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Influence of dentin pretreatment with 2.5% titanium tetrafluoride on inhibiting caries at the tooth-restoration interface *in situ*

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A R T I C L E I N F O

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A B S T R A C T

Objective: Investigate the effects of dentin pretreatment with 2.5% titanium tetrafluoride (TiF_4) aqueous solution followed by two-step self-etching (CLE/Clearfil SE Bond) and one-step self-etching adhesive systems (SBU/Single Bond Universal) on carious lesion inhibition at the tooth-restoration interface using an *in situ* model.
Design: Sixty-four cavities at the enamel-dentin junction of dental fragments were randomly distributed according to groups (n = 16): 1) TiF_4 + CLE; 2) TiF_4 + SBU; 3) CLE; 4) SBU. Cavities were restored using resin composite, and placed in intraoral palatal devices used by 16 volunteers for 21 days, to induce caries formation *in situ*. The fragments were then ground-flat to perform Knoop microhardness tests. Nine indentations were performed on each enamel and dentin substrate, subjacent to the restoration. Analysis of variance and Tukey's test were applied.
Results: Enamel: groups receiving TiF_4 dentin pretreatment (regardless of adhesive system and tooth-restoration interface distance) presented higher hardness means at a depth of 25 µm from the outer tooth surface ($p < 0.0001$). Dentin: groups receiving CLE presented higher means when applying TiF_4 pretreatment, whereas groups restored with SBU presented higher means without pretreatment ($p = 0.0003$).
Conclusions: Dentin pretreatment with TiF_4 inhibited demineralization of the enamel interface *in situ*, regardless of the adhesive, and TiF_4 pretreatment followed by CLE application showed higher potential for inhibiting dentin demineralization at the interface.

1. Introduction

The presence of secondary carious lesions has been reported as the main reason for the replacement of restorations (Lai, Zhu, Li, & Wang, 2013; Mjör, 2005). One of the more promising ways of inhibiting development or influencing lesion progression is by incorporating fluoride compounds into restorative materials or adhesive systems in either ionizable or non-ionizable form. Fluoride compounds act to reduce the solubility of the dental substrates in acidic media and/or potentialize the remineralization of these tissues (Naik, Subba Reddy, & Shashikiran, 2017; Robinson et al., 2000; Wiegand, Buchalla, & Attin, 2007). Some restorative materials containing fluoride-ion release include ionomeric materials, adhesive systems and pretreatment solutions. These have been found to be effective in inhibiting secondary carious lesions *in vitro* (Itota et al., 2005; Mayanagi, Igarashi, Washio, Domon-Tawaraya, & Takahashi, 2014; Naik, Naik, Subba Reddy, & Shashikiran, 2017; Shinohara et al., 2009), for a period of up to one year (Shiozawa, Takahashi, & Iwasaki, 2014).

Titanium tetrafluoride (TiF_4) is a fluoride compound used mainly to prevent primary carious lesions, and thus decrease demineralization *in vitro* (Magalhães et al., 2008; Medeiros et al., 2016; Tezel, Ergucu, & Onal, 2002). It also prevents these lesions from developing *in situ* and *in vivo* (Alcântara, Alexandria, Souza, & Maia, 2014; Büyükyılmaz, Ogaard, Duschner, Ruben, & Arends, 1997; Comar et al., 2012; Magalhães et al., 2008; Reed, & Bibby, 1976). It is available in the form of a gel, varnish or aqueous solution. In sound enamel, TiF_4 has proven effective in reducing demineralization after severe cariogenic challenge *in situ*, regardless of varnish or solution formulations (Comar et al., 2012). When applied topically on the enamel and dentin surface, TiF_4 has been found to prevent erosion and abrasion lesions (Hove, Holme, Young, & Tveit, 2008; Wiegand, Magalhães, & Attin, 2010; Wiegand et al., 2008).

When TiF_4 is applied as a dentin pretreatment, there is a lower potential risk of secondary carious lesions, achieved by promoting

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modification of the smear layer prior to application of the adhesive system. This pretreatment solution has been found to form a vitreous layer on the dentin (Basting et al., 2015; Sen & Büyükyilmaz, 1998), which considerably increases the nanohardness of this substrate *in vitro* and the resistance to erosive challenge (Basting et al., 2015). Nonetheless, self-etching and conventional adhesive systems are still able to infiltrate this modified layer and promote hybrid layer formation (Basting et al., 2017; Bridi, Amaral, França, Turssi, & Basting, 2013; Domingues et al., 2014; Tranquilin et al., 2016), thus maintaining (Basting et al., 2017; Bridi, Amaral, França, Turssi, & Basting, 2013; Domingues et al., 2014) and even improving bond strength values (Tranquilin et al., 2016).

In a previous study, Bridi et al. (Bridi, do Amaral, França, Turssi, & Basting, 2016) observed that pretreatment with TiF_4 followed by self-etching adhesive systems did not influence the anticariogenic potential *in vitro*. However, the calcium and phosphate concentrations present in the demineralization and remineralization solutions used to induce artificial carious lesions, as well as the pH values for caries induction *in vitro*, are not always similar to an intraoral situation (Moron et al., 2013). On the other hand, protocols for induction of carious lesions *in situ* are more similar to the clinical situation, due to the presence of saliva, biofilm and exposure to sucrose (Ogaard, & Rølla, 1992; da Silva, França, Flório, & Basting, 2010; Souza et al., 2016). Another consideration is that *in vitro* induced lesions are not capable of simulating biological events, such as bacterial penetration and collagen degradation (Marquezan et al., 2009; Moron et al., 2013). Overall, the *in situ* potential of inhibiting carious lesion development at the tooth-restoration interface with TiF_4 pretreatment should be evaluated further.

