with an explorer. The ICDAS method for the diagnosis of caries will be used. In the case of caries evolution, patients will be referred for restorative treatment, if necessary.

2.9.2 Indirect assessment of the functional properties of the material

The treated teeth will be molded with addition silicone in the baseline and in all the assessment periods. These moldings will be cast in epoxy resin and the models will be evaluated in a stereomicroscope. Aspects such as marginal adaptation, presence of cracks, fissures, and fractures of the material will be assessed through indirect clinical evaluation.

2.10 Data analysis

The analysis will be performed following the intention-to-treat protocol and will involve all the patients who will be randomly assigned (Schulz et al., 2011).

In the case of data loss, the last observation will be taken forward. The statistician will be masked for the study groups.

Qualitative variables will be analyzed by a Chi-square test of independence. For all tests applied, the alpha value will be considered at a 5% - 95% confidence interval. The statistical program used will be IBM SPSS Statistics 23.

3. Discussion

Caries lesions control is an issue that deserves constant investigation, due to the importance of preserving dental structure (Giacaman, 2017) and maintaining patient quality of life (Barbosa & Gavião, 2008). Thus, a thorough and adequate diagnosis is essential to check the clinical condition of the carious lesion, which can be active or inactive. Inactive lesions may clinically leave a “scar” or show radiographically residual radiolucent image but this is not necessarily a problem or risk to the patient's health (Innes et al., 2019).

Strategies with minimal intervention associated with adhesive techniques may soon be replaced or associated with biomimetic remineralizing materials, biofilm modulators, and tissue engineering (Innes et al., 2019). Thus, the investigation of bioactive materials that helps in the remineralization and decrease demineralization processes has gained great prominence.

Muñoz-Sandoval et al., 2019 compared using non-invasive conservative treatments in ICDAS (International Caries Detection and Assessment System) 3 lesions using a randomized clinical trial. After 24 months of follow-up, they found low progression rates of carious lesions in sealed teeth (Muñoz-Sandoval et al., 2019). Therefore, evaluating the application of bioactive materials in ICDAS 3 and 4 lesions seems to be a promising alternative.

For the analysis of caries lesion stages the ICDAS presents itself as a sensitive and widely available instrument (Ekstrand et al., 2018). This instrument guarantees greater flexibility regarding the category of caries disease stage, subdividing lesions into cavitated or non-cavitated (Ismail et al., 2007). The caries assessment methods must be accurate and able to check the manifestations of the caries process at any stage and differentiate the behavior of lesions (progression, arrest, and/or regression) (NB Pitts & Stamm, 2004). Visual examination is the most widely used method for diagnosing caries. However, auxiliary techniques for the final diagnosis, such as bite-wing radiographs, can also be employed (NB Pitts & Stamm, 2004). For this purpose, in the methodology of this study protocol, in addition to the clinical examination, complementary exams that promote better patient monitoring will be used.

The carious process is the result of an imbalance in the cycle of demineralization. When the demineralization is more frequent, dentin matrix degradation occurs, affecting its mechanical and tissue properties (Bertassoni et al., 2011). However, in
the early stages, this process may be reversible (Featherstone, 2004). The reincorporation of minerals in demineralized structures can promote improvements in the mechanical properties of tissues such as enamel and dentin (Lavigne et al., 2018). Thus, it is interesting and recommended to use materials that promote the release of minerals in the environment (Qvist et al., 2010).

The resin-modified glass ionomer cement has proven to be a viable option in preventing the evolution of caries disease (Yengopal & Mickenautsch, 2011). Due to its good adhesion to the tooth, besides the ability to release fluoride, the use of these materials may explain the lower progression of carious lesions (Muñoz-Sandoval et al., 2019). And this prevention can also be observed on the proximal surface of the teeth adjacent to those restored with fluoride-releasing materials (Qvist et al., 2010). Furthermore, it is an effective restorative material for primary teeth, presenting a satisfactory retention rate (Jassal et al., 2018) of 84% success after two years of follow-up (Dermata et al., 2018). Patients from six to eight years of age with a high risk of caries did not present a greater loss of retention of RMGIC when this material was used to prevent occlusal caries (Tagliaferro et al., 2017).

The use of 45S5 glass seems to be able to contribute to the treatment of initial carious lesions, due to the ability to release calcium, and phosphorus ions on the surface of the treated tooth, also, to assist in the medium alkalinization (Bauer et al., 2019). In cases of enamel erosion, the 45S5 bioglass formed a layer of calcium phosphate crystals on the surface that showed loss of hydroxyapatite crystals (Bakry et al., 2014).

The retention is highly dependent on the adaptation of the material to the dental substrate and preservation of adhesion over time, besides being a common reason for the failure of the adhesive processes (Söderholm, 1995). Thus, in the present study, the marginal adaptation and retention of the material will be assessed through microscopy. The analysis by microscopy allows the verification of the presence of gaps, cracks, or even partial or total losses of the material, factors that may justify the occurrence of the evolution of the carious process or not.

Marginal discoloration, marginal adaptation, surface texture, and anatomical shape will be the criteria analyzed. Monitoring of sealed teeth with bioactive and conventional materials will be performed through radiographic bite-wing shots, besides the clinical examination.

This will be one of the preliminary clinical studies evaluating the use of a 45S5-based bioactive material in 3 and 4 ICDAS lesions. The clinical performance of the 45S5 association with the RMGIC is of great importance, as it may be a viable material option for caries lesions with enamel microcavities. With this, it will contribute to the advancement of minimally invasive dentistry, conserving dental structures, as well as possibly making this dental structure more mineralized.

4. Conclusion

The use of bioactive materials has shown satisfactory results in in vitro studies regarding the ability to remineralize the dental structure and to promote the alkalinization of the environment. Thus, our hypothesis is that the use of these materials will be an interesting strategy capable of promoting the prevention of caries disease progression.

Acknowledgments

Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Finance Code 001”.

Foundation for the Support of Research and Scientific and Technological Development of Maranhão – Brazil (FAPEMA)

Foundation for the Support of Research and Scientific and Technological Development of Maranhão – Universal – 00829/19
Note
The percentage of participation of each author in the article was as follows: Ana Carolina Soares Diniz - 35%; Geyna Aguia do Couto – 7.5%; Thais Bordinassi da Silva – 7.5%; José Roberto Bauer – 15%; Leily Macedo Firoozmand – 35%.

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