Clinical Oral Investigations (2022) 26:1889–1902
https://doi.org/10.1007/s00784-021-04166-2

ORIGINAL ARTICLE

Two-year randomized clinical trial of different restorative techniques in non-carious cervical lesions and MMP activity in gingival crevicular fluid

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Received: 14 December 2020 / Accepted: 24 August 2021 / Published online: 10 September 2021

Abstract

Objectives To evaluate different restorative techniques for non-carious cervical lesions (NCCLs) and the activity of matrix metalloproteinases (MMPs) in gingival crevicular fluid.

Materials and methods Two hundred restorations were performed in 50 patients using resin composite restorative system without (I) and with selective enamel conditioning (II) and resin-modified glass-ionomer cement without (III) and with EDTA pretreatment (IV). Gingival crevicular fluid samples were collected in 15 patients. Restorations were evaluated using USPHS criteria at baseline and after 2 years. Percentages of MMP activity were assessed by zymography as a surrogate outcome. Equality tests of two proportions, logistic regression analysis, survival analysis, ANOVA repeated measures, and Fisher tests were used.

Results No differences in clinical performance were found among groups. Group I had lower retention at 2 years than at baseline. Decreased alpha scores for marginal integrity and marginal discoloration were observed for all groups after 2 years. MMP-2 decreased after 1 year, and its activity increased back to the initial level after 2 years, mainly for groups I, II, and III. MMP-9 increased after 1 year, and it was reduced to the initial level after 2 years, mainly for group I.

Conclusions All restorative techniques performed similarly in NCCLs after 2 years with initial marginal defect alterations. MMP-2 reestablished its initial levels after 2 years, and MMP-9 had few alterations over time in crevicular fluid.

Clinical relevance

The different restorative techniques are equally successful in NCCLs after 2 years of clinical functioning and have similar effects on MMPs present in crevicular fluid.

Keywords Clinical trial · Gingival crevicular fluid · Glass-ionomer cement · Matrix metalloproteinases · Non-carious cervical lesions · Resin composite

Introduction

Non-carious cervical lesions (NCCLs) are characterized by loss of dental tissue near the cementum-enamel junction, occurring predominantly in premolars, and having multifactorial etiology with an overall prevalence of 46.7% [1]. Recurrent restoration failures of NCCLs occur due to hypermineralized dentin and denatured collagen, which is not the ideal combination for a bonding substrate [2]. Moreover, unbalanced occlusal forces and patient habits contribute to that scenario [3].

NCCL restoration follow-ups have been the purpose of several clinical studies that evaluate the performance of various types of restorative materials, being resin composite and/

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or resin-modified glass-ionomer cement the most preferred restorative materials for NCCLs [4, 5]. For resin composite, different adhesive systems may be used [6]. An option may be multimode or universal adhesive systems due to the chemical interaction of hydroxyapatite with bifunctional acid monomer, such as the 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) [7]. This adhesive system can be used with or without prior treatment of the dental substrate with phosphoric acid [7–10]. In this context, the activation of MMP-2 and MMP-9 are related to collagen degradation after acid conditioning [14], since these enzymes are activated by low pH [11]. Glass-ionomer cements exhibit chemical adhesion to the dental substrate, which makes them suitable for NCCL restorations [4–6]. Fagundes et al. [12] demonstrated in an in vitro study that dentin pretreatment with ethylenediaminetetraacetic acid (EDTA) prior to the insertion of resin-modified glass-ionomer cements is able to increase bond strength values. This may be justified since EDTA dissolves the dentin mineral phase without altering the fibril structure, allowing better adhesion of the glass-ionomer cement to the intrafibrillar mineral content [12], being also considered a possible agent to inactivate endogenous matrix metalloproteinases (MMPs) [13]. Inhibition of the collagenolytic activity and the use of cross-linking agents are the main strategies to increase the resistance of the hybrid layer to enzymatic degradation [14].

MMPs are present in radicular dentine and gingival crevicular fluid [15–19], and their activation in dentine could be associated with the presence of NCCLs, mainly when the cause of these lesions is erosion [18, 19]. Moreover, materials used for dental restoration can induce biochemical changes in the oral cavity, since a suppressive effect of metal alloy components on MMP-2 production was found in oral fluid of patients with metal restorations [20]. Also, NCCLs are in areas where gingival fluid is released, allowing for the relationship between MMPs in this oral fluid and cervical lesions to occur.

This study is innovative in terms of clinically using EDTA as a pretreatment of ionomer and semi-quantitating MMP levels in gingival fluid of patients with NCCLs. This study evaluated the clinical performance of NCCL restorations using two adhesive restorative materials and two forms of pretreatment of dental substrates for each material. In addition, the activity of MMPs in gingival fluid near NCCLs was determined from part of the participants as a surrogate outcome. The following hypotheses were established: (I) there is no difference in clinical performance between the four restorative techniques after 2 years and between the evaluation periods when the same restorative technique is evaluated; (II) there is no difference in the MMP activities between the four restorative techniques after 2 years and between the evaluation periods when the same restorative technique is evaluated.

Materials and methods

This was a prospective randomized study that evaluated resin composite and resin-modified glass-ionomer cement in NCCLs using different dental substrate treatments. The following groups were established: (I) application of the multimode adhesive system (Single Bond Universal, 3 M ESPE, St. Paul, USA) in the self-etching technique + nanoparticulate resin composite (Z350XT, 3 M ESPE, St. Paul, USA); (II) application of the multimode adhesive system (Single Bond Universal, 3 M ESPE, St. Paul, USA) with pretreatment of the enamel with phosphoric acid 37% for 15 s + nanoparticulate resin composite (Z350XT, 3 M ESPE, St. Paul, USA); (III) insertion of resin-modified glass-ionomer cement (Vitremer, 3 M ESPE, St. Paul, USA), according to manufacturer's instructions; and (IV) application of 0.1 M EDTA solution for 60 s prior to insertion of resin-modified glass-ionomer cement (Vitremer, 3 M ESPE, St. Paul, USA). Characteristics of restorative materials can be seen in Table 1.

After approval by the local Ethics Committee (# 668.963) and trial registration (RBR-655c3z), patients were selected as described below.

Sample size calculation and selection of patients

The sample size calculation was performed considering the retention rate of 94% of the predecessor of this multimode adhesive from the same manufacturer in the follow-up at 18 to 24 months [21, 22]. Using an alpha of 0.05, a power of 80%, and a two-sided test, the minimal sample size was 50 restorations in each group in order to detect a difference of 20% among groups.