Therefore, the objective of the present study was to evaluate the inhibition of *in situ* caries formation in enamel and dentin at the tooth-restoration interface after using a 2.5% TiF_4 solution, followed by the application of two self-etching adhesive systems. The null hypothesis tested was that application of the TiF_4 solution prior to the adhesive systems evaluated would not promote an inhibitory effect on secondary caries formation *in situ* in enamel or dentin at the restoration margins.

2. Materials and methods

2.1. Experimental design

After approval by the Research Ethics Committee (CAAE no. 30721114.6.0000.5374), 64 cavities located at the enamel-dentin junction were prepared on the vestibular and lingual surfaces of 32 human third molars. The cavities were randomly separated and restored according to different treatments: application of dentin pretreatment with 2.5% TiF_4 or no application, and two different self-etching adhesive systems. All restored dental fragments were placed in intraoral palatal devices to be used by 16 volunteers. Each device received one fragment from each group, according to the treatment; each volunteer constituted a complete experimental block (four fragments for each device, with different treatments, as described below).

The experimental factors were: 1) application of a TiF_4 dentin pretreatment on two levels: present and absent; 2) application of a self-etching adhesive system, on two levels: two-step self-etching adhesive system (Clearfil SE Bond/Kuraray Medical Incorporation) and one-step self-etching adhesive system (Single Bond Universal/3 M ESPE); 3) the depth from the outer tooth surface where the indentations were performed: 25 μm, 50 μm, 75 μm and 100 μm from tooth surface; 4) the distance vertically away from the tooth-restoration interfaces where the indentations were performed: 50 μm, 100 μm and 150 μm from the adhesive interface. The response variable was assessed quantitatively by the Knoop microhardness test.

2.2. Cavities preparation and adhesive procedures

Thirty-two completely unerupted human third molars without coronal cracks or enamel malformations, stored in 0.1% aqueous thymol solution, were used in this study. The teeth were submitted to debridement with scalpel blades and periodontal curettes.

The cervical portions of the buccal and palatal/lingual surfaces of the teeth were used. The tooth was cut mid-region to separate the buccal from the palatal/lingual surfaces. Both sides were reduced to obtain a fragment measuring 5 mm x 5 mm x 3 mm thick, which included the cervical region of the tooth. A digital caliper (Mitutoyo Corporation, Kawasaki, Japan) was used to verify the measurements.

The fragments were sterilized in an autoclave (1 atm pressure–121 °C for 20 min) to ensure the absence of any type of microorganism that could interfere in the results, and to avoid contamination (da Silva, França, Flório, & Basting, 2010).

Sixty-four class V cavity preparations measuring 2.0 mm in diameter and 2 mm in depth were made with a standardized flat cylindrical bur #2292 (KG Sorensen Ind. e Com. Ltda, Barueri, SP, Brazil) with a stop, at high speed and under cooling with distilled water spray on both tooth surfaces (vestibular and lingual surfaces). The occlusal region of the preparation margins was located in the enamel, and the cervical region was located in the dentin. The burs were changed after every 10 cavity preparations to maintain uniformity of the preparations.

The treatments were randomly applied to four groups (n = 16), according to the presence or absence of dentin pretreatment with TiF_4, and to the adhesive system to be used. In the groups receiving the dentin pretreatment, TiF_4 pro-analysis was dissolved in deionized distilled water to achieve a concentration of 2.5% (wt/v; pH 1.2) (Basting et al., 2017; Bridi, Amaral, França, Turssi, & Basting, 2013; Bridi, do Amaral, França, Turssi, & Basting, 2016; Dündar, Ozcan, Cömlekoglu, & Sen, 2011). The TiF_4 solution was applied to the cavity walls actively with a disposable brush for 60 s, followed by air drying briefly for 5 s. The application was restricted to the cavity walls and limited to the cavosurface angle.

The adhesive systems were applied according to their corresponding group, following the manufactures' instructions. The primer of the Clearfil SE Bond two-step self-etching adhesive system was applied for 20 s, followed by application of the bond and photoactivation for 10 s. The adhesive of the Single Bond Universal one-step self-etching adhesive system was applied for 20 s and light-cured for 10 s. Description of the materials used and their compositions are presented in Table 1.

After applying the adhesive system, a nanoparticle resin composite (Filtek Z350) was placed using the incremental insertion technique (two oblique layers), followed by polymerization of each increment for 20 s. The light-curing appliance used was a LED device (Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein) with a light intensity of 1400 mW/cm^2. The resin restorations were polished using aluminum oxide discs of medium and fine granulation (Sof-Lex, 3 M, Saint Paul, MN, USA) to avoid residues of the resin composite and the adhesive system, and application of TiF_4 beyond the cavity margins.

2.3. Selection of volunteers and in situ caries formation

The participants in the experiment ranged between ages 18 and 30 years old. They were undergraduate or Master of Science students. The inclusion criterion comprised volunteers who did not have active carious lesions, who had low risk of developing caries or periodontal disease, and were not users of orthodontic appliances, removable prostheses or medications, nor could they be pregnant, breastfeeding or smokers. The risk of caries was evaluated by a clinical exam, and those who ultimately participated in the experiment received information about the research, risks and benefits. Additionally, all selected volunteers received a tube of dentifrice without fluoride (Sensodyne Original, Glaxosmithkline Brasil Ltda, Rio de Janeiro, RJ, Brazil), a