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Histological and ultrastructural evaluation of remaining dentin after selective caries removal in primary molars: Ex-vivo study

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Highlights

Mineralization characteristics were apparent in dentin after selective removal, demonstrating effective remineralization.

Both low-cost and high-cost glass ionomer cements (GICs) showed satisfactory performance with selective caries removal.

No progression of the carious lesion was observed, confirming the effectiveness of selective removal technique.

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Abstract

Aim: In this study, ultrastructural, chemical, and histological analyses of extracted deciduous teeth were performed to analyze the effect of two different types of glass ionomer cements used in association with selective caries removal in deep lesions of deciduous teeth. **Methods:** A clinical, ex vivo, longitudinal study was conducted. A total of 19 deciduous molars from 9 volunteer patients were used. The teeth were randomly restored with one of two types of glass ionomer cement following the selective caries removal technique. The groups were divided into a control group, which used Fuji IX (GC- Europe, Leuven, Belgium), and a test group, which used Maxxion R (FGM- Joinville, Santa Catarina, Brazil). After exfoliation, the teeth were stored at 4°C and subjected to scanning electron microscopy (SEM), optical microscopy (OM), and energy dispersive spectrometry (EDS). **Results:** EDS analysis revealed that calcium (Ca) and phosphorus (P) were present in 100% of the specimens in both the control and test groups. Fluoride was found in 60% of the control group specimens and 63% of the test group specimens. OM images showed areas of mineralized dentin in both treatment groups. **Conclusions:** Selective caries removal for deep lesions in deciduous teeth was shown to be a safe therapeutic protocol, demonstrating signs of dentin mineralization and control of disease progression.

Keywords: Carious, Dental Caries Removal; Glass Ionomer Cement; Scanning Electron Microscopy

INTRODUCTION

The principles of minimal intervention dentistry represent a break from the classical restorative model and are supported by strong scientific evidence, building new principles for educational and preventive measures and decisions related to clinical interventions.1

In addition, the use of minimally invasive methods can reduce the losses in dental tissue, affected carious tissue, and limit the possibility of pulp exposure.2

In this vein, the selective removal of carious tissue is extremely relevant in clinical practice since this philosophy includes all the requirements that ensure the functionality and longevity of teeth in an oral cavity.3

Studies on the selective removal of carious tissue with outcomes concerning the bacterial count, clinical survival of restorations, radiographic control, disease progression, and dentin hardness have demonstrated the effectiveness and safety of this technique compared to the complete removal of carious dentin.4,5

Likewise, studies involving comparisons of gradual dentin removal techniques, including two sessions with selective removal, have indicated substantial advantages for the selective removal approach.6,7

Considering all these assertions, it is necessary to emphasize the importance of the insertion process when selectively removing carious tissue in both private and public pediatric dental clinics.8

Children will certainly benefit from the reduced risk of pain during the procedure, as also the duration of clinical intervention is reduced. Thus, decreasing the patient's degree of anxiety.9

Considering the gap in the literature regarding the report of the ultrastructural, chemical, and histological characteristics of dentin after selective caries removal, since the majority of studies only report the success of these methods through clinical follow-up using radiographic methods.10–12 Also, the deeper understanding of the stimulated response of dentin, to support this fundamental technique in pediatric dentistry. The aim of this study was analyzing the characteristics of dentin subjected to selective removal of carious tissue and treated with two different types of glass ionomer cement. The null hypothesis adopted in this study was: H01 - the different types of glass ionomer did not change the proprieties of dentin subjected to selective caries removal.

METHODS

This study was an *ex vivo*, longitudinal clinical research project submitted to and approved by the Research Ethics Committee (REC) of the São Leopoldo Mandic Research Center according to protocol number 4,538,773/2021. Also, the study was previously registered in clinicaltrials.gov, receiving the ID NCT02778503. This study adhered to Consolidated Standards of Reporting Trials (CONSORT) guidelines.

