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Full Length Article

Comparison of deproteinization agents on bonding to developmentally hypomineralized enamel

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ABSTRACT

Objective: To compare bonding of dental adhesive to hypomineralized enamel (HE) after pre-treatment with either 5% sodium hypochlorite (NaOCl) solution or papain-based papacarie gel.

Methods: Normal enamel (NE) and HE obtained from hypomineralized first permanent molars were acid-etched with 32% phosphoric acid and randomly allocated into no deproteinization, deproteinization using 5% NaOCl, or deproteinization usping papacarie gel groups. Subsequently, the specimens were bonded, packed with composite resins and subjected to micro-shear bond strength (MSBS) testing and the data analysed using 2-way ANOVA and Tukey tests. Furthermore, specimens from all groups were subjected for qualitative analysis using scanning electron microscope.

Results: Two way-ANOVA showed that the factor "enamel substrate" was significant ($p < 0.001$), "enamel pre-treatment" was not significant and interaction of the two factors was significant ($p = 0.005$). HE produced inferior bonding with dental adhesive compared to NE. Enamel pre-treatment with deproteinization agents enhanced bonding to HE. No significant difference in MSBS was evident between the two deproteinization agents ($p > 0.05$). Qualitative analysis of acid-etched moderate HE showed barely visible enamel rods with irregular etching pattern. Following acid etching and deproteinization, Type I and II etching patterns were observed in moderate HE; while a porous enamel surface with more profound etching patterns in severe HE.

Conclusions: Papain-based papacarie could be an alternative deproteinization agent for bonding dental adhesive to HE.

Clinical significance: Papain-based papacarie, a natural deproteinization agent and a proven chemo-mechanical caries removal agent could be an alternative to NaOCl for enhancement of bond durability of adhesive restorations to HE.

1. Introduction

Molar-Incisor Hypomineralization (MIH) is a condition of systemic origin that involves one to four first permanent molar teeth and often associated with affected incisors [1]. It has been reported that approximately 1 in 5–6 children are affected with this condition [2]. Second primary molars, tips of permanent canine cusps, second permanent molars and premolars have also found to be affected in patients presenting with MIH [3]. The affected teeth exhibit demarcated enamel opacities, which vary in colour and extent. The affected enamel represents the degree of hypomineralization and can be either white (mild), creamy or yellow (moderate) or brown (severe) in colour. The extent of the defective enamel can vary from a very small to a large area, and in some cases involving an extensive part of the crown.

When compared to normal enamel (NE), hypomineralized enamel (HE) exhibited a mean 28% reduction in its mineral content, 80% more carbonated apatite and 3- – 15- fold increase in its protein content [4–6]. The hardness of HE was also significantly lower than NE [4]. The chemical analysis of HE has demonstrated a reduction in both the Ca, P concentrations and mean Ca/P ratio; while C, Mg and K concentrations were increased compared to NE [7,8].

Bonding to HE from teeth affected with MIH is very challenging due to the altered physical and chemical characteristics. The increased protein content in HE is the main challenge for bonding to this altered

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substrate [9,10]. Recently, a systematic review on bonding to HE [11] concluded that resin dental adhesives achieved an inferior bonding to HE when compared to NE. Furthermore, the review also reported that enamel deproteinization with 5% sodium hypochlorite (NaOCl) before adhesive application procedure may potentially enhance the bonding of resin dental adhesives to HE [11].

Five percent NaOCl is an effective deproteinization agent and several concentrations (5.25%, 2.5%, 1% and 0.5%) of NaOCl have been used for irrigation during root canal preparation. Sodium hypochlorite removes the excess protein content from the HE and thereby, improving bonding of dental adhesive to that substrate [12]. "Papacarie®" is a papain-based natural deproteinizing agent synthesized from leaves and fruits of green adult papaya. It is a papain-based gel containing chloramine, toluidine blue, salts, preservatives, stabilizers, thickener and deionized water [13]. It has antibacterial, proteolytic and anti-inflammatory properties and therefore have been used in chemomechanical caries removal [14]. Recently, enamel pre-treatment with Papacarie® has been shown to increase bond strength of orthodontic brackets [15]. However, to date, there are no published reports comparing the deproteinization effect of 5% NaOCl and Papacarie on HE and bonding of dental adhesives. Therefore, the present study aimed to compare bonding of dental adhesive to HE after pre-treatment with either 5% NaOCl solution or papain-based Papacarie gel. The hypothesis tested were: (1) There is no difference in the bond strength between NE (obtained from MIH-affected teeth with no visible sign of hypomineralization) and HE when bonded with dental adhesive, (2) pre-treatment of HE with de-proteinization agents would not have an effect on bonding with dental adhesive and (3) there is no difference in the bonding performance of dental adhesive to HE after pre-treatment with either 5% NaOCl solution or Papacarie gel.

