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ADHESIVE STRENGTH OF POSTERIOR BIOLOGICAL RESTORATIONS SUBMITTED TO MICROTRACTION

RESISTÊNCIA ADESIVA DE RESTAURAÇÕES BIOLÓGICAS POSTERIORES SUBMETIDAS À MICROTRAÇÃO

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ABSTRACT: This study evaluated the adhesive strength of biological restorations compared to conventional porcelain restorations through a microtraction test and fracture areas evaluation. 24 recently extracted and planed third molar crowns were randomly divided into a control (n = 12; porcelain restorations) and an experimental group (n = 12, biological restorations). Biological restorations were made from fragments of the teeth and porcelain crowns with the same dimensions using composite resin. After 24 hours, the teeth were submitted to thermal cycles and to microtraction. Fracture analysis was performed with a stereoscopic magnifying glass; samples were characterized by SEM. Data analysis was performed with the Student's t-test and the chi-squared test ($p \leq 0.05$). A significant difference was found regarding the type of fracture ($p = 0.015$), with adhesive fracture rate of 75% in the experimental group. Biological restorations constitute a viable option for the re-establishment of function and esthetics on posterior teeth.

KEYWORDS: Adhesion. Dental Restoration Wear. Mechanical stress.

RESUMO: Este estudo avaliou a resistência adesiva de restaurações biológicas comparadas com restaurações convencionais de porcelana. 24 coroas de terceiros molares extraídas e alisadas foram divididas aleatoriamente em grupo controle (n = 12; restaurações de porcelana) e grupo experimental (n = 12, restaurações biológicas). Restaurações biológicas foram confeccionadas a partir de fragmentos dos dentes e coroas de porcelana e coladas com resina composta. Após 24 horas, os dentes foram submetidos a ciclos térmicos e à microtração. A análise da fratura foi realizada com lupa estereoscópica; as amostras foram caracterizadas por MEV. A análise dos dados foi realizada com o teste t de Student e o teste qui-quadrado ($p \leq 0,05$). Houve diferença significativa quanto ao tipo de fratura ($p = 0,015$), com taxa de fratura adesiva de 75% no grupo experimental. As restaurações biológicas constituem uma opção viável para o restabelecimento da função e estética em dentes posteriores.

PALAVRAS-CHAVE: Adesividade. Desgaste de Restauração Dentária. Estresse mecânico.

INTRODUCTION

For many years, restorative dentistry has been evolving and seeking more esthetic solutions to satisfy the personal needs of patients.[1] The use of composite resins and the evolution of associated adhesive systems to the enamel and dentin acid etching technique allows for more conservative aesthetic restorative treatments.[2,3]

Although restorative dental material has achieved a high level of development and stability, no material in restorative dentistry can completely substitute the requirements that reestablish the loss of dental structure in terms of esthetics, mechanical and biologic properties.[4,5] Therefore, one alternative technique involves fragment collage ("Biological Restoration"), which, when well-planned, has several advantages over indirect restorations using resin compounds or porcelain. This technique reestablishes shape, function, esthetics, alignment and contour, shine, surface smoothness, and natural physiological wear, and can be considered a relatively simple, conservative, and inexpensive technique. Another advantage of biological restorations involves the patient recovering their emotional well-being and the sensation of having their healthy teeth back.[1,4]

The term biological restoration was proposed by Santos and Bianchi in 1991[6] and refers to a restorative technique that uses a fragment of an extracted tooth as a restorative material. This technique can be used in anterior and posterior teeth and the tooth fragment may be obtained from the fractured tooth itself or from a tooth that's been extracted from the own patient, autogenous bond, or from a donated tooth, homogenous bond.[6,7]

A critical analysis of the literature only confirmed one set of case reports that involved the use of fragment collage, particularly in the posterior region[1,4,8-10] and one study reported the use of biological restoration in primary posterior teeth.[11] Several motives support the use of biological restorations. Unlike porcelain or resin compounds, enamel and dentin interact normally with the oral environment, with constant ion exchanges in the processes of demineralization and remineralization.[7] The excellent biomechanical properties of the amelodentinal junction can divert severe cracks in enamel by considerable plastic deformation and substantial and long-lasting resistance to traction, enabling synergy between enamel and dentin.[12]