The sample size calculation was based on the number of children receiving care at the public health care center in the city of Livramento de Nossa Senhora – BA, Brazil. With this, a confidence level of 95% and a margin of error of 5% were estimated, resulting in a sample size of 29 children. The initial sample group used in this study consisted of 40 deciduous molars, involving 20 from children aged 4 to 9 years. The final sample group consisted of 19 deciduous molars, including 11 molars from children. In the course of the study, 9 children did not attend the control appointments, and as an exclusion criterion, they were excluded from the study, leading to a sample loss of 21 deciduous molars.

The samples were categorized and selected according to the International Classification of Clinical Safety Standards (ICDAS) indices 5 (enamel cavitation with exposure of the underlying dentin involving less than half of the crown of the tooth in question) and 6 (enamel cavitation with exposure of the underlying dentin involving at least half of the crown of the tooth in question).[13]

All the caries lesions were treated by selective removal of carious dentin and immediate posterior restoration using glass ionomer cements (GICs) of different compositions (Table 1), with clinical and radiographic control measures being conducted every three months for a mean period of 36 months.[14]

Therefore, after the selective removal of carious tissue the samples were stratified in two groups, according to the glass ionomer cement used in the treatment. The first group was treated with Fuji IX (GC - Europe, Leuven, Belgium), which is a gold standard GIC with high viscosity and high cost; thus, this was the control group. The second group was the test group, for which the GIC Maxxion R (FGM - Joinville, Santa Catarina, Brazil) was selected for treatment, it is a low viscosity product. This represents a widely used GIC in Brazil, given its relatively low cost.

Table 1. Compositions of the ionomeric restorative cements used in the test

Brand of glass ionomer cement	Composition	
	Powder	Liquid
Fuji IX (GC - Europe, Leuven, Belgium) - High viscosity - High cost	- 95% fluorine–aluminum–silicate glass - 5% polyacrylic acid - Pigments	- 40% polyacrylic acid - 50% distilled water - 10% carboxylate polybasic acid
Maxxion R (FGM - Joinville, Santa Catarina, Brazil) - Low viscosity - Low cost	- Micromized glass ionomer - Iron oxide - Silica - Zirconia - Potassium fluoride - Strontium glass	- Polyacrylic acid - Deionized water

The deciduous teeth subjected to the research were collected immediately after natural shedding. The parents of the children were instructed to keep them in a physiological solution contained in hermetically sealed containers, refrigerated at 4°C. The researcher herself was responsible for collecting these teeth from the children's homes and forwarding them for laboratory procedures. The samples were labelled based on the type of glass ionomer cement (GIC) used as a restorative material, the survival time of the material in the oral cavity and the respective ICDAS index.

Laboratory Procedures

The teeth were sectioned in the mesiodistal direction with a double-sided diamond disc (Odonto Mega, Ribeirão Preto, SP, Brazil). The disc had a thickness of 0.17 mm and a diameter of 22 mm at a rotational speed of 5000–20000 rpm (micromotor 500 – Kavo intramatic, Joinville, SC, Brazil), where the restoration–dentin interface was exposed. Half of the specimens were subjected to scanning electron microscopy (SEM) analysis, and the other half were subjected to optical microscopy (OM) analysis to prepare histological slides.

To prepare bone marrow (BM) slides, the specimens were kept in 10% buffered formalin (fixation-48 hours), decalcified (10% nitric acid solution for 60 days, with replacement every 48 hours), and cut into sagittal sections. The sections were subsequently washed to remove excess acid. In addition, the specimens were dehydrated in an alcohol series (for 1 hour in 80% alcohol, 1 hour in 90% alcohol, 1 hour in absolute alcohol, and another hour in absolute alcohol), diaphanized (for 1 hour in xylene I and 1 hour in xylene II), and paraffinized at the melting point. The samples were cut at 58 °C for 2 hours in a rotary microtome (4 specimens were lost due to fragmentation) and stained (hematoxylin and eosin (HE) and Masson's trichrome). To acquire images for histological analysis, 15 slides were scanned using a slide scanning microscope (Olympus© VS-110, Tokyo, Japan) coupled with a digital camera (XC10 Olympus©, Tokyo, Japan). Japan) and Virtual Slide System 2.5 software (Olympus©, Tokyo, Japan). The analyses of the images generated in 2× and 4× were performed by a histopathologist.

