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Effect of sodium hypochlorite on primary dentin—A scanning electron microscopy (SEM) evaluation

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ABSTRACT

Objective: The aim of this study was to evaluate the alterations of etched deciduous dentin when submitted to different time and concentrations of sodium hypochlorite (NaOCl), using scanning electron microscopy (SEM).

Material and methods: Forty deciduous anterior teeth were selected, cleaned and ground until expose a flat dentin area on the buccal surface. The specimens were randomly distributed into eight groups ($n = 5$), according to dentin surface treatment (35% phosphoric acid etching for 7 s—AE and/or NaOCl application), NaOCl solution concentration (5% or 10%), and time of application (0, 30, 60, and 120 s), as follows: G1: control (without AE and NaOCl); G2: only AE; G3, G4, and G5: AE + 5% NaOCl for 30, 60, and 120 s, respectively; G6, G7, and G8: AE + 10% NaOCl for 30, 60, and 120 s, respectively. All specimens were prepared for SEM analysis and the photomicrographs (three for each specimen) were classified according to a score as follow: 0: presence of smear layer (SL); 1: absence of SL + non-altered collagen fibrils; 2: absence of SL + collagen fibrils slightly altered; 3: absence of SL + collagen fibrils severely altered; and 4: absence of SL and absence of collagen fibrils. Data were submitted to Kruskal–Wallis and Mann–Whitney tests ($p < 0.05$).

Results: All groups treated with NaOCl solution were significant different from G1 and G2, and showed alterations on the collagen fibrils network. Collagen complete removal was only observed when a 5% NaOCl solution was applied for 120 s and 10% NaOCl solution for 30, 60, and 120 s.

Conclusions: The NaOCl action produced significant changes in the etched deciduous dentin. The higher NaOCl concentration, the lower the time required to completely removing the collagen fibrils network in deciduous dentin.

1. Introduction

Acid etching on dentin substrate increases the micromechanical retention and decrease the marginal leakage[1] by removing the smear layer,[2] and exposing the collagen fibrils network. Due to the hydroxyapatite dissolution, the demineralized substrate become a rough and high surface energy substrate.[3]

The concept of dentin adhesion is based on micromechanical retention: monomers impregnate the exposed collagen network of demineralized superficial dentin, and upon polymerization, result in the formation of a hybrid layer.[4–7]

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For some authors, the formation of a hybrid layer is an essential condition for improving the bond strength between composite resin and dentin.[6,8–12]

Although the hybrid layer has been described being responsible for the restoration's longevity, some studies[13,14] have shown leakage within the hybrid layer. This phenomenon might be due to hydrolytic degradation of the exposed collagen fibrils. This has been related to the poor infiltration of monomers into the collagen fibrils, at the bottom of the hybrid layer, remaining after dentin acid etching, creating a weak zone. This zone is vulnerable to degradation after a long-term exposure to water.[6,13,14]

Dissolution of the collagen fibrils after acid etching has been proposed,[4,15–19] and may result in better monomer diffusion by increasing dentin permeability and changing its composition.[18]

Because of its non-specific deproteinizing and disinfecting action,[20] sodium hypochlorite solution (NaOCl) is widely used in various dental procedures, such as chemomechanical treatment in endodontic preparations, chemomechanical removal of carious lesions in dentin (e.g., Carisolv™), and in dentin adhesion procedures.[4,7,9,15–19,21–23]

The dissolution of organic tissues by sodium hypochlorite solutions is based on the action of chloride over the proteins, forming chloramines, which are soluble in water. This reaction is directly proportional to the active chloride concentration present in the solution.[24] Sodium hypochlorite solution alters the configuration or removes the organic components of dentin; especially, the collagen fibrils.

The deproteinized dentin has higher hardness, modulus of elasticity,[20] wettability,[25] and permeability[26] than the demineralized dentin. The dentin substrate is transformed, after deproteinization, in a very porous structure with multiples irregularities and anastomoses, which could not be seen only by the normal demineralization process.[22] These substrate characteristics could promote an increase on bond strength of adhesive systems applied over the deproteinized substrate.[4,19,21]

However, depending on the adhesive system used, dentin surface treatment with sodium hypochlorite can increase,[4,16,18,19,21,23,27] decrease,[7,11] or not interfere[9,15,27] in the bond strength between composite resin and dentin.