In the absence of clinical longitudinal data and clinical trials, it is known that laboratory data (*in vitro)* helps to predict the performance of different materials in the oral cavity and to suggest hypotheses for more adequate technical studies for each case. Due to the scarcity of scientific studies that prove the laboratory and clinical efficiency of "Biological restorations", the present study aimed to use the microtraction test to compare the adhesion strength of biological restorations after cementation in dentin substrate with conventional esthetic ceramic restorations, as well as to characterize the fracture area.

METHODOLOGY

ETHICAL ASPECTS

The present study received approval from the Human Research Ethics Committee of the Universidade Federal dos Vales do Jequitinhonha e Mucuri, approval number 095/12.

CALCULATION OF THE SAMPLE SIZE

The sample size was calculated based on a pilot study, with a confidence interval of 95%, a standard deviation of 4.9[13] and a difference of 1.4 points between groups, with a total of 24 teeth determined for the present study.

SELECTION AND CLEANING OF THE TEETH

In total, 24 healthy, human, recently extracted third molars were used. Before manipulation, the teeth were stored for a week in a 10% formalin solution[14,15] for sterilization and kept in distilled water until the moment of their use. Initially, the teeth were cleaned with periodontal curettes, before being polished at a low rotation (Dentflex, Ribeirão Preto, SP, Brazil) with a Robinson brush, a pumice stone paste and water.

PREPARATION OF THE TEETH

The teeth selected were randomly divided into two groups (n=12). One group was the control (Ceramic system IPS e.max ZirPress) and the other was the experimental group (biological restorations). After sterilization and cleaning, each tooth was sectioned transversely using a precision metallographic cutter (ELSAW, ELQUIP, São Carlos, SP, Brazil), a high-concentration diamond disk (Buehler, São Paulo, Brazil) and constant irrigation with distilled water. Two cuts were performed: the first was done to remove cusps and flatten the remaining surface; the second cut was made to obtain 2.0 mm thick dentin disks (Fig. 1A), which were used as a definitive restoration (Fig. 1B).

In the control group, the dentin disks taken from the occlusal cut were discarded and the remaining teeth were molded with addition-type silicone (3M EXPRESS XT, Sumaré, SP, Brazil) to design the dyes with type IV special stone plaster (Herostone - Vigodent, Rio de Janeiro, RJ, Brazil). The dyes were sent to the laboratory to design the IPS e.max ZirPress ceramic inserts (Ivoclar Vivadent, Barueri, SP, Brazil), which had a standardized thickness of 2.0 mm (Fig. 1C).

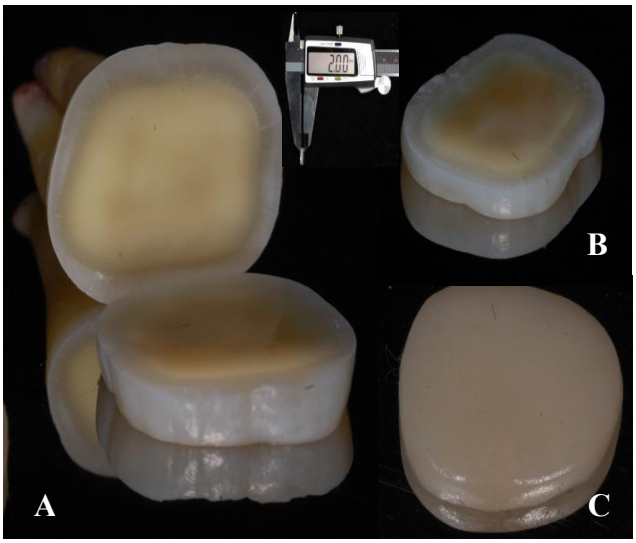

Figure 1. Tooth sectioned transversely to obtain a 2.0 mm thick dentin disc (A), biological fragment (B) and ceramic tablet (C). Source: The authors.