Fifty volunteers were selected for this study from the local undergraduate clinic, requiring the presence of at least four NCCLs per patient, regardless of their location in the dental arch and dominant etiology. The inclusion criteria were good health, no clinical history of allergies to dental products and medicines, and adequate oral hygiene, that is, without active periodontal disease. Exclusion criteria were pregnancy or nursing, presence of active carious lesions, usage of desensitizing agents or fluorine, undergoing orthodontic treatment, and presence of severe bruxism with more than 50% wear. The oral conditions were analyzed using the DMFT, visible plaque (VPI), and gingival bleeding (GBI) indexes.

Selection of teeth

The teeth used in this study had NCCLs with depths equal to or greater than 1.0 mm and with a maximum depth of

Table 1 The technical characteristics of the materials investigated

Materials	Manufactures	Compositions
Universal Single Bond	3 M ESPE, St Paul, MN, USA	MDP phosphate monomer, dimethacrylate resins, Vitrebond copolymer, filler, ethanol, water, initiators, silane
Filtek Z350 XT		Bis-GMA, UDMA, TEGDMA, and bis-EMA; non-agglomerated/non-aggregated silica filler (20 nm), non-agglomerated/non-aggregated zirconia filler (4 to 11 nm), and aggregated zirconia/silica cluster filler (comprised of 20 nm silica and 4 to 11 nm zirconia particles)
Vitremer		Powder/liquid: methacrylate polyacids, water, aluminum fluorosilicate glass, methacrylate monomers, and initiators
Finishing Gloss		Copolymers, HEMA, ethanol, and initiators
0.1 mol/l ethylenediaminetetraacetic acid	Aphoticario, Araçatuba, SP, Brazil	Ethylene diamine tetracetic disodium acid, water, sodium hydroxide, and deionized water

MDP methacryloyloxydecyl dihydrogen phosphate, *Bis-GMA* bisphenol A-glycidyl methacrylate, *UDMA* urethane-dimethacrylate, *TEGDMA* triethylene glycol dimethacrylate, *bis-EMA* ethoxylated bisphenol A-dimethacrylate, *HEMA* 2-hydroxyethyl methacrylate

3.0 mm, with at least 50% of the margins without enamel. The characteristic of the dentine according to the degree of sclerosis was evaluated as follows: (1) no evident sclerosis, the dentine is opaque in appearance, being yellow or whitish in color discoloration; (2) more than one but less than 50% between categories 1 and 4; (3) less than 4, but more than 50% between categories 1 and 4; and (4) significant sclerosis present, the dentine has a vitreous, dark yellow, or even discolored (brown) appearance and significant translucency or transparency is evident in dentine [23]. NCCLs are also categorized according to the internal cavity as: 45–90°, 90–120°, or > 120°. Details from these procedures can be seen in our previous paper [23].

Sample collection for MMP analysis

Fifteen patients were randomly chosen for this analysis. Gingival fluid was obtained according to the protocol proposed by Sorsa et al. [24]. Buccal and proximal surfaces of the four teeth with NCCLs were cleaned with a rubber cup, pumice stone, and water to eliminate the presence of plaque. After relative isolation and before the restorative procedures, teeth were dried with air, and samples were obtained from 3 sites (mesial, distal, and buccal surfaces). For this purpose, absorbent paper cones were gently inserted into the insertion regions, approximately 1 mm inside the gingival sulcus, without causing bleeding. The sample collection time was 30 s. For each tooth, gingival crevicular samples were stored in Eppendorf microcentrifuge tubes containing 300 µl buffer, 50 mM Tris, 0.2 M NaCl, 10 mM $CaCl_2$, and pH 7.4 and frozen at − 80 °C.

The samples were then centrifuged (Jouan Centrifuge, Paris, FR) at 13,400 rpm for 20 min. The supernatant was separated and stored in new microcentrifuge tubes, discarding the precipitate. The supernatant was put in water bath (CSE Lab-Line Instruments, IL, USA, serial Nº. 0069, cat # 13,000) at 50 °C for 30 min. After 30 min, the samples were slightly shaken. After 30 min, samples were frozen again before being submitted to zymographic analysis. Sample collection for MMP analysis was also performed after 1 and 2 years.

Gelatin zymography

Total protein concentrations were determined by the Bradford assay. Purified human MMP-2 (Calbiochem, Millipore Corp., Billerica, MA, USA) and MMP-9 (Abcam, Cambridge, MA, USA) were used as positive control. The purified enzymes and the samples of different strategic group (5 µg) were diluted in Laemmli sample buffer at 4:1 ratio and electrophorized under non-reduced conditions on 10% sodium dodecyl sulfate–polyacrylamide gel (SDS-PAGE) containing 1 mg/ml gelatin from porcine skin (Sigma Chemical, St. Louis, MO, USA). Prestained low-range molecular-weight SDS-PAGE standards (Bio-Rad Laboratories, Hercules, CA, USA) were used as molecular-weight markers (MWM). After electrophoresis, the gels were washed for 1 h in 2% Triton X-100 and incubated for 18 h at 37 °C in activation buffer (50 mM Tris HCl, 5 mM $CaCl_2$, 1 µM $ZnCl_2$, 0.02% (w/v) NaN_3, pH 7.4). Negative control gel was incubated with the same buffer solution containing 2 mM of 1,10-phenanthroline (specific MMP inhibitor). After incubation, gels were stained in 0.1% Coomassie Brilliant Blue R-25 for 30 min and distained (30% methanol, 10% acetic acid diluted in distilled water). The gelatinolytic activity could be detected as clear bands. The gels were scanned (Imagescanner, Amersham Biosciences, Uppsala, Sweden) and bands evaluated by densitometry (arbitrary units) using the software ImageJ (Research Services Branch, National Institutes of Health, Bethesda, MA, USA). Experiments were performed in triplicate, and the results were expressed as percentage according to MMP activity.

Randomization and restorative procedures

After color selection, other procedures such as initial photography, local anesthesia when necessary, and isolation with cotton rolls and suction were performed. NCCLs were not submitted to any type of cavity preparation. The restorative procedures were performed by two operators (postgraduate students) trained by a faculty member specialized in restorative dentistry. For calibration, each operator performed two restorations for each group in patients not selected for the research. One person not directly involved with the study prepared opaque sealed envelopes with each group identified as I–IV, which were employed to conceal the randomization sequence. The treatment was allocated regarding the group, where the first tooth restored was raffled for one treatment, while the next teeth were assigned to the other treatments, according to the split-mouth design. It is important to emphasize that each operator performed the same number of restorative treatments, both for control and test groups.