For SEM, the specimens were taken to an oven (Lab-line©, Model 3608-1, Mumbai, India) for drying at 50 °C and fixed in stubs with 8-mm double-sided carbon tape (Electron Microscopy Sciences©, Lansdale, Pennsylvania, USA). Then, the specimens were blasted with gold (Dentom Vacum Desk IV- New Jersey, USA) and electrophotomicrographed by SEM (JEOL-JSM 6390 LV-Scanning Electron Microscope©, JEOL Ltd., Tokyo, Japan). This microscope featured thermionics and a tungsten filament as the electron source. During the analysis, an acceleration voltage of 15 kV was applied, and no filter was used. The images obtained by SEM showed the surface morphologies of the samples, which were evaluated with a secondary electron (SE) detector at magnifications of 100×, 200× and 550×. Subsequently, the samples were bombarded with X-ray beams for qualitative and quantitative microanalyses of the chemical elements present in the dentin through energy dispersive spectrometry (EDS). By conducting EDS analyses, images, graphs and tables of the chemical elements present in each sample and their corresponding concentrations were generated. For data analysis, descriptive statistics were used with the absolute values of EDS data.

RESULTS

SEM analyses of the 19 samples showed that the dentin had irregular edges and grooves of varying sizes, likely due to removal via manual instruments. Fewer dentinal tubules were observed compared to dentin closer to the pulp, with dense intertubular dentin and peritubular dentin present in some dentinal tubules; in addition, some bacteria and deposits present in the mouths of the tubules were observed (residual smear layer and restorative materials) (Figure 1).

In the quantitative and qualitative analyses of the chemical elements present in dentin, calcium (Ca) and phosphorus (P), the majority of which were present in sound dentin, were found in both the control and test groups. Mean values of 19,381 (100%) and 12,970 (100%) for Ca and P, respectively, were observed in the control group and 17,158 (100%) and 12,707 (100%) for Ca and P, respectively, were observed in the test group. The element fluorine (F) was present in 60% of the samples in the control group (915) and 63% of the test group (374). Potassium (K) was observed in 42.1% of the total sample, as was zirconium (26.3%), silicon (73.68%), aluminum (73.68%) and iron (21%), which were present in the composition of the restorative material. Carbon (C), a chemical element present in demineralized dentin, was isolated in 100% of the samples and was present in greater proportions in the test group (7297) than in the control group (2843). This phenomenon reinforced the possibility that the remaining affected dentin would remain intact and was less mineralized than sound dentin (Table 2).