RESTORATIVE PROCEDURES

The sequence of restorative procedures started with four randomized blocks (n=6). All the remaining teeth received the same form of substrate treatment and biological fragments. Prophylaxis of the surfaces was conducted with pumice paste and water, using Robinson's brush at a low rotation. Afterwards, acid conditioning was performed with phosphoric acid 37% (Condac FGM, Joinville, SC, Brazil) on the remaining occlusal surface and the biological fragments obtained for the experimental group (15 seconds for dentin and 30 seconds for enamel), followed by rinsing for 30 seconds and drying with absorbent paper. Immediately after drying, two fine consecutive layers of the Adper Single Bond 2 (3M ESPE, Sumaré, SP, Brazil) adhesive were applied to the conditioned surface, before being dried with mild jets of air for 5 seconds to evaporate the solvent. Finally, photoactivation was conducted for 20 seconds. (Ultraled; Dabi Atlante, Ribeirão Preto, SP, Brazil).

The IPS e.max ZirPress ceramic inserts were conditioned with hydrofluoric acid at 10% (DENTSPLY, Petrópolis, RJ, Brazil) for 4 minutes, following the manufacturer's instructions. They were then washed for 30 seconds and dried with mild jets of air for 5 seconds before the application of two layers of the silane bonding agent primer/activator (DENTSPLY, Petrópolis, RJ, Brazil). They were dried again for 5 seconds after each application.

The dual resin cement RelyX ARC (3M ESPE Dental Products. ST. Paul, MN, USA) was used in the cementation of the ceramic inserts and biological fragments. A fine layer was applied to the internal face of each ceramic insert and biological fragment, which was then positioned on the corresponding occlusal surface and held for 30 seconds under mild digital pressure, simulating clinical conditions. Excess was removed from the edges using an exploratory probe before photoactivation for 40 seconds on each side. Afterwards, the restored teeth were immersed in distilled water and stored in an incubator at 37ºC ± 1ºC for 24 hours.

THERMOCYCLING

In the next stage, all teeth were submitted to thermocycling (MSCT/3e, Elquip, Piracicaba, SP, Brazil), totaling 10,000 cycles, with 30-second baths in temperatures of 5º C, 37º C and 55º C,[10] which is the same as one year of thermal aging.

MICROTRACTION TEST AND ANALYSIS OF THE FRACTURE AREA

To design the specimens using the precision metallographic cutter (ELSAW, ELQUIP, São Carlos, SP, Brazil) and constant irrigation with distilled water, serial cuts were performed mesiodistally, buccolingually and parallel to the edge of each restored tooth, obtaining parallel sections of approximately 1.0 x 1.0 mm (Figs. 1D and E). Only specimens that exhibited dentin structure were used in the tests. Specimens that exhibited an enamel structure were discarded.

The specimens obtained were fixed by their extremities with cyanoacrylate glue (Super Bond gel, Loctite, Henkel Corp, Brazil) using the grips of the Geraldelli microtraction device (*Dispositivo Odeme Equipamentos Médicos e Odontológicos*, São Carlos, SP, Brazil) and coupled to the EZ Test - L universal testing machine (Shimadzu, Tokyo, Japan) (Fig. 1F). The test was conducted at a speed of 0.5 mm/min, using a load cell of 5 Kg, until the rupture of the specimens in the bond interface or in proximity, obtaining the bond strength values of each section in megapascals (MPa). The mean values of the specimens representing each tooth were then calculated.

The resulting fractured areas were observed using a stereomicroscope (SZ40, Olympus Corporation, Tokyo, Japan) with a zoom of 40x to analyze the type of fracture that occurred. The types of fractures were determined based on the percentage of substrate-free material and then classified as adhesive, cohesive (restorative material or remaining teeth) or mixed flaws. The specimens representative of the types of fracture in each group were characterized using scanning electron microscopy (SEM) (TM 3000 Hitachi, Japan). Using an electron beam of 15 to 20 kV, the surfaces were covered with a fine layer of Gold-Palladium (Au-Pd) by sputtering to enable the transmission of electrons.