The patients received a restoration from each group, as follows: (I) multimode adhesive system (Universal Single Bond) was applied under agitation for 20 s, a light air jet was applied, and the adhesive system was photoactivated (Radii-cal, 1200 mW/cm^2, SDI, Victoria, Australia); (II) conditioning with 37% phosphoric acid (Total Etch – Ivoclar Vivadent, Liechtenstein) was applied in the enamel margin for 15 s, washed with a water jet, and slightly dried, and then the adhesive system was applied as described for group I; both groups I and II were restored with a nanoparticulated resin composite (Z350XT); (III) following manufacturer's instructions, the primer was applied and photocured before resin-modified glass-ionomer cement (Vitremer); (IV) 0.1 M EDTA was applied for 60 s with a brush and washed with water, and the surface was dried and restored with resin-modified glass-ionomer cement without primer application; for both groups III and IV, the surface protection was applied and photocured (Finishing Gloss, 3 M ESPE St. Paul, USA). The complete procedures are described in our previous study [23].

Clinical evaluation

The evaluation was performed by direct clinical observation through visual and tactile inspection using a flat mouth mirror, periodontal probe, and illumination by dental reflector. Data from indexes (DMFT, VPI, and GBI) and MMP analysis were again collected at 1- and 2-year evaluations. Restorations were evaluated immediately (baseline), after 1 and 2 years using USPHS (Table 2) by two calibrated evaluators, and when there was disagreement, a common agreement was reached.

Statistical analysis

Kappa test was used to evaluate agreement between examiners, while Friedman's nonparametric test was used to compare the four groups regarding DMFT, VPI, and GBI. Multiple logistic regression analysis was performed to verify the influence of some factors (tooth type, degree of sclerosis, and internal angles) in the survival of the restoration with the retention-dependent variable. The chi-square test was used to verify the distribution of the sample in relation to the initial characteristics and for the comparison between the four groups at each time. The Cochran test was used for the comparison between the four periods of evaluation within each group. The retention criterion was used to compare the survival rate of the four groups by the Wilcoxon (Gehan) test.

MMP percentages were $\log_{10}$ transformed and subjected to two-way ANOVA repeated measures and Fisher test for multiple comparisons to analyze differences between groups and periods for both types of MMP-2 and MMP-9 present in the gingival crevicular fluid. A significance level of 5% was adopted. Statistical procedures were performed in SPSS version 13 for data of clinical performance and in SigmaPlot version 12 for MMP results.

Results

The sample consisted of 34 (68%) men and 16 (32%) women with an average age of 61.8 years. Forty-eight patients returned after 2 years. The number of patient losses and restorations in each evaluation is presented in Fig. 1, and each reason for patient loss was described in the respective follow-up, according to the Consolidated Standards of Reporting Trials (CONSORT).

The Kappa test showed an excellent degree of agreement between examiners: baseline = 0.993, 1 year = 0.994, and 2 years = 0.991. The initial characteristics (DMFT, VPI, GBI) referring to 15 (MMPs collected) and 48 patients are presented in Table 3. The initial characteristics of 200 NCCLs can be seen in our previous study [23], and no statistical difference in the distribution of tooth type, degree of sclerosis, and internal angles of the cavities among the four groups was found. The DMFT index increased over time, and both VPI and GBI decreased after 1 year, with a small increase after 2 years. None of the initial characteristics of the NCCLs influenced the retention of restorations after 1 and 2 years according to the multivariate logistic regression test ($p \geq 0.05$, Table 4).

Data from the clinical evaluation of all restorations following the USPHS criteria can be seen in Table 5. Lower marginal discoloration was found for group IV when compared to group I at 1 year ($p \leq 0.05$). Group I had lower

Table 2 Modified USPHS criteria rating system for clinical evaluation of the restorations

Retention
Alfa (A): presence of the restoration
Charlie (C): partial or total absence of restoration
Marginal integrity
Alfa (A): there is no visual evidence of marginal fracture, and the dental probe's tip is not trapped in the tooth/restoration interface
Bravo (B): there is visible and tactile evidence of the presence of cleft, but the dentin and/or base are not exposed nor the restoration presents mobility
Charlie (C): the dental probe penetrates the tooth/restoration interface, presenting exposed dentin and/or base, but the restoration is not mobile, fractured, or lost
Marginal discoloration
Alfa (A): there is no visual evidence of marginal discoloration at the tooth/restoration interface
Bravo (B): there is visual evidence of marginal discoloration at the tooth/restoration interface, which can be removed with polishing
Charlie (C): there is visual evidence of deep marginal discoloration at the tooth/restoration interface, which cannot be removed with polishing
Surface texture
Alfa (A): smooth and shiny, similar to enamel
Bravo (B): slightly rough
Charlie (C): high roughness, not reflective
Wear
Alfa (A): no wear, continuous interface
Bravo (B): discontinuous interface, no exposed dentin
Charlie (C): discontinuous interface, exposed dentin
Secondary caries
Alfa (A): there is no visual evidence of tooth decay at the tooth/restoration interface
Charlie (C): there is visual evidence of tooth decay at the tooth/restoration interface
Anatomical form
Alfa (A): the restoration presents continuity with the anatomical form of the existing tooth
Bravo (B): the restoration has a slight over-contour or sub-contour
Charlie (C): there is loss of restorative material leading to exposure of dentin and/or base
Surface staining
Alfa (A): absent
Bravo (B): present
Charlie (C): severe staining
Color
Alfa (A): non-apparent interface with the tooth
Bravo (B): subtle visualization between tooth and restoration
Charlie (C): clear visualization between tooth and restoration
Gingival tissue
Alfa (A): without inflammation
Bravo (B): mild inflammation
Charlie (C): severe inflammation

retention at 2 years compared to baseline period ($p \leq 0.05$). Four restorations of group I and one of other groups lost their retention at 2 years. Decreased alpha scores for marginal integrity were observed for group I comparing 1 year with baseline; this difference was found only after 2 years for other groups ($p \leq 0.05$). However, increased bravo scores for marginal discoloration were observed for groups I and II comparing 1 year with baseline; this difference was found only after 2 years for groups III and IV ($p \leq 0.05$). There were differences for surface texture of groups II and IV comparing 2 years with baseline ($p \leq 0.05$). Photographs of the evaluated restorations can be seen in Figs. 2, 3, 4, and 5. No statistically significant differences for cumulative survival rate were observed, as shown in Table 6 ($p \geq 0.05$).