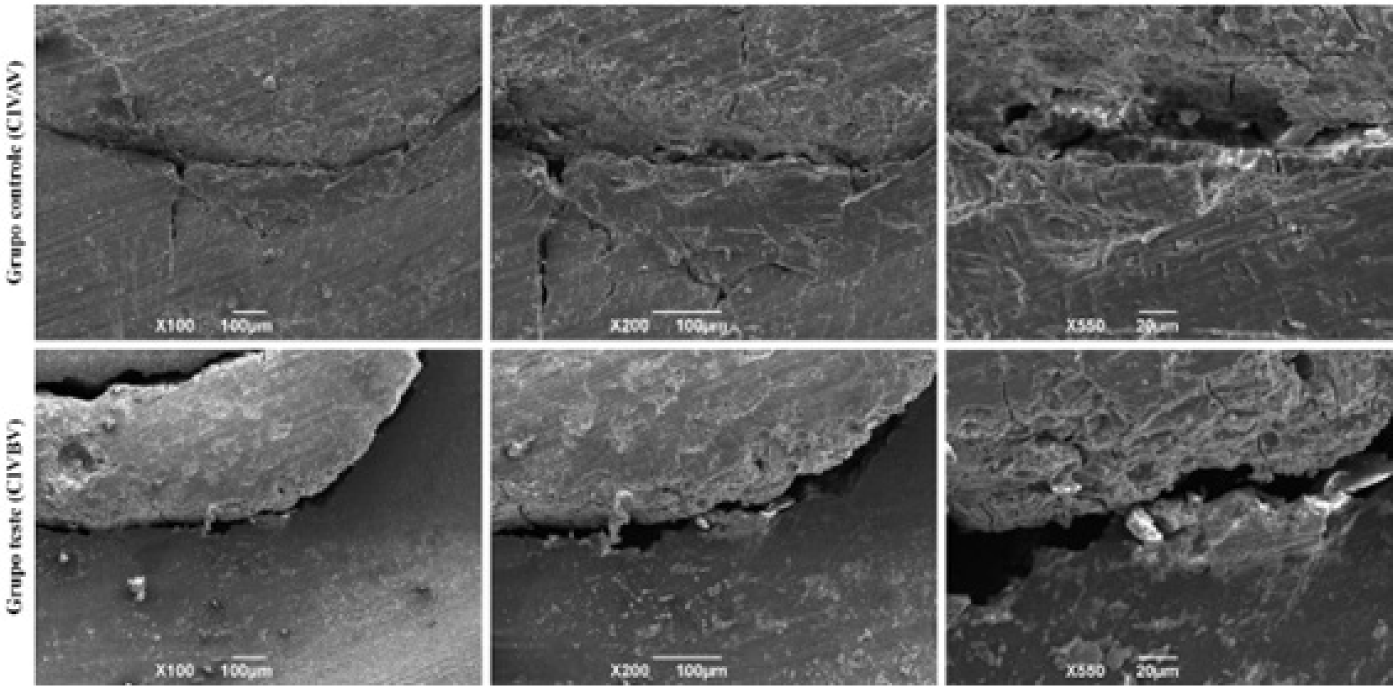

Figure 1. Representative SEM images of the dentin of deciduous molars from both groups

Table 2. Chemical elements present in the affected dentin of the collected sample identified through EDS

Chemical element	Sample number/ % of the total sample		Average Concentration
Potassium (K)	08	42,1	230
Zirconium (Zr)	05	26,3	2251
Iron (Fe)	04	21	26,5
Chlorine (Cl)	09	47,37	345,33
Niobium (Ni)	12	63,1	9440,16
Fluoride (F)	11	57,9	554,5
Yttrium (Y)	04	21	705
Magnesium (Mg)	07	36,8	257,57
Silica (Si)	14	73,68	1964,44
Sodium (Na)	18	94,73	226,6
Oxygen (O)	19	100	2306,0
Carbon (C)	19	100	5745,6
Aluminum (Al)	14	73,68	1999,71
Nitrogen (N)	01 Maxxion R group	5,26	69
Zinc (Zn)	01 Maxxion R group	5,26	820
Barium (Ba)	01 Maxxion R group	5,26	565
Calcium (Ca)	19	100	16.665
Phosphorus (P)	19	100	13.522,8

According to the optical microscopy results, the histological examination revealed dentin with mineralized characteristics and the presence of dentinal tubules (Figure 2). The edges of this dentin were irregular, with projections and slopes of varying sizes, probably due to the removal of caries with manual instruments (dentin curettes). The lesions were stained more intensely than the underlying dentin. In some samples, tertiary dentin was present, reducing the lumen of the pulp chamber.

DISCUSSION

Despite the remarkable decline in dental caries in the global population, a substantial number of individuals are affected by this disease, which challenges researchers, public managers and clinicians in designing interventions and decision-making that can mitigate the sequelae of carious lesions.[15] The construction of contemporary treatment models involves the selective removal of carious tissue, which is a technique included in the concept of minimal intervention dentistry.[16]