Although the efficiency of sodium hypochlorite on bonding procedures has been proved on permanent dentin,[19,21,23,27] there is no standardization for sodium hypochlorite application parameters (concentration, time of application) on deciduous dentin in the literature. Wakabayashi et al.[21] established that a 10% sodium hypochlorite solution applied for 60 s is the ideal treatment for the complete removal of the collagen fibrils. But, this study was performed using bovine permanent dentin.

Due to the morphological and constitutional differences of deciduous vs. permanent teeth,[28] their bonding behavior differs. The dentin of deciduous teeth is more susceptible to acid etching,[29,30] and it is likely that the application of NaOCl solution to that substrate would produce different alterations when compared with those observed in permanent teeth.

The aim of this study was to evaluate the micromorphological alterations promoted by the application of sodium hypochlorite solution, in different concentration and time of action, on deciduous dentin. The hypothesis tested was that the higher the concentration of the sodium hypochlorite solution, the lower the time of application of this solution for total depletion of exposed collagen fibrils.

2. Material and methods

A 35% phosphoric acid gel (3M/ESPE) and 5% or 10% sodium hypochlorite solutions (NaOCl) pH 12.4, prepared one day before the use (Proderma Farmácia de Manipulação LTDA, Piracicaba, SP, Brazil), were used in this study for demineralization and deproteinization protocols, respectively.

2.1. *Specimen preparation*

Forty sound human anterior deciduous teeth were selected, cleaned and stored in a 0.5% Chloramine T solution at 4 °C for no more than a week. After removing the roots or root remains, 1 mm below the cemento–enamel junction, the crowns were ground on a water-cooled mechanical grinder and polisher (Metaserv 2000, Buehler, UK LTD, Lake Bluff, IL, USA) using 320-, 400-, and 600-grit silicon carbide (SiC) abrasive paper (Carbimet Disc Set, #305178180, Buehler, UK LTD) in order to expose a flat dentin area on the buccal surface. The specimens were observed in a stereomicroscope (Zeiss, Manaus, AM, Brazil), at 25× magnification, to verify if the enamel was completely removed.

The specimens were randomly assigned into eight groups ($n = 5$), according to the treatment of the substrate (acid etching—AE and/or NaOCl application), the concentration (5% or 10%) and the time of application of the NaOCl solution (0, 30, 60, and 120 s). The distribution of the groups is shown in Table 1.

The 35% phosphoric acid gel (3M/ESPE) was applied for 7 s,[31] copiously rinsed for 15 s and blot-dried in all groups, except G1 (control) in which no treatment was carried out over the smear layer. After the demineralization protocol, the specimens were deproteinized by a 5% or 10% NaOCl solution application for 30, 60, or 120 s, according to the distribution of the groups (Table 1). Next, the specimens were copiously rinsed with distilled water in a continuous flow for 30 s and carefully dried, to avoid the contamination of the specimens.

After the demineralization and the deproteinization protocols, the specimens were prepared for the scanning electron microscopic (SEM) evaluation. All specimens were fixed by immersion in 2.5% glutaraldehyde/2% paraformaldehyde in 0.1 M sodium cacodylate buffer at pH 7.4 for 1 h with three changes, followed by deionized water for 1 min. They were then dehydrated in ascending grades of ethanol (25% for 20 min, 50% for 20 min, 75% for 20 min, 95% for 30 min, and 100% for 60 min). After the final ethanol step the specimens were dried by immersion in hexamethyldisilazane (HMDS) for 10 min, placed on a filter paper inside a covered glass vial, and air-dried at room temperature.[32]

The specimens were mounted on aluminum stubs with double-sided carbon tape (SEM, NISSHIN EM Co. Ltd., Tokyo, Japan), and sputter coated at 10 mA for 2 min (SCD050 sputter coater, Balzers, Liechtenstein). They were observed under a Scanning Electron Microscope (JSM 5600LV, JEOL, Tokyo,