No patient recruited for MMP analysis dropped out, and all MMP data can be seen in Graphics 1 and 2. The percentages were transformed to $\log_{10}$ to decrease the inter-subjects' variability. A difference between groups was found only for group I differing from other groups at 12 months ($p \leq 0.05$) for MMP-9. MMP-2 decreased between baseline and 1 year; and after 2 years, the expression of this MMP increased to the initial level, with statistical differences for groups I, II, and III ($p \leq 0.05$). MMP-9 statistically increased between

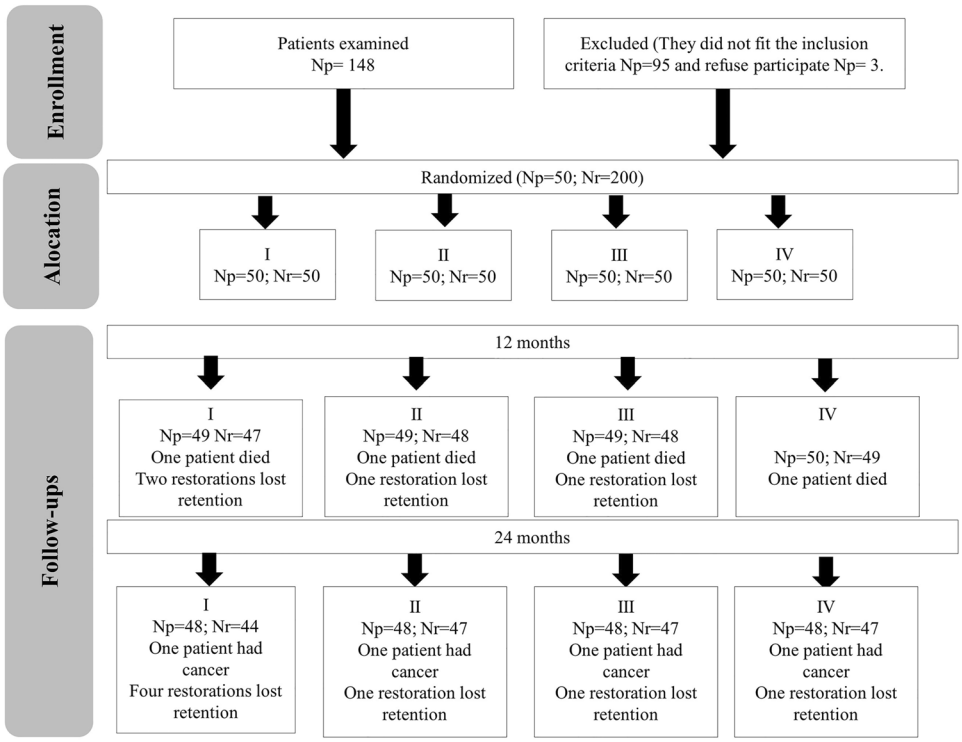


Fig. 1 Consort flowchart

baseline and 12 months and was reduced to the initial level after 2 years for group I ($p \leq 0.05$).

Discussion

In this study, the satisfactory performance of all techniques evaluated over the 2 years shows that the restorative materials used, when applied correctly, are effective and have clinical durability. Then, the evaluation period was not enough to detect differences between the four restorative strategies. However, it was noted that four restorations from the group without selective enamel conditioning lost retention.

Table 3 Mean and standard deviation (SD) of patients' indexes

Characteristics	15 patients		48 patients	
	Mean	SD	Mean	SD
Baseline DMFT	17.7	5.2	18.0	4.9
12 months DMFT	21.1	3.3	21.0	3.4
24 months DMFT	22.0	4.0	21.4	3.5
Baseline VPI	44.6	28.0	41.2	24.9
12 months VPI	32.0	22.3	49.8	25.9
24 months VPI	35.0	12.0	44.0	22.0
Baseline GBI	4.3	9.1	13.5	16.4
12 months GBI	1.6	5.2	3.7	6.9
24 months GBI	2.0	6.0	4.3	9.8

The increase in the DMFT index occurred due to the fact that the patients received all the necessary complementary dental treatments. There was an improvement in the VPI and GBI indexes after 1 year, with a slight increase after 2 years. This observation is due to the fact that patients were motivated during clinical evaluations in relation to oral hygiene instructions. It is also noted that between 1 and 2 years, most patients had already finished their complementary dental treatment, without constant supervision by dentists, leading to this minimal increase in rates.

The logistic regression test showed that the initial characteristics of the NCCLs did not influence the retention of restorations. Tooth location influenced the retention rates of NCCL restorations, and occlusal force loading induces tensile or compressive strain at the occlusal and gingival sites of NCCLs; then, the presence of wear facets is a risk factor for the retention of these restorations [3, 25]. All restorative procedures were performed using isolation with

Table 4 Influence of initial NCCL characteristics on the restoration retention after 2 years (multivariate logistic regression)

Initial characteristics	Odds ratio	SE	Wald	*p*-value
Degree of sclerosis	66.927	5.789	0.527	0.468
Internal angles	423.228	6.159	0.964	0.326
High	0.004	6.115	0.846	0.358
Width	0.001	7.911	0.737	0.391
Deep	1175.445	5.357	1.742	0.187

Table 5 Results in numbers of restorations per group according to each criterion at baseline, 1 and 2 years after the procedure