ANALYSIS OF NANOLEAKAGE

Two representative specimens were selected from each control and experimental group to conduct the nanoleakage process. These specimens were immersed in ammoniacal silver nitrate solution 50% ($AGNO_3$ NH_4) and stored in an incubator for 24 hours at 37°C ± 1°C. Afterwards, they were washed in distilled water for 2 minutes and then immersed in a revealing solution (Kodak – *Revelador* D-76 – Kodak Brasileira, Ind. E com. Ltda, São José dos Campos, São Paulo, Brazil) for 8 hours. Next, they were exposed to direct fluorescent illumination using a luminaire, as well as indirect light from the lighting of the environment, to reduce the silver ions to metallic silver grains in the empty spaces along the bond interface.

The specimens were then processed to visualize the nanometric spaces inside the hybrid layer using SEM. To do this, the specimens were included in polystyrene resin. After inclusion, they were worn sequentially with different grits of sandpaper (600, 1200 and 2000) in a sanding machine and a metallographic polisher (PLFDV – Fortel Ind. e Com. Ltda – São Paulo, SP, Brazil). Afterwards, they were polished with felt disks and diamond paste of a decreasing granulation (3.1 and 0.25 µm). Between each grain of sandpaper, the specimens were immersed in distilled water and placed in an ultrasound unit (*Cuba de Ultrassom Cristófoli – Cristófoli Equipamentos de Biossegurança Ltda*, Campo Mourão, PR, Brazil) for 10 minutes to remove debris.

The specimens were then dried with absorbent paper and the demineralization process was initiated with phosphoric acid (50%) to remove the inorganic dentin matrix. Deproteinization was also conducted in a solution of sodium hypochlorite 10%. The specimens were then dehydrated in ethyl alcohol, using increasing concentrations (25%, 50%, 75%, 90% and 100%), for 10 minutes at each concentration. To finish the process, the specimens were coated with carbon wire through sputtering (Leica EM SDC 500, Leica Microsystems, Switzerland) and fixated in stubs for SEM observation (Quanta FEG 200, FEI, 2006, Oregon, USA), operating at a high vacuum and a power setting of 20 kV, which provided backscattered electron images.

STATISTICAL ANALYSIS

The data obtained were tabulated using the Statistical Package for Social Science (SPSS for Windows, version 20.0; SPSS Inc., Chicago, Ill., USA) and then submitted to statistical analysis to compare the control and experimental groups in terms of adhesion strength and the types of fractures. Resistance strength was associated with the strength/area (MPa) applied to the specimens. These data revealed a normal distribution (Shapiro-Wilk test) and similar variance (Levene test). Thus, the parametric Student's t-test ($p>0.05$) was used for independent specimens to determine the occurrence of differences in the bond strength between groups.

The chi-squared test was used to determine the association between the dependent variable, the type of restorative material, the independent variable and the fracture pattern.

RESULTS

Although there was a statistical difference in the adhesion strength values obtained in megapascals (MPa) during the microtraction test (Table 1), no statistically significant difference was found between the ceramic system and biological restorations (p=0.136).

Table 1. Mean microtensile strength, standard deviation and p-value according to restorative material.

Type of restorative material	N	Adhesion resistance (MPa) Mean	Standard deviation	P
Ceramic System	12	12,15	1,68	0,136 [T]
Biological Restoration	12	10,22	3,99	

[T] Student's t-test for independent samples. Source: The authors.

With regards to the type of fracture that occurred in the groups tested, there was a statistically significant difference (p=0.015), with a predominance of mixed fractures (75.0%) in the control group and adhesive fractures (75.0%) in the experimental group (Table 2).

Table 2. Types of fractures according to the restorative material.

Type of fracture	Type of restoration		P
	Ceramic System n (%)	Biological Restoration n (%)	
Adhesive	2 (16,7)	9 (75,0)	
Cohesive	1 (8,30)	0 (0,00)	0,015[C]
Mixed	9 (75,0)	3 (25,0)	

[C] Chi-square test (p ≤0.05). Source: The authors.

