

Effect of caries infiltration technique and fluoride therapy on the bond strength of the demineralized enamel

Efeito da técnica de infiltração de cárie e fluoroterapia na resistência adesiva ao esmalte desmineralizado

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ABSTRACT

Different techniques are employed to control de caries progression, such as fluoride remineralization and resin infiltration (ICON®). However, the interference of these techniques on further adhesive procedures on the treated tissue is still controversial. The aim of this study was to evaluate the bond strength (BS) of the bovine enamel demineralized and treated with either fluoride or ICON®. Material and Methods: The tooth fragments were randomly divided into 4 groups: Group Sound samples (control); Group Demineralized samples (DS); Group Remineralized samples (NaF- 0.05% /8 weeks); Group ICON® samples. The samples were etched and next, a total etch bonding system was applied followed by resin composite. Then, they were submitted to microtensile test in a universal testing machine (10 Kg 1 mm/min). Results: Data were evaluated by ANOVA. There were statistically significant differences among groups ($p = 0.28$), with the following mean values (MPa): Group: Sound samples ($20,20 \pm 2,97$), Group: Demineralized Samples ($21,99 \pm 4,25$), Group: Remineralized Samples ($23,48 \pm 4,03$), Group: ICON® samples ($22,10 \pm 3,37$). Conclusion: The treatments did not interfere in bond strength of the composite resin to enamel, providing values similar to those of the control group.

KEYWORDS

Dental caries; Dentine adhesive; Tooth remineralization; Bovine enamel; Caries infiltration; Artificial caries.

RESUMO

Diferentes técnicas são empregadas como forma de controle da progressão da lesão cáriosa, como a remineralização com flúor e a infiltração com resina (ICON®). No entanto, a interferência destas técnicas sobre futuros procedimentos adesivos no tecido tratado ainda mostra-se controversa. O objetivo do estudo foi avaliar a resistência adesiva (RA) em esmalte bovino desmineralizado e tratado com flúor ou com ICON®. Materiais e Métodos: Fragmentos dentais foram divididos aleatoriamente em 4 grupos: Grupo Espécimes íntegros (controle); Grupo Espécimes desmineralizados (ED); Grupo Espécimes Remineralizados (NaF- 0,05% /8 semanas); Grupo Espécimes ICON®. Sobre os espécimes tratados foi feito condicionamento ácido, aplicação de adesivo total etch e resina composta, sendo submetidos ao teste de microtração em máquina de ensaio universal (10Kg 1mm/min). Resultados: Os dados foram avaliados pelo teste ANOVA. Não houve diferença significante entre os grupos ($p=0,28$) sendo que, os valores médios em MPa obtidos para os diferentes grupos foram: Grupo: Espécimes Íntegros ($20,20 \pm 2,97$), Grupo: Espécimes Desmineralizados ($21,99 \pm 4,25$), Grupo: Espécimes Remineralizados ($23,48 \pm 4,03$), Grupo: Espécimes ICON® ($22,10 \pm 3,37$). Conclusão: os tratamentos não interferiram na RA da resina composta ao esmalte, fornecendo valores semelhantes ao controle.

PALAVRAS-CHAVE

Cárie dentária; Adesivos dentários; Remineralização dentária; Esmalte bovino; Infiltração da lesão cáriosa; Cárie artificial.

INTRODUCTION

The effect of the conservative treatments to stop the development of the initial caries on further adhesive restoration, in cases of caries re-agudization is still controversial. The etiological factors of the dental caries comprise the association among the bacterial agents, substrate, dietary habits, over time. Caries lesion on enamel are characterized by mineral loss under a superficial layer apparently sound [1].

The demineralization and remineralization processes which involve mineral loss and gain dynamic should be well understood and may be conducted through in vitro models, in which artificial caries lesions may be created. The advantages of these models are: the simulation of the mineral loss and gain dynamic, ease execution and standardization of the lesions [2].

Clinical studies have demonstrated that non-cavitated caries lesions may be repaired by the saliva, mainly when there is a combination of the dietary control, removal of bacterial plaque and fluoride treatments [3,4].