Periods	Groups	Scores	Retention	Marginal integrity	Marginal discoloration	Surface texture	Wear	Secondary caries	Anatomical form	Surface staining	Color	Gingival tissue
Baseline	***I***	***A/B***	50/-^{Aa}	49/1^{Aa}	50/-^{Aa}	50/-^{Aa}	50/-^{Aa}	50/-^{Aa}	49/1^{Aa}	50/-^{Aa}	36/14^{Aa}	47/3^{Aa}
		C	0	0	0	0	0	0	0	0	0	0
	II	***A/B***	50/-^{Aa}	49/1^{Aa}	50/-^{Aa}	50/-^{Aa}	50/-^{Aa}	50/-^{Aa}	50/-^{Aa}	50/-^{Aa}	41/9^{Aa}	47/3^{Aa}
		C	0	0	0	0	0	0	0	0	0	0
	III	***A/B***	50/-^{Aa}	50/-^{Aa}	50/-^{Aa}	49/1^{Aa}	50/-^{Aa}	50/-^{Aa}	47/3^{Aa}	50/-^{Aa}	37/13^{Aa}	47/3^{Aa}
		C	0	0	0	0	0	0	0	0	0	0
	IV	***A/B***	50/-^{Aa}	48/2^{Aa}	50/-^{Aa}	50/0^{Aa}	50/-^{Aa}	50/-^{Aa}	48/2^{Aa}	50/-^{Aa}	39/11^{Aa}	44/6^{Aa}
		C	0	0	0	0	0	0	0	0	0	0
1 year	***I***	***A/B***	47/-^{Aab}	38/9^{Ab}	41/6^{Ab}	45/2^{Aa}	47/-^{Aa}	47/-^{Aa}	46/1^{Aa}	45/2^{Aa}	36/11^{Aa}	46/1^{Aa}
		C	2	0	0	0	0	0	0	0	0	0
	II	***A/B***	48/-^{Aa}	43/5^{Aab}	43/5^{ABb}	43/5^{Ab}	48/-^{Aa}	48/-^{Aa}	48/-^{Aa}	47/1^{Aa}	38/10^{Aa}	48/-^{Aa}
		C	1	0	0	0	0	0	0	0	0	0
	III	***A/B***	48/-^{Aa}	44/4^{Aab}	45/3^{ABab}	47/1^{Aa}	48/-^{Aa}	48/-^{Aa}	46/2^{Aa}	48/-^{Aa}	33/15^{Aa}	48/-^{Aa}
		C	1	0	0	0	0	0	0	0	0	0
	IV	***A/B***	49/-^{Aa}	44/5^{Aa}	48/1^{Ba}	47/2^{Aab}	49/-^{Aa}	49/-^{Aa}	46/3^{Aa}	49/0^{Aa}	38/11^{Aa}	49/-^{Ab}
		C	0	0	0	0	0	0	0	0	0	0
s	***I***	***A/B***	44/-^{Ab}	35/9^{Ab}	35/9^{Ab}	43/1^{Aa}	44/-^{Aa}	44/-^{Aa}	43/1^{Aa}	43/1^{Aa}	36/8^{Aa}	43/1^{Aa}
		C	4	0	0	0	0	0	0	0	0	0
	II	***A/B***	47/-^{Aa}	40/7^{Ab}	37/10^{Ab}	43/4^{Ab}	46/1^{Aa}	47/-^{Aa}	47/-^{Aa}	45/2^{Aa}	38/9^{Aa}	47/-^{Aa}
		C	1	0	0	0	0	0	0	0	0	0
	III	***A/B***	47/-^{Aa}	38/8^{Ab}	42/5^{Ab}	43/4^{Aab}	46/1^{Aa}	47/-^{Aa}	45/2^{Aa}	46/1^{Aa}	33/14^{Aa}	47/-^{Aa}
		C	1	1	0	0	0	0	0	0	0	0
	IV	***A/B***	47/-^{Aa}	37/10^{Ab}	41/6^{Ab}	42/5^{Ab}	47/-^{Aa}	47/-^{Aa}	44/3^{Aa}	47/-^{Aa}	35/12^{Aa}	47/-^{Ab}
		C	1	0	0	0	0	0	0	0	0	0

Distinct uppercase letters compare groups at the same evaluation; distinct lowercase letters compare a same group over time

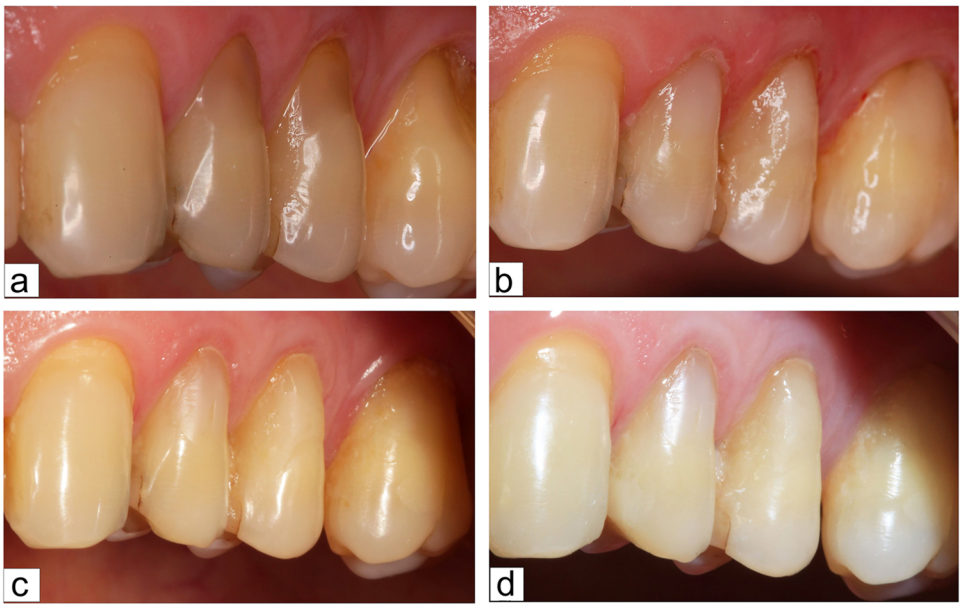


Fig. 2 Tooth 24 from group I and tooth 25 from group III, considered successful over time (alpha score for all criteria): **a** initial NCCL, **b** baseline, **c** after 1 year, and **d** after 2 years

cotton rolls and suction. A number of authors concluded that the usage of rubber dam in NCCL restoration procedures may have little or no effect on the survival rates of the restorations compared to cotton rolls after 12 and 18 months, but it should be emphasized that those evidences are very uncertain [26]. In future clinical evaluations of the present study, it will probably be possible to identify which factors will be influenced in the retention of restorations.

The literature presents several clinical studies that evaluate NCCL restorations; however, despite the lack of a standard clinical trial protocol, some systematic reviews and meta-analyses have suggested more well-designed clinical trials [4–6, 27]. In general, chemical bonding is important for the quality and durability of adhesion in NCCLs, with the best results in the clinical retention efficacy obtained by glass-ionomer cements and the milder categories of self-etching adhesive systems [4–6, 27], but a higher roughness was observed in the ionomer when compared to resin composite [5]. A systematic review of selective enamel conditioning for self-etching adhesives in NCCLs, including studies from 1 to 5 years of clinical follow-up, concludes that selective enamel conditioning before applying this type of adhesives can improve the clinical performance of cervical composite resin restorations [27]. In addition, these authors revealed lower loss of retention with selective enamel conditioning occurring after 3 years of follow-up [27].