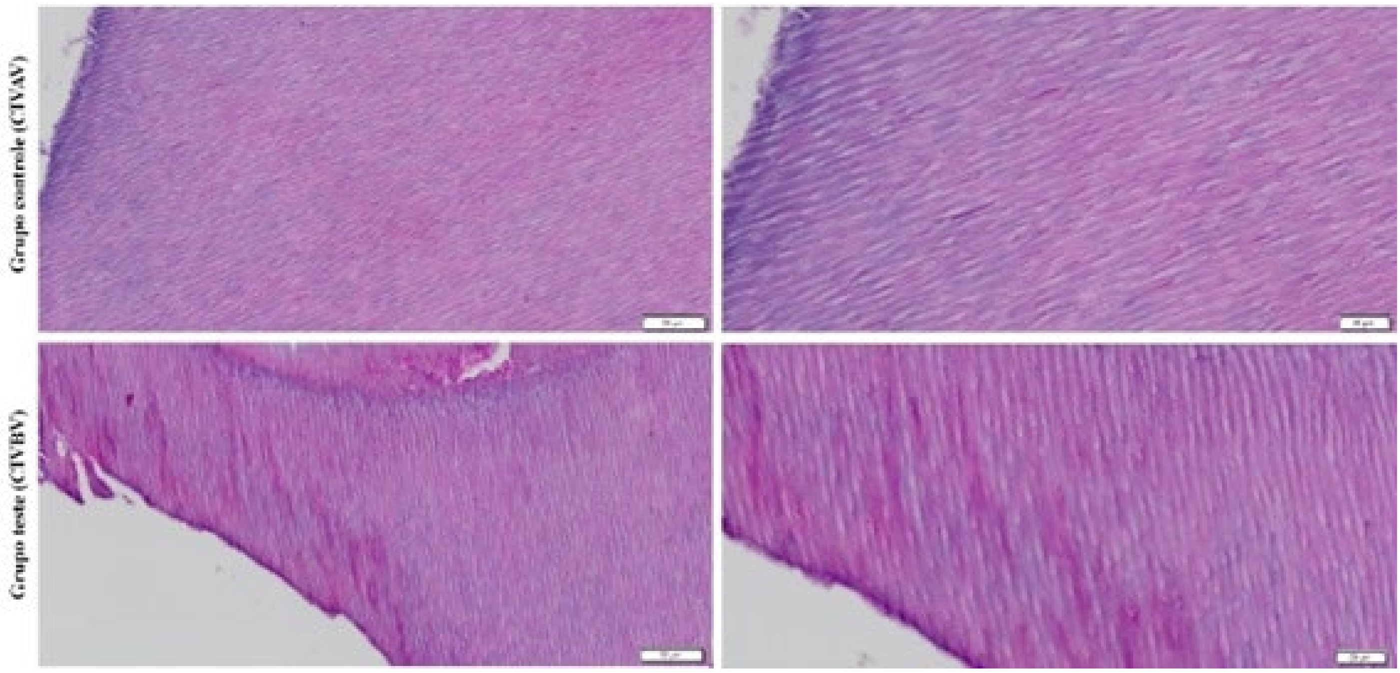

Figure 2. Representative photomicrographs of the dentin of deciduous molars from the control group (GICVAV Fuji IX –GCe) and from the test group (GICBV Maxxion R – FGM)

The selective removal of carious tissue is recognized for preserving a healthy tooth structure, preventing tooth decay and reducing the risk of pulp exposure; these aspects are qualities and virtues that inspired the development of the present study.[17]

In addition, the selective removal of carious tissue allows for a simplification of dental procedures, suppressing caries disease based on the framework of preventive dentistry amd the promotion of oral health.[18] In general, the results of the present study show that dentin has mineralization characteristics according to histological and ultrastructural perspectives. Based on the radiographic data of the randomized clinical study, a hindering of caries development and consequent clinical success is perceptible, taking as a parameter the criteria adopted in the methodology.

Notably, the presence of dentinal tubules and dense intertubular dentin associated with chemical components typical of sound dentin, such as Ca and P, and with trace elements, such as Fe, Al and K, in addition to the chemical components integrating the restorative material, such as F, Si, Zr and Mg; these results are similar to those of another study.[19]

The presence of C, especially in the samples of the test group, is indicative of the possibility of less mineralized dentin than healthy dentin, suggesting that the affected dentin remaining in the pulp wall is not completely mineralized; this result that is consistent with that of another study.[20]

However, dentin stability can be observed, with no signs of progression of the caries lesion, which is similar to the findings of a previous study.[21] This phenomenon can be explained by the ion exchange between the restorative material and dentin, which occurs due to the presence of F, Mg, Si and Zr according to the EDS analysis.