The SEM micrographs of the specimens analyzed by light microscopy showed a fine layer of adhesive on the surface of dentin on the remaining teeth after both restorations, suggesting adhesive fractures (Figs. 2A and B). The EDS spectrum of the surface of dentin (Figs. 2a and b) showed elevated peaks of calcium (Ca) and phosphorus (P), corresponding to the dental structure, peaks of silicon (Si) and the inorganic load of the single component adhesive system.

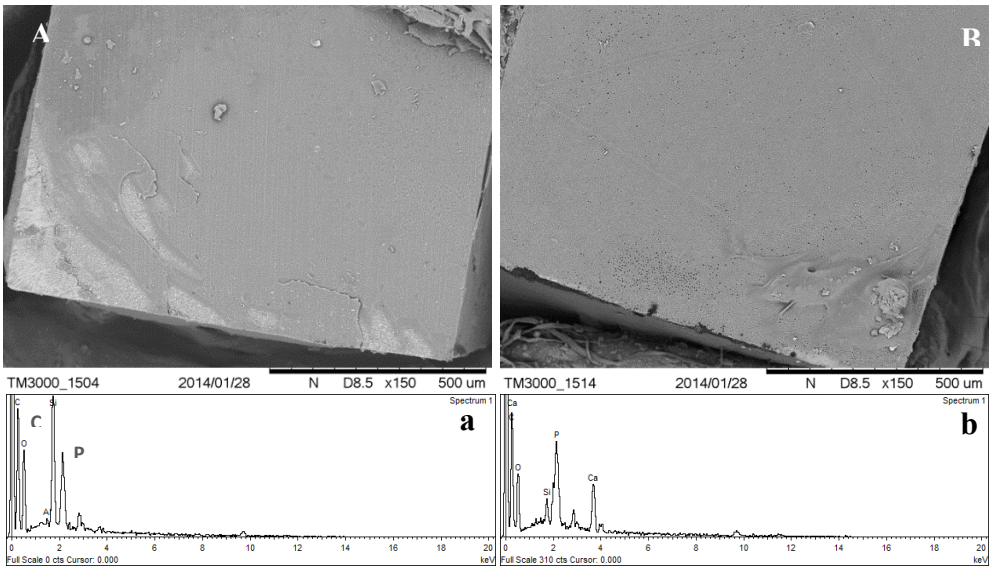

Figure 2. SEM image of the adhesive fracture area of the biological (A) and ceramic (B) restoration and respective EDS spectra of the fracture areas in the tooth structure (a) and (b). Source: The authors.

A cohesive fracture of the restorative material was only observed for the ceramic restoration (Fig. 3A). The EDS spectrum exhibited peaks of zirconia (Zr) and Si, as well as low-intensity peaks of potassium (K), corresponding to the ceramic structure (Fig 3a).

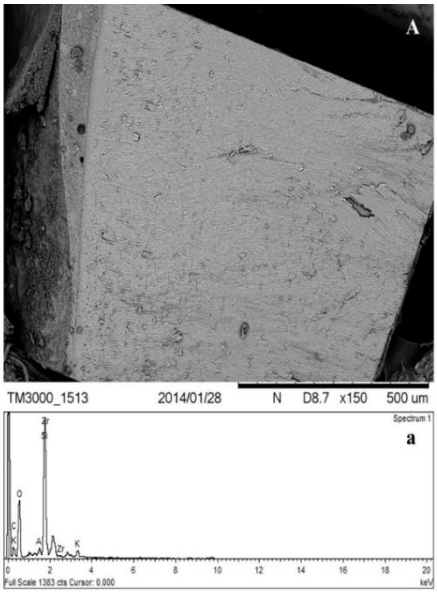

Figure 3. SEM image of the cohesive fracture area of the ceramic restorative material (A) and EDS spectrum of the fracture area of the ceramic structure (a). Source: The authors.

The mixed fracture of the biological restoration (Fig. 4A) exhibited peaks of Ca, P and Si (Figs. 4a and b). The EDS analysis of the mixed ceramic fracture (Fig. 4B) exhibited a spectrum with peaks of Ca, P, Zr, Si and K (Figs. 4c and d). Peaks of gold and palladium, caused by metallization, were not identified in the EDS spectra.