Fluoride is still the most important therapy currently available to promote caries remineralization. It acts as chemotherapeutic agent within oral cavity through three mechanisms: improvement in the enamel resistance to acids; remineralization of the initial lesions; interference on the bacterial metabolism; and interference on the enzymatic processes [5].

Different calcium-based formulations have been developed and the previous data have suggested that they could have remineralizing properties; however, these products are still in development with lack of clinical evidence [6].

New remineralizing therapies have been studied attempting to increase the efficacy, once laboratorial data suggested that the fluoride threshold required to remineralize cavitated lesions could be higher than the threshold required to avoid the onset of the caries lesions [7].

The increased porosity within the carious lesion body results in a whitish appearance of the enamel, so-called the white spot lesions [8].

The resin infiltration in the caries lesion is a therapeutical alternative to avoid the enamel caries progression, because deeper lesions tend to remineralize only superficially [9]. This treatment aims to close the microporosities inside the lesion body by means of infiltration with low-viscosity light-curing resin, which were improved to penetrate fastly into the porous enamel [10]. This technique's goal is to create a diffusion barrier not only onto the

lesion surface but also inside the carious lesion [11]. A positive effect of the infiltration of white spot lesions with these resin is the loss of the whitish appearance, once the refractive index of the enamel is recovered when the microporosities are filled [8].

Notwithstanding, many aesthetical procedures may be required after either the resin infiltration or the fluoride remineralization, such as: tooth recontour, orthodontic brackets bonding, tooth whitening, and even a cavity preparation followed by restoration because of the caries re-agudization. Based on the aforementioned considerations, the aim of this study was to evaluate the bond strength of teeth with demineralized enamel which were submitted to caries blockage through either fluoride remineralization or resin infiltration (ICON®) to understand better whether these methods would interfere on further adhesive procedures.

MATERIAL AND METHOD

Preparation of the specimens

Forty bovine incisors were used, coming from the same batch of animals to ensure the same type of feeding and degree of calcification of the teeth. After extractions, the teeth were kept into distilled water and 0.1% thymol solution and submitted to manual scaling with size [11] periodontal curettes (Hu Fridedy - USA) and polished with the aid of a Robinson brush with pumice and water paste (Viking-KG Sorensen, Barueri, SP, Brazil) at low speed (Kavo do Brasil SA, Joinville, SC, Brazil). The teeth were stored into closed flasks containing distilled water under refrigeration (Brastemp, 4 °C) until the moment of their use, which was no longer than 7 days.

The roots of the teeth were removed with the aid of carborundum discs (Dentauro, Pforzheim, Germany), at low speed. The enamel of the labial surfaces were flattened. The fragments were embedded into colorless self-curing resin (Jet, Campo Limpo-SP, Brazil) with the aid of a putty silicon matrix (Rhodorsil, Artigos Odontológicos Clássico, Campo Limpo Paulista, SP, Brazil). The bases of the resin blocks were flattened with the aid of 400-grit sandpapers, for 3 min [12] in a polisher (Polipan 2 - Pantec, São Paulo, SP, Brazil) under refrigeration. Following, the labial surface was polished with the aid of 600-grit sandpapers for 3 min to remove possible self-curing resin excesses, to ensure an exposed enamel surface. Then, the specimens were stored into distilled water in a bacteriological incubator at 37° C for 24 h.

Division of the experimental groups and Treatment of the enamel surface

The specimens were randomly divided into the experimental groups, according to the type of treatment to be performed onto the enamel surface (n = 10):