Selective etching application modes of multimode adhesives also tended to provide better clinical outcomes

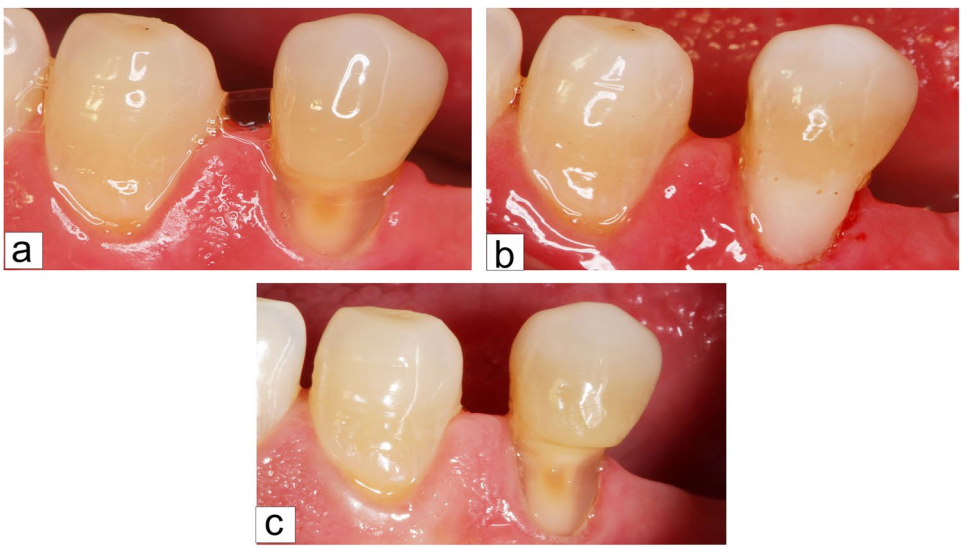


Fig. 3 Tooth 34 from group I considered unsuccessful for the retention criterion at 12 months: **a** initial NCCL, **b** baseline, and **c** after 1 year

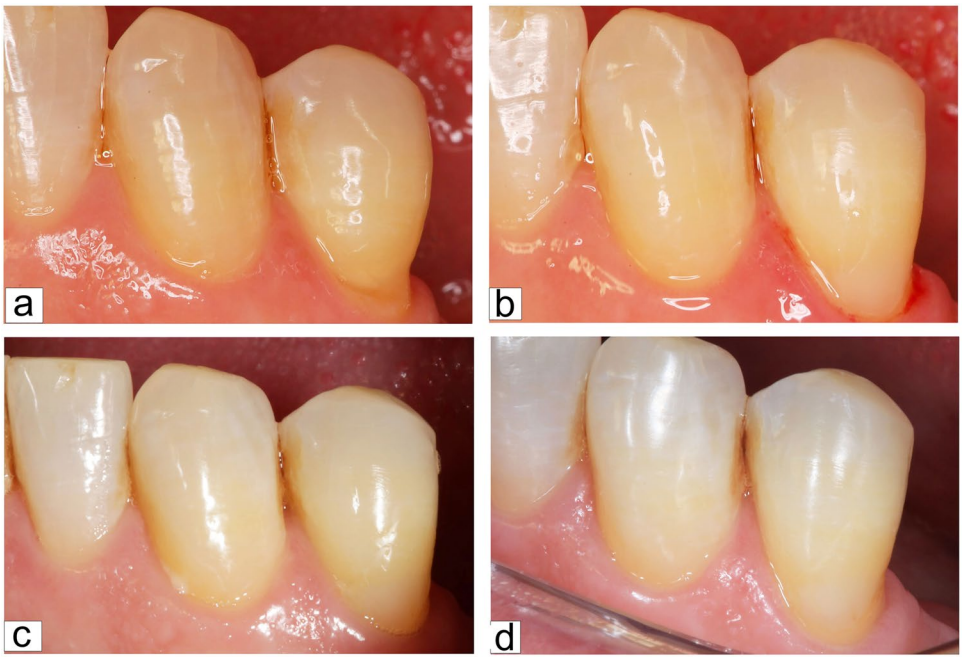


Fig. 4 Tooth 34 from group II considered successful (A for all criteria): **a** initial NCCL, **b** baseline, **c** after 1 year, and **d** after 2 years

[7, 28–30]. It is worth noting that in the self-etch technique, adhesives containing 10-MDP showed a higher retention rate when compared to a single bottle of adhesive system that does not contain this monomer [29, 31, 32]. This fact reinforces the importance of this monomer in the clinical performance of multimode adhesives when used in the self-etch technique [29]. However, another monomer 2-hydroxyethyl methacrylate (HEMA) is present in the adhesive system used in the current study. Although HEMA negatively influences hydrolytic stability and the durability of the adhesive interface [33], no significant difference in retention effectiveness between a HEMA-rich and one-step adhesive without HEMA was found [34]. Furthermore, the active application of multimode adhesive systems, as performed in the present study, increases the chemical interaction of the material with the dental substrate and the degree of conversion of the adhesive [32].

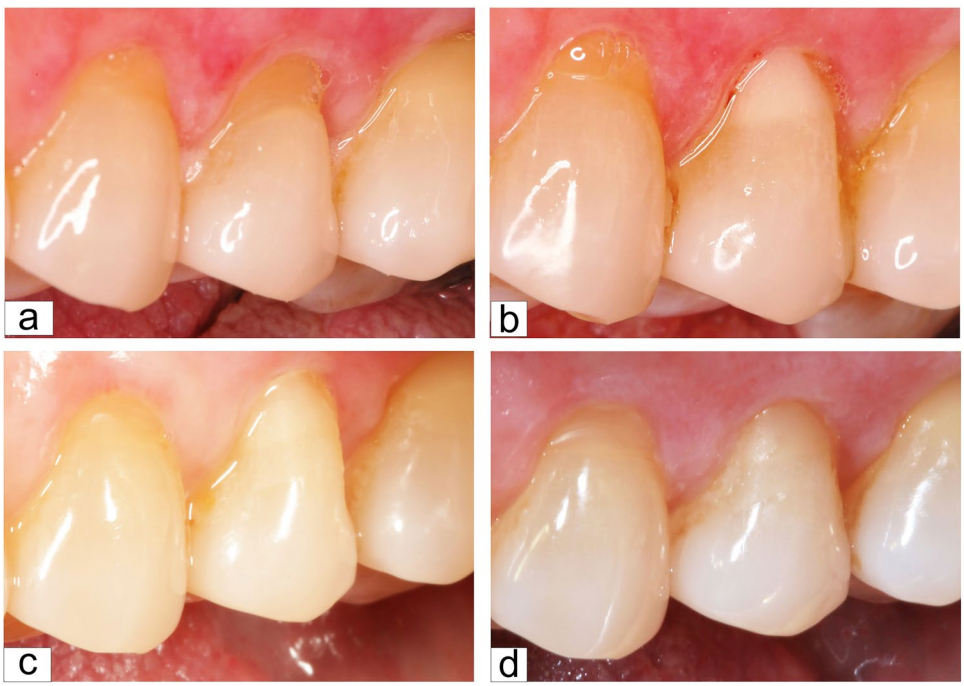


Fig. 5 Tooth 24 from group IV considered successful (A for all criteria): **a** initial NCCL, **b** baseline, **c** after 1 year, and **d** after 2 years