In summary, the results of the present ex vivo study are in line with those of other studies,[22–24] demonstrating that low-cost GICs have substantial flaws in the restorative process, probably because they have low mechanical strengths. In addition, the use of this ionomeric cement standard requires frequent repairs during clinical control; this finding is also observed in this study.[25] Therefore, the null hypothesis was denied.

The clinical reinterventions during the follow-up period of a study, to maintain the integrity of a superficial seal, seem to justify the results presented by SEM and OM. These findings, which, similar to those of other studies,[4,26] suggest that sealing the cavity ensures control of the progression of a carious lesion, considering the reduction in the microbial density of the dentin.The numerically small sample of the present study is a limitation of the study; however, the lack of clinical control and the follow-up time of 3 years surely circumvents this limitation, enhancing the results presented.

An outstanding aspect of the present study is that, in addition to being a milestone in the evolution of the field of dentistry, the selective removal of carious tissue reaffirms new paradigms in decision-making in dental procedures. In addition, this approach allows for operational simplification, economic viability, problem indication, execution of minimally invasive procedures and the ability to resolve the disease in a curative and/or preventive manner.

In principle, all these requirements have been included in this study, and based on them, we suggest their applicability in private clinical activities and in public health systems.

CONCLUSIONS

With this study, it can be concluded that the use of both glass ionomer cements, Fuji IX and Maxxion R, proved to be efficient in association with selective caries removal. Moreover, the ultrastructural, chemical, and histological analyses suggest that the groups show signs of dentin mineralization and control of disease progression. Therefore, the selective removal of caries for deep lesions in deciduous teeth in association with the tested glass ionomer cements showed to be a safe clinical protocol.

REFERENCES

1. BaniHani A, Santamaría RM, Hu S, Maden M, Albadri S. Minimal intervention dentistry for managing carious lesions into dentine in primary teeth: an umbrella review. Eur Arch Pediatr Dent 2022;23:667-693

2. Dawett B, Deery C, Banerjee A, Papaioannou D, Marshman Z. A scoping literature review on minimum intervention dentistry for children with

dental caries. Br Dent J 2022. https://doi.org/10.1038/s41415-022-4038-8

3. Souza TF de, Martins ML, Jural LA, Maciel IP, Magno MB, Coqueiro R da S, et al. Did the use of minimum interventions for caries management change during the COVID-19 pandemic? A cross-sectional study. Caries Res 2023;57:459-469

4. Bitello-Firmino L, Soares VK, Damé-Teixeira N, Parolo CCF, Maltz M. Microbial Load After Selective and Complete Caries Removal in Permanent Molars: A Randomized Clinical Trial. Braz Dent J 2018;29:290–295

5. Jardim JJ, Mestrinho HD, Koppe B, de Paula LM, Alves LS, Yamaguti PM, et al. Restorations after selective caries removal: 5-Year randomized trial. J Dent 2020;99:103416

6. Lim ZE, Duncan HF, Moorthy A, McReynolds D. Minimally invasive selective caries removal: a clinical guide. Br Dent J 2023;234:233–240

7. Ricketts D, Lamont T, Innes NP, Kidd E, Clarkson JE. Operative caries management in adults and children. Cochrane Database of Systematic Reviews. 2013;28:CD003808

8. BaniHani A, Deery C, Toumba J, Munyombwe T, Duggal M. The impact of dental caries and its treatment by conventional or biological approaches on the oral health-related quality of life of children and carers. Int J Paediatr Dent 2018;28:266–276

9. Aravinda VSS, Kandregula CR, Y M, M MK, BS N, Sree BM. Minimal Extension for Maximal Results - Review on Minimal Intervention Paediatric Dentistry. Acta Sci Dent Sci 2022;1:102–114