- Group Sound samples (control). The sound specimens did not undergo any previous treatment. They only received the conventional adhesive treatment comprising acid etching with 37% phosphoric acid for 30 s (s) and adhesive agent (Ambar - FGM, Joinville, SC, Brazil). The acid etching was performed for 30 s onto the enamel surfaces and washed for 10 s. Then, they were air dried for 10 s. Two layers of the adhesive system were vigorously applied for 10s. Then the sample was air dried and light-cured for 10s (XL 3000 light-curing unit, 3M / ESPE, St. Paul, Mn, USA) at 500 mW/cm².
- Group Demineralized Samples: The sound samples were immersed into a demineralizing solution prepared according to Queiroz et al. 2008 [13]. Firstly, 0.05 M of acetate buffer pH 5.0, 50% saturated in relation to enamel was prepared. In this solution, 1.28 ± 0.058 mmol / L of Ca, 0.74 ± 0.005 mmol / L of inorganic Phosphorous (iP) and 0.023 ± 0.006 ug F / mL were detected. From these results, a acetate buffer solution 0.05 mol / L, pH 5.0 containing 1.28 mmol / L of Ca, 0.74 mmol / L and 0.03 iP F ig / mL was prepared from the following salts: Ca (NO₃) 2.4H₂O, KH₂PO₄ and NaF, respectively to induce caries (AWS- active white spot). After 24 h, the specimens were washed in deionized water and then received the adhesive treatment according to the group Sound samples.
- Group Remineralized samples: Demineralized samples + fluoride. The samples with active white spot induced as aforementioned described (Group Demineralized Samples), undergone a remineralizing process through daily immersion in 0.05% sodium fluoride solution for 1 min, during a 8-week period. The specimens were kept into artificial saliva [14] during all remineralizing period. Following, they received the adhesive treatment as described in the group Sound samples.
- Group ICON® samples: Demineralized samples + ICON®. ICON®(DMG, Hamburg, Germany) application was executed as the

manufacturer's instructions, onto the AWS surfaces. Firstly, 15% chloridric acid was applied onto the sample surface for min, followed by the surface washing with water-air spray. The surface was air dried and then an ethanol solution was applied to remove the remnant water followed by a new air jet. Next, ICON® was applied and light-cured. After the final light-curing, the samples were polished with the aid of a polisher and 6000-grit sandpapers for 5 s (s), and kept into deionized water at 37° C for 24 h. Then, they received the adhesive treatment according to the Group Soun samples.

Next to each adhesive treatment performed onto the enamel surface of the samples of each group, a block was constructed with a light-curing composite resin, shade D2 Venus (Heraeus Kulzer, Hanau, Germany), similarly to all experimental groups. The standardization of the test area was executed with the aid of a silicon matrix of 4x4x4mm.

The silicon matrix was centered onto the tooth surface and the light-curing composite resin was inserted with the aid of a steel spatula in two increments of 2 mm each, light-cured for 40 s. The matriz was removed and a block of resin composite was obtained, bonded to the tooth surface. This block was again light-cured for more 20 s at each side, after the matrix removal.

Microtensile test (µTBS)

Following the distilled water storage at 37 oC for 24 h, the samples were longitudinally sectioned at ‘x’ and ‘y’ directions, at the adhesive interface, with the aid of a Labcut machine (Extec Corp, Enfield, CT, USA) and diamond disc, at 300 rpm under refrigeration to obtain sticks (Figure 1) with a cross-sectional are of approximately 1 mm². Each sample provided 9 sticks. The samples were measured with the aid of a caliper to calculate the bonding area and then glued to the microtensile machine with the aid of cianoacrylate gel (Figure 2) to execute the tensile test in the universal testing machine (DL-1000, EMIC, São José dos Pinhais, PR, Brazil), with a load cell of 10 kg, at a crosshead speed of 1 mm/min, according to ISO TR 11405 guidelines. Bond strenght values were calculated in MPa.

The samples fractured were also analyzed in stereomicroscope (X20 Stemi 2000 C, Zeizz, Carl Zeiss, Jena, Alemanha) at x50 magnification to determine

the fracture type: Cohesive in tooth substrate– fracture predominantly (about 75%) within the tooth structure; Cohesive in resin - fracture predominantly (about 75%) within the resin composite; Adhesive – fracture at either the adhesive/tooth structure interface or adhesive/resin composite interface, at most of 75% of the area analyzed; Mixed – fractures without predominance greater than 75% of any type.

Only the data resulting from the adhesive and mixed fractures were analyzed (table 2).

The following hypotheses were tested:

- a) The demineralizing process do not interfere on the bond strength;
- b) The remineralization do not interfere on the bond strength;
- c) Resin infiltration do not interfere on the bond strength.