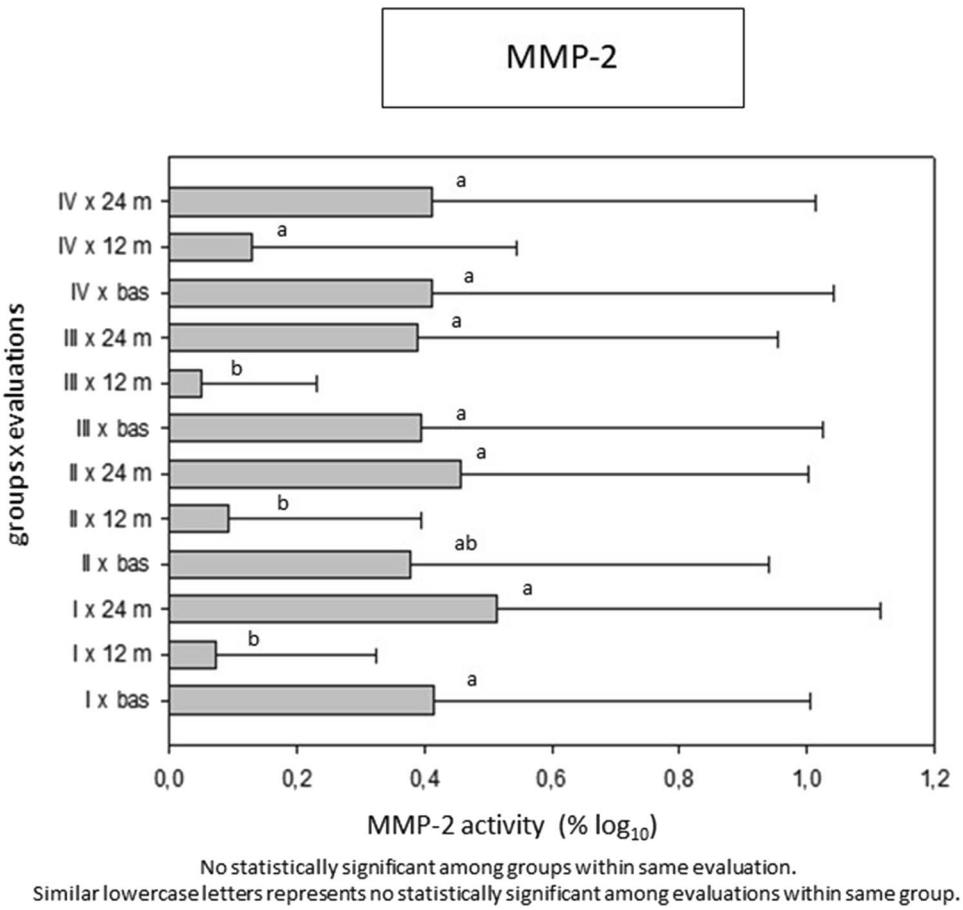
Table 6 Survival analysis comparing the four groups

Groups	Interval	Number of Restorations			Survival (%)	Accumulated survival (%)
		Initial	**Final**	**Failures**		
I	0–12 months	50	47	2	98.0	97.0
	12–24 months	46	46	2	95.7	91.8
II	0–12 months	50	47	1	99.0	98.0
	12–24 months	47	47	0	100.0	98.0
III	0–12 months	50	47	1	99.0	98.0
	12–24 months	47	47	0	100.0	98.0
IV	0–12 months	50	48	0	100.0	100.0
	12–24 months	48	48	1	97.9	97.9

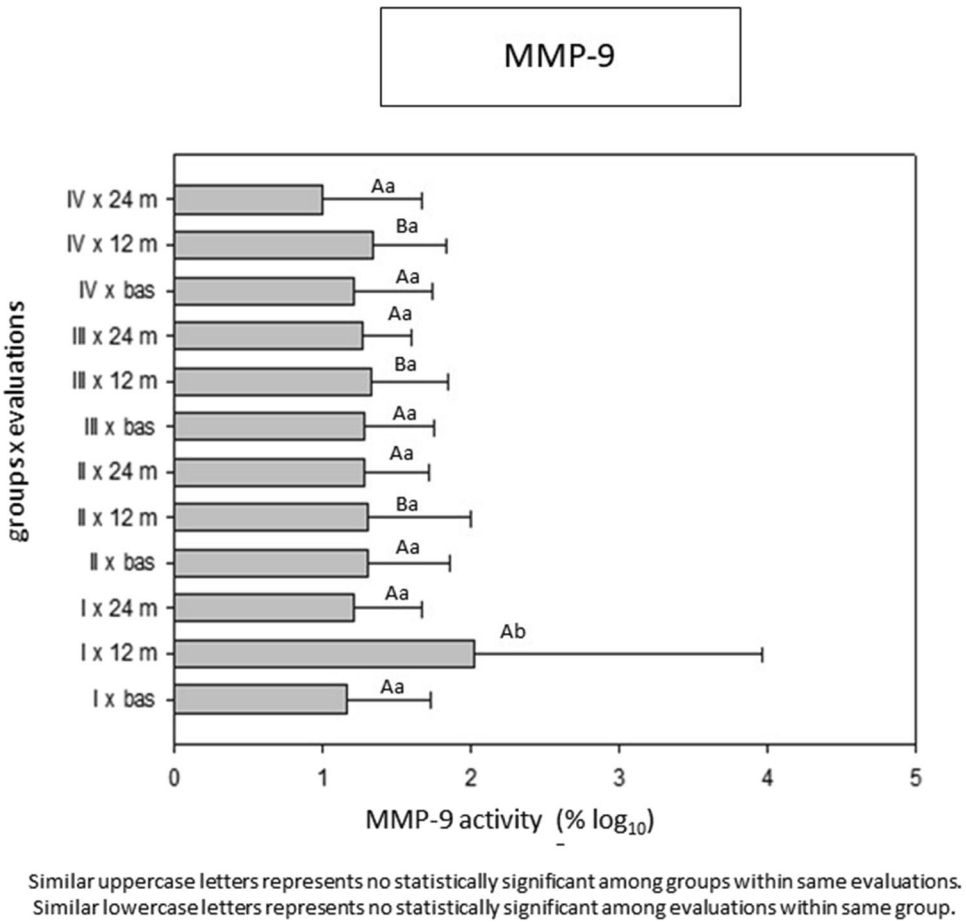
Wilcoxon (Gehan) $p = 0.280$ ns

Corroborating with the present study, other clinical studies showed that approximately 20% of restorations restored with multimode adhesives developed marginal degradation after 36 months [35]. A greater number of bravo scores were also observed for marginal integrity and marginal discoloration in groups I and II, but without statistical difference between groups. Laboratory studies have also shown that multimode adhesives have better adhesion and improved interfacial quality after acid enamel conditioning, especially after the adhesive interfaces are subjected to an acid challenge [36–38]. However, other authors observed that the performance of multimode adhesives did not depend on the adhesive strategy when applied on eroded dentine [10].