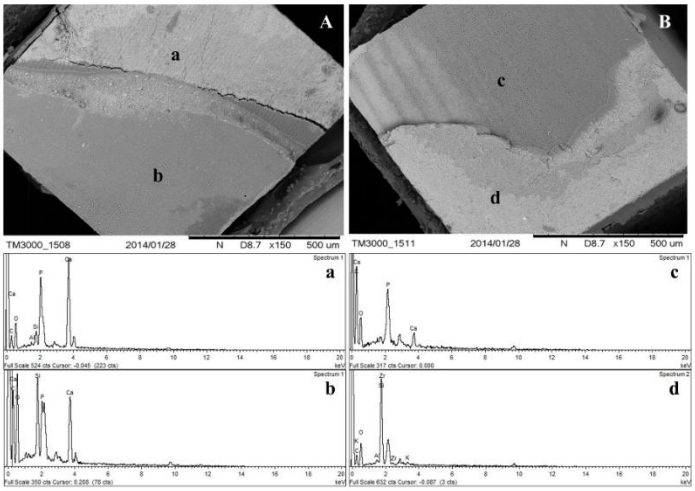

Figure 4. SEM image of the mixed fracture area of the biological (A) and ceramic (B) restoration and respective EDS spectra of the fracture areas (a), (b), (c) and (d). Source: The authors.

Extensive expression of nanoleakage was found in the adhesive interface of the hybrid layer, whereas a lower quantity was found in the dentinal tubules in the ceramic group (Fig. 5A). Upon comparison of the groups, there was a comparatively lower expression of nanoleakage along the hybrid layer and greater nanoleakage in the dentinal tubules in the biological restoration group (Fig. 5B).

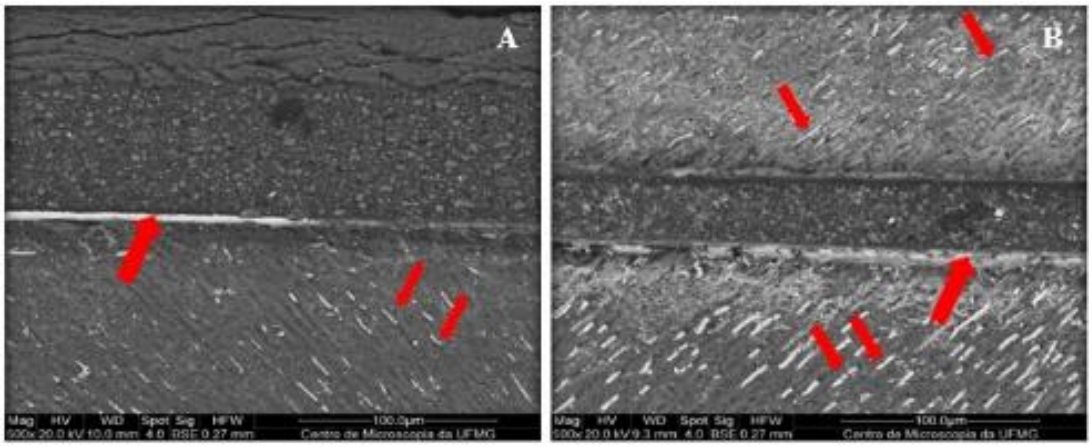

Figure 5. Representative SEM images of the group of ceramic restorations (A) showing extensive expression of nanoleakage at the bonding interface of the hybrid layer and to a lesser extent inside the dentinal tubules. And biological restorations (B) showing less nanoleakage along the hybrid layer and greater inside the dentinal tubules.
Source: The authors.

DISCUSSION

In the present study, both groups exhibited similar adhesion strength. However, regarding the pattern of the fractures, there was a difference between the groups. The adhesion strength value found in the present study was lower than the values found in the literature, which range from 12.94 Mpa to 49.8 Mpa for zirconia-based ceramic restorations.[16,17] The divergence between the values found could be explained by the low concentration of zirconia in the composition of the ceramics used and the residual tension introduced during the preparation of each specimen.[16]Therefore, the different brands of ceramic may have different surface roughness once the duration and the particle size of blasting affect the roughness of the zirconia.[18] However, no previous studies have been carried out to assess the adhesive strength of biological restorations, which hinders a direct comparison of the microtraction values in relation to other restorative materials.