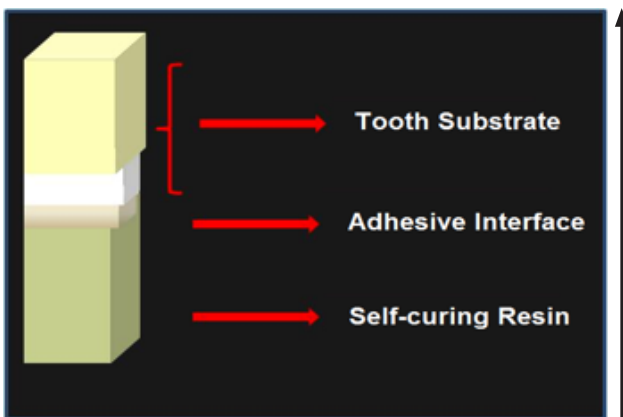


Figure 1 - Illustrative scheme of the sticks obtained.

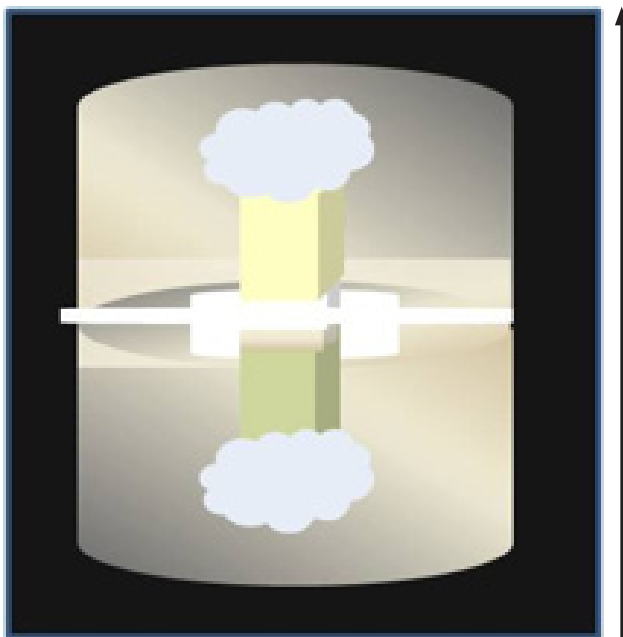


Figure 2 - Stick glued with cianoacrylate to the microtensile device.

RESULTS

The descriptive statistics comprised the calculus of the means and standard deviations. Inferential statistics comprised the analysis of the variance.

The level of significance choose was 5% ($p < 0.05$) to verify whether there would be significant differences among the conditions tested.

ANOVA was used to evaluate the influence of the treatment type ($p \leq 0.05$), showing no statistically significant differences ($p = 0.28$).

It could be observed that the demineralized samples did not exhibited statistically significant differences in relation to the sound samples, that is, the sound and the demineralized samples and the samples where ICON® was applied, the bond strenght (MPa) was similar.

The remineralization process did not significantly interfere on the adhesion. Table 2 demonstrates that a greater number of adhesive and mixed fractures occurred, indicating a good condition of the microtensile test. A high number of cohesive fractures in the substrate was observed in the group demineralized, indicating the fragility of the substrate undegoing demineralization as well as its effectiveness.

DISCUSSION

The aim of this study was to evaluate the bond strength of demineralized bovine teeth to enamel which undergone the blockage of the caries lesions either by fluoride remineralization or resin infiltration (ICON®).

The dental caries originates with the demineralization of the enamel, dentine or cementum, through acidogenic bacterias which alter the dynamic of the demineralization-remineralization process. When appropriate methods are employed, the caries lesion may be stopped at its initial phase or it may even be reversed [15].

At physiological conditions, the oral fluids (saliva, biofilm fluid) have calcium (Ca) and phophate (PO4) at supersaturated concentrations in relation to the mineral content of the enamel. Consequently, these ions are continuously depositated onto either the sound surface of the enamel or the areas undergoing mineral loss. This may be considered a natural defense phenomenon promoted by the saliva to preserve the mineral structure of the enamel within the oral cavity. Therefore, the remineralization would be better defined as the redeposition of the minerals lost by the

enamel, and this term has been used as synonym for enamel repair [16].

When the white spot lesions were recognized as carious lesion which can evolve to cavities, a clinical discussion on the treatment through a non-invasive method was initiated in Dentistry [16].

Currently, there is a consensus on that the local, topical fluoride presence may interfere on the carious process [17]. If the enamel undergoes demineralization, the saliva has the capacity of remineralize it [17], but in the fluoride presence, this effect is potentialized [18].