10. Stafuzza TC, Vitor LLR, Rios D, Cruvinel Silva T, Machado MAAM, Oliveira TM. Clinical and Radiographic Success of Selective Caries Removal to Firm Dentin in Primary Teeth: 18-Month Follow-Up. Case Rep Dent 2018;2018

11. Casagrande L, Seminario AT, Correa MB, Werle SB, Maltz M, Demarco FF, et al. Longevity and associated risk factors in adhesive restorations of young permanent teeth after complete and selective caries removal: a retrospective study. Clin Oral Investig 2017;21:847–855

12. Ali AH, Koller G, Foschi F, Andiappan M, Bruce KD, Banerjee A, et al. Self-Limiting versus onventional Caries Removal: A Randomized Clinical Trial. J Dent Res 2018;97:1207–1213

13. Ismail AI, Sohn W, Tellez M, Amaya A, Sen A, Hasson H, et al. The International Caries Detection and Assessment System (ICDAS): an integrated system for measuring dental caries. Community Dent Oral Epidemiol 2007;35:170–178

14. Schwendicke F, Frencken JE, Bjørndal L, Maltz M, Manton DJ, Ricketts D, et al. Managing Carious Lesions. Adv Dent Res 2016;28:58–67

15. Jain N, Dutt U, Radenkov I, Jain S. WHO's global oral health status report 2022: Actions, discussion and implementation. Oral Diseases. John Wiley and Sons Inc; 2023.

16. Innes NPT, Chu CH, Fontana M, Lo ECM, Thomson WM, Uribe S, et al. A Century of Change towards Prevention and Minimal Intervention in Cariology. J Dent Res 2019;98:611–617

17. Leal SC, Dame-Teixeira N, Barbosa CDB, Kominami PAA, Raposo F, Nakagawa EMT, et al. Minimum intervention oral care: defining the future of caries management. Braz Oral Res 2022;36:e135

18. Chiu HHC, Lam PPY, Yiu CKY. The Impact of Minimal Intervention Dentistry on Patient-Reported and Observation-Based Outcomes in the Pediatric Population: A Systematic Review and Meta-Analysis. Healthcare 2023;11:2241

19. Sarna-Boś K, Skic K, Boguta P, Adamczuk A, Vodanovic M, Chałas R. Elemental mapping of human teeth enamel, dentine and cementum in view of their microstructure. Micron 2023;172:103485

20. Zain S, Davis GR, Hill R, Anderson P, Baysan A. Mineral exchange within restorative materials following incomplete carious lesion removal using 3D non-destructive XMT subtraction methodology. J Dent 2020;99:103389

21. Arnold WH, Konopka S, Kriwalsky MS, Gaengler P. Morphological analysis and chemical content of natural dentin carious lesion zones. Annals of Anatomy - Anatomischer Anzeiger. 2003;185:419–424

22. Calvo AFB, Kicuti A, Tedesco TK, Braga MM, Raggio DP. Evaluation of the relationship between the cost and properties of glass ionomer cements

indicated for atraumatic restorative treatment. Braz Oral Res 2016;30: S1806-83242016000100201

23. Rocha MFED, Fujimaki M, Pascotto RC, Mendes LLC, Lacerda CM, Pereira OC, et al. Survival analysis of ART restorations in primary molars of preschool children: 1 year follow-up. Rev Odontol UNESP 2018;47:112–118

24. Olegário IC, Pacheco AL de B, de Araújo MP, Ladewig N de M, Bonifácio CC, Imparato JCP, et al. Low-cost GICs reduce survival rate in occlusal ART restorations in primary molars after one year: A RCT. J Dent 2017;57:45–50

25. Moura MS, Sousa GP, Brito MHSF, Silva MCC, Lima MDM, Moura LFAD, et al. Does low-cost GIC have the same survival rate as high-viscosity GIC in atraumatic restorative treatments? A RCT. Braz Oral Res 2019;33: e125

26. Singhal D, Acharya S, Thakur A. Microbiological analysis after complete or partial removal of carious dentin using two different techniques in primary teeth: A randomized clinical trial. Dent Res J (Isfahan). 2016;13:30-40

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Declarations

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