The adhesion strength between restorative adhesion material and the dental substrate is commonly assessed by micromechanical laboratory trials. The microtraction trial allows a better distribution of tension and fewer cohesive errors in the substrat.[19] According to the study conducted, the fractures were predominantly mixed in the control group, similar to the results of previous studies[16,20,21] possibly due to the stronger adhesion between resin cement and the ceramic surface. However, the result found in the present study was the opposite of those reported in previous studies[22,23] in which adhesive flaws were predominance. Mixed fractures were characterized by the presence of EDS spectra with peaks of Ca and P coming from the dental structure and of Zr and Si referring to the chemical composition of the ceramics used.

Regarding the biological restoration group, there was a greater percentage of adhesive fractures, confirmed by the presence of Ca and P in the elemental analysis (EDS) of the fracture surface of the remaining teeth, coming from the dentin structure. The greater percentage of adhesive fractures can be explained by the organic composition and humidity contained in dentinal tubules, as well as the fact that it is a more elastic substrate than the ceramic used.[24] The clinical consequences of the flaws that can occur depend on the location in the bond interface. When the flaw occurs between the hybrid layer and the layer of the cementing agent, the dentin can remain sealed and protected, minimizing the risk of demineralization, bacterial invasion, sensitive teeth, and pulp irritation.[25]

One of the aims of dental restorations is to rehabilitate esthetics and function while preserving the greatest possible quantity of the remaining dental structure. Thus, the restorative material should promote the sealing of exposed dentin, impeding the passage of fluids and bacteria that cause a relapse of caries and pulpal damage.[26,27] The longevity of restorative procedures is associated with the attainment of a perfect adaptation, among other factors, and the formation of a stable and long-lasting bond between the restorative material and the dental structure. This prevents and/or minimizes the processes of microleakage and, consequently, staining, recurrent carious lesions, and sensitivity.[27]

The present study analyzed the quality of the adhesive interface of ceramic and biological restorations by observing nanoleakage in the adhesion interface, characterized by the infiltration of silver nitrate ions through submicron porosities in the hybrid layer that were not adequately filled by the adhesive, or where the adhesive was poorly polymerized.[28] This region represents a weak point, which enables the introduction of enzymes, bacteria and oral fluids to the interface, thereby degrading adhesion.[27] The resulting porosities are too small for bacteria to penetrate but are big enough for enzyme penetration.[28]

Recently, it has been noted that metalloproteinases from the extracellular matrix, or enzymes capable of degrading the collagen of dentin, are naturally present in the structure of the dentin pulp complex. These enzymes can be activated by the fall in the pH caused by treatment of the surface with a primer and adhesive or the biochemistry of the carious process, leading to greater degradation of collagen fibers and a higher risk of nanoleakage.[29]

In the present study, it was possible to confirm the expression of interfacial nanoleakage in both groups through the SEM analysis. Silver nitrate deposits were found along the adhesive interface and the dentinal tubules, demonstrating that the quality of the hybrid layer is essential to the longevity of the restorative process.[30] This type of infiltration enables the observation of the location of the flaw in the adhesive interface and an understanding of how the degradation of the adhesive occurs over time.[28]

Although the "biological restorations" exhibited similar characteristics as the ceramic restorations in terms of adhesion strength, further laboratorial and clinical studies should be developed to assess their performance and other properties with greater accuracy.

The use of biological restorations as a restorative technique can be considered viable since they can reestablish the esthetic and functional aspects without laboratory costs. They are an option for many patients, particularly when focusing on cost-benefit. The technique exhibits highly satisfactory results through conservative, simple procedures that can be performed by the professional. They also provide another option for the restoration of teeth with extensive coronel destruction.

CONCLUSION

Considering the limitations of the present study and according to the tests applied, it is possible to conclude that biological restorations exhibit similar characteristics, in terms of adhesion strength, to ceramic restorations and thus, can be indicated as an alternative for the esthetic and functional reestablishment of teeth with extensive destruction.

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