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Microhardness of dentin underneath fluoride-releasing adhesive systems subjected to cariogenic challenge and fluoride therapy

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ABSTRACT

Objectives: The aim of this study was to evaluate the microhardness of dentin subjacent to the bonding interface of composite restorations using fluoride-releasing adhesive systems submitted to pH-cycling regimen associated or not to fluoride therapies.
Methods: Thirty human third molars were prepared with class V cavities with dentin cervical margins. The adhesive systems One-Up Bond F Plus (OU), Prime&Bond NT (NT), Clearfil Protect Bond (CF), Optibond Solo Plus (OP) and also the controls [−] Single Bond 2 (SB) and [+] Ketac Molar (KM) were used previously to composite resin restorations. The restorations were sectioned into four slabs and submitted to different storage media for 15 days: distilled water, pH-cycling, pH-cycling associated to NaF 0.05% and associated to NaF 1.23%. The Knoop microhardness test was performed in dentin at 50, 100, 150 and 300 μm from the adhesive interface. Data was analyzed by three-way ANOVA and Tukey HSD test ($p < 0.05$).
Results: KM resulted in significantly higher microhardness when compared to all the adhesive systems at 50 μm, with the exception of OU, that was similar to KM when submitted to pH-cycling alone or associated to 1.23% NaF. Microhardness of dentin was significantly higher with all the tested materials, when pH-cycling was associated to NaF 0.05%, at 50 μm and 100 μm depths. OU resulted in similar dentin hardness at all depths and storage media.
Conclusions: The incorporation of NaF 0.05% fluoride therapy to the cariogenic challenge was capable to recover the original microhardness of dentin at 50 and 100 μm with all the tested materials.

1. Introduction

Restorations with cervical margins in dentin and cementum are more susceptible to microleakage, postoperative sensitivity and secondary caries.[1] According to Mjör et al.,[2] secondary caries can be defined as lesions that are limited to margins of existing restorations, where the microbiota is very similar to that found in the primary caries.

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Studies have shown that secondary caries are the most important etiologic factor in restoration failure and the most common reason for replacing restorations.[3–6] According to Burgess,[7] secondary caries are often found in the cervical margins of restorations because of the difficulties involved in getting access to the root margin, ensuring sufficient isolation, removing carious tissue and inserting the restorative material. Factors such as these inevitably lead to micro gaps at the interface, which are associated with a greater probability of secondary caries developing as a result of the penetration of fluids and cariogenic microorganisms into this region.[4] In addition, an in vitro study showed that secondary caries are found to develop more often in root dentin, since minerals can be lost twice as fast in the root as in enamel.[8]

Fluoride has been incorporated in some restorative materials so that they can release this ion, which is incorporated by the tooth hard tissue, preventing secondary caries in cavity margins.[1] The ability of restorative materials to release fluoride and of the adjacent dentin to incorporate it are important factors which affect the cariostatic potential of fluoride.[9,10] According to Hahn et al.,[11] this potential is not sufficient to completely prevent secondary caries, as the fluoride concentration and the length of time during which fluoride is released depend on the materials, with a greater amount normally being released during the first 24 h.[9,6,12,13] Itota et al.[14] reported that adhesive systems that release fluoride are effective at preventing lesions in cavity walls but are not able to reduce the depth of the lesion. They concluded that the formation of secondary caries can only be prevented by a combination of a fluoride-releasing adhesive system and a restorative material that also releases fluoride.[14] Furthermore, while the fluoride released by adhesive systems is able to ensure the integrity of the cavity wall, it does not prevent secondary lesions.[15] Hence, the question whether the fluoride found in restorative materials and adhesive systems is able to reduce the incidence of caries continues to be subject of debate.

Restorative materials that contain fluoride include glass ionomer cements, resin-modified glass ionomer cements, compomers, some composite resins, surface sealants and dental amalgam.[16] Recently, a number of manufacturers of adhesive systems have included fluoride-releasing components in their products. However, the remineralizing effect of these materials appears to be insufficient, since the depth of penetration of fluoride ions released from the adhesive systems may be limited to the superficial dentin adjacent to the fluoride-releasing source, as the ions concentrates at the base of the hybrid layer.[17]

A very large number of in vitro models of cariogenic challenges have been used to simulate the pH of the oral cavity and produce artificial caries in enamel and dentin. These models can be static chemical models involving immersion in solutions such as acetic acid with a pH of 4.5,[1,18] artificial saliva with a pH of 7.0[15,19] or acidified gels with a pH of 4.25.[20] However, most studies use a dynamic chemical model with demineralization and remineralization cycles involving immersion in acid and neutral solutions for specific lengths of time.[11,21–30] Some studies have used solutions with pHs of 5.0 and 7.0 for 6 and 18 h, respectively, to simulate the cariogenic challenge.[15,22,24,25,27,30] Biological models that expose the substrate to one or more species of cariogenic microorganisms as a source of acid are also used.[9,31–33] Nevertheless, pH cycling is believed to be the method that most closely reflects the natural development of caries, as it simulates periods of demineralization and remineralization[34] and is able to produce caries similar to those produced in vivo.[26,35]

Different methods have been used to evaluate the degree of demineralization of root dentin. In a review, Featherstone[35] reported that both microradiograph and microhardness tests can be used to evaluate subsurface carious lesions quantitatively.[34] Microhardness testing is the method of choice for detecting changes in the consistency of the surface. Use of the Knoop microhardness test in the caries inhibition zone in root dentin adjacent to restorations with fluoride-releasing materials has also been reported in the literature.[21,24,25,30,31,36–40] However, there is a lack of studies in the literature regarding the effect of fluoridated therapies in association with fluoride-releasing materials following a cariogenic challenge.

Thus, the aim of this in vitro study was to evaluate the microhardness of dentin underneath the adhesive interface of composite resin restorations using fluoride-releasing adhesive systems subjected to a cariogenic challenge with and without daily and weekly fluoride therapy.

The first hypothesis to be tested in this study was that differences between the microhardness of dentin underneath fluoride-releasing adhesive systems and under conventional glass ionomer cement would not be detected. The second hypothesis to be tested was that topical fluoride therapies would increase dentin microhardness, irrespective of the material used.

2. Materials and methods

2.1. *Preparation of the specimens*

This study was approved by the local Research Ethics Committee (#0001312/07). Thirty healthy human third molars that had been extracted and stored in the tooth bank were used. The teeth were cleaned immediately after extraction using manual curettes and stored in 0.05% chloramine-T solution at 4 °C for a maximum of 6 months.

Class V cavities with a depth of 2 mm, mesiodistal width of 4 mm and gingival/occlusal height of 3 mm were made on the lingual and vestibular surfaces of the molars, with the occlusal margins in enamel and cervical margins in dentin (Fig. 1A). The cavities were prepared with a spherical carbide (#4 - KG Sorensen, São Paulo, SP, Brazil) burr rotating (Dabi Atlante, Ribeirão Preto, SP, Brazil) at high speed with constant air and water cooling. After five teeth had been prepared, the burr was replaced with a new one. The teeth were then divided at random into six groups of five teeth (i.e., 10 restorations) each and sectioned mesiodistally with a diamond disc (Fig. 1B and C) (#7020 - KG Sorensen, São Paulo, SP, Brazil) fitted to a chuck and a straight handpiece (Dabi Atlante, Ribeirão Preto, SP, Brazil) under a constant water spray.

The materials used for the control and experimental groups are described in Table 1. The bonding systems were used in accordance with the manufacturers' instructions. The cavities