EDTA can be an alternative for pretreatment of NCCLs, since it may allow a selective dissolution of hydroxyapatite [39], leading to a better bond of ionomeric cements to sclerotic dentine, as occurs with self-etching adhesive systems when this pretreatment was used [40]. Group IV had the greatest cumulative survival rate in



Graphic 1 The mean $\log_{10}$, standard deviation (SD) values, and results of repeated measures ANOVA and Fisher tests for comparison between $\log_{10}$ MMP-2 measurements in all groups at different time periods. Similar uppercase letters represent no statistically significant among groups within same evaluations. Similar lowercase letters represent no statistically significant among evaluations within same group



Graphic 2 The mean log_{10}, standard deviation (SD) values, and results of repeated measures ANOVA and Fisher tests for comparison between log_{10} MMP-9 measurements in all groups at different time periods. Similar uppercase letters represent no statistically significant difference among groups within the same evaluations. Similar lowercase letters represent no statistically significant differences among evaluations within the same group

percentage, since the retention failures for this restorative treatment occurred only at 2 years. EDTA is also considered a potential inhibitor of MMPs [41]. MMPs are zinc- and calcium-dependent endopeptidases, and, consequently, the chelating capacity of Zn^{2+} Ca^{2+} could have kept these enzymes in an inactive state [42]. Besides MMPs, cysteine cathepsins (CCs) can also break down unprotected type I collagen fibrils in dentine matrix; in this context, potassium fluoride significantly inhibited the catalytic activity of CCs [43]. A systematic review of clinical studies that evaluated the use of pretreatments inhibiting the degradation of the hybrid layer concludes that there is still not enough evidence to the beneficial effects of these treatments [44].

In summary, the advantages cited above about the use of EDTA as pretreatment adjunct with chemical bond properties of ionomers may explain lower marginal discoloration for group IV when compared to group I at 1 year [39, 40]. Furthermore, the literature supports the use of acid enamel conditioning prior to multimode adhesives, justifying lower retention and decreased alpha scores for marginal integrity for group I [36–38].

The literature is scarce on studies evaluating the presence of MMPs in gingival crevicular fluid in teeth with NCCLs; so it is difficult to discuss our results with previous studies. Based on the results presented, different adhesive strategies did not influence the activity of MMPs from gingival fluid of patients with NCCLs, except for group I that had greater amount of MMP-9 than other groups at 1 year. The analysis of MMP activity by means of zymography is an excellent tool for the study of gelatinases in active biological systems [45]. EDTA is a chelator, widely used as a negative control in zymographic tests for its effectiveness in inhibiting MMPs [46, 47]. MMP-9 levels are related to the inflammatory response [20], and few alterations were found for this MMP over time, because patients with active periodontal disease were excluded from sample. MMP-2 is related to normal tissue [20, 48] and was reduced after 1 year returning to initial levels after 2 years for all groups, probably due to the aggression related to restorative procedures, with stabilization after 2 years. However, the only group in which EDTA (group IV) was applied prior ionomer no statistical alterations among the evaluation periods were found. In

this context, this type of pretreatment may be suggested as a potential agent prior ionomer restorations in NCCLs.

Different activity was also observed between MMP-9 and MMP-2 in a clinical study in which the presence of these MMPs was evaluated in carious cavities, and it was observed that MMP-9 differed between deep and superficial cavities, while MMP-2 did not show this difference in dentine fluid [48]. Results of in situ zymography showed that hybrid layers of tested adhesives exhibited intense collagenolytic activity, while almost no fluorescence signal was detected when specimens were pre-treated with other type of MMP inhibitor [46]. Furthermore, the MMP-2 enzyme from human coronal and radicular dentine was dynamically influenced by pH because the extracted enzyme activates this latent form at low pH, whereas collagen degradation by the matrix-bound enzyme was only observed when pH is close to neutral [11].

Inter- or intra-arch characteristics (e.g. occlusion factors, antagonist, and teeth selection regardless of their location in the dental arch) can also play an important role in studies of NCCL restorations. The dominant etiology of NCCLs should also be emphasized. It is known that demineralized substrate present in eroded NCCLs may activate the MMPs in dentine [18, 19]. However, in case of abfraction and abrasion lesions, it is not well acknowledged. It is difficult to recognize the dominant etiology of NCCLs by clinical examination. In a study evaluating the prevalence and risk factors of NCCLs in a Brazilian population of workers exposed and not exposed to acid mists and chemical products, it was observed that a multifactorial process occurs in the development of NCCLs, with no clearly dominant factor [49]. Other limitation of the present study was the high variability between MMPs present in the gingival fluid, but it was expected since in a clinical trial is not possible to control the individuals' variables such as occur in an in vitro study. The mean level of MMPs measured in gingival crevicular fluid also varied greatly between patients as shown in other study about orthodontic tooth movement [50]. Moreover, these preliminary results can guide the planning of future trials on that issue.

Further clinical studies should investigate different adhesion strategies, for example, the usage of phosphoric acid containing MMP inactivators [51, 52] and multimode adhesives in the self-etch mode with the addition of cross-linking agents, since improved adhesive performance has been observed with these components [14, 52, 53]. More studies are also needed to evaluate the activity of other MMPs, such as MMP-8, since its inhibitors have been studied to prevent collagen degradation and extend the longevity of adhesive restorations [54].

Conclusion

Within the limitations of the present study, the four different adhesion strategies in NCCLs showed similar clinical performance at 2 years with initial marginal defect alterations. MMP-2 reestablished its initial levels after 2 years, and MMP-9 had limited alterations over time in crevicular fluid, with no clear relation with the type of restorative materials.

Funding Funding for this study was provided by the São Paulo Research Foundation – FAPESP (grant number 2014/07086–0) and the Brazilian National Council for Scientific and Technological Development – CNPq (grant number 447616/2014–5).

Declarations

Ethics approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare no competing interests.

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