Results of in vitro studies already conducted on the determination of the fluoride effectiveness on preventing enamel demineralization have clearly indicated that the effects observed are highly dependent on the experimental conditions used [19]. In this present study, the concentration of the sodium fluoride solution was of 0.05%. This group showed the highest bond strength, however, without statistically significant difference. Such finding suggests that remineralization through 0.05% fluoride solution did not pose risks of reduction of adhesivity when a new restorative procedure is required onto the same tooth. In the other groups, the fluoride absence did not significantly interfere on the adhesion, therefore not contra-indicating the use of other therapies regarding to the adhesion process, as shown in table 1.

Since the 70s of the 20th century, the infiltration of low-viscosity resins into the carious lesions aiming to stop them has been studied [20,21]. Reports evidenced that the action of sealants which penetrate up to 95% into the lesion body [22], and reduce significantly the volume of the accessible porous within the lesions [23]. Additionally, it has been observed that the sealants are capable of inhibiting the lesion progression, mainly in demineralizing conditions [20,23,24].

According to the results of this study, ICON® application did not alter the bond strength whether a further restorative treatment was needed, therefore indicating its use in cases of active white spot lesions.

Rocha et al., in 2011 [1] concluded that ICON® is effective in both stopping the caries lesion and devolving the natural color to the teeth, mimetizing the whitish aspect of the white spot by the filling of the porous of the lesion's body [1]. Thus, after a conventional remineralizing treatment, although the remineralized lesions exhibited a size decrease (depth and width), they can still become clinically visible. This occurs because the most part of the detection signal is sent by the lesion's body which can not be completely remineralized [25-27]. In this

present study the remineralization effectiveness was not assessed. Only the post-remineralization bond strength was evaluated. However, it was found that ICON® application did not either make impossible or damage a further adhesive procedure onto the same area. It should be considered that the model of the artificial lesion of the enamel in bovine teeth employed in this study should not be directly applied to daily clinics, because the human enamel lesions are deeper and other dynamic factors exist in in vivo conditions; notwithstanding, it is extremely viable as initial results of assessment [28].

In this present study, the mechanical test of microtensile bond strength was executed similarly to other researches, and its results may contribute for the comparative evaluation of the bond strength [29].

By observing the table 2, it can be seen that the group demineralized samples exhibited a very greatest number of cohesive fractures of the tooth substrate. Thus, it can be inferred that although the demineralization process had not interfered on the bond strength, such condition weakened the tooth structure, which can put all the restorative process at risk.

TABLE 1: MEAN (MPa) AND STANDARD DEVIATION OF THE BOND STRENGTH VALUES ACCORDING TO THE EXPERIMENTAL GROUPS.

GROUPS	MEAN	STANDARD DEVIATION
Sound	20.20	2.97
Demineralized	21.99	4.25
Remineralized	23.48	3.37
ICON®	22.10	4.03

TABLE 2: DATA FROM THE FRACTURE TYPES OF THE EXPERIMENTAL GROUPS.

Group	Fracture types			
	Mixed	Adhesive	Cohesive tooth substrate	Cohesive material
1- Sound	14	13	9	15
2- Demineralized	14	15	18	-
3- Remineralized	12	8	12	11
4- ICON®	14	13	4	2

ICON® infiltration showed a marked reduction in the number of cohesive fractures of either the material or substrate, displaying that the bond strength in such condition is really dependent on the capacity of the adhesive agent. Also, the substrate is more strengthened after ICON® treatment than after fluoride, probably because it fills the caries lesion.

Therefore, considering the conditions tested in this study, all hypotheses were accepted.

Further studies on both the fluoride concentration required to promote an effective deeper remineralization at clinical conditions and on the longitudinal assessment of ICON® effectiveness should be conducted. However, this present study

showed that even facing the lesion reagudization and need of a more radical restorative treatment, both conditions tested - fluoride and ICON® - did not affected negatively on the adhesion of the future restoration.

CONCLUSION

a) the enamel demineralization did not interfere on bond strength;

b) the remineralization did not interfere on bond strength;

c) the resin infiltration into the demineralized enamel did not interfere on bond strength.

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