2. Materials and methods

2.1. Teeth collection

Twenty-seven hypomineralized first permanent molars that were extracted from patients below 18 years of age for reasons other than for use in this research project were collected over 12 months period after getting an informed consent approved by The University of Hong Kong/Hospital Authority of Hong Kong West Cluster (Reference number UW 15-381). The collected teeth were stored in 0.5% Thymol solution at 4 °C until use.

2.2. Identification of normal (NE) and hypomineralized enamel (HE) substrates

Three authors of this study (ME, CY and GSR) independently inspected the hypomineralized teeth and included only those teeth that fulfilled the MIH judgement criteria specified by Weerheijm et al. [16]. The included teeth were visually inspected under day light conditions in order to differentiate HE and NE. Demarcated areas of discoloured enamel were identified as HE and enamel with no apparent discolouration was identified as NE. Furthermore, HE was carefully examined based on its colour in order to differentiate the severity of the defect (Fig. 1). Accordingly, the demarcated areas of HE were categorized as "creamy-white (CW)" or "yellow-brown" (YB) [17]. In order to obtain samples with adequate thickness, NE was obtained only from the occlusal half of the tooth. For HE, enamel specimens less than 2–3 mm wide were excluded [17]. The number of HE specimens obtained per tooth was dependent on the extent of severity for that particular tooth. Accordingly, specimens per tooth varied from one to four in number.

2.3. Enamel specimen preparation for bond strength testing

The roots of the teeth were removed from the crown using a slow-speed diamond impregnated disc (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under water-cooling. The crown was further sectioned along the long axis of the tooth in different planes to prepare the specimens. The sectioned specimens were minimally ground on 600-grit SiC paper under running water for 10 s to remove the surface prismless layer and create a flat surface suitable for bonding. The prepared enamel specimens were carefully embedded in acrylic cylindrical molds using self-cure acrylic resin. The specimens were randomly allocated (n = 10) to the experimental groups for bonding. For experimental groups with HE, the number of specimens with CW and YB were similar between the groups.

The specimens were divided into the following six experimental groups:

Group 1 – NE; no deproteinization
Group 2 – NE + deproteinization using 5% NaOCl
Group 3 – NE + deproteinization using Papacarie gel
Group 4 – HE (5 CW, 5 YB); no deproteinization
Group 5 – HE (5 CW, 5 YB) + deproteinization using 5% NaOCl
Group 6 – HE (5 CW, 5 YB) + deproteinization using Papacarie gel

2.4. Enamel pre-treatment and bonding procedure

Enamel specimens from Groups 1 and 4 were etched with 32% phosphoric acid gel (Scotchbond™ Universal Etchant, 3 M ESPE, St. Paul, MN, USA) for 10 s, followed by rinsing with de-ionized (DI) water for 10 s. The enamel specimens from Groups 2 and 5, after the acid etching and rinsing procedures, were treated with 5% NaOCl (Henan Hairen Biotechnology Co. Ltd, China) as a deproteinizing agent for 60 s (applied using microbrush), followed by rinsing with DI water for 10 s. The enamel specimens from experimental Groups 3 and 6, after the acid etching and rinsing procedures, were treated with papain-based Papacarie gel (Papacarie Duo®, F & A Pharmaceutical Laboratory Ltd, Sao Paulo, Brazil) as a deproteinizing agent for 60 s (applied using microbrush), followed by rinsing with DI water for 10 s. The specimens from all the groups were air-dried, bonded with 2 coats of Adper Single Bond 2 (3 M ESPE, St. Paul, MN, USA), following manufacturer's instructions and were subsequently light-cured for 10 s using a quartz-halogen light-curing unit (Optilux, Demetron-Kerr, Orange, CA, USA) with a constant output intensity of 600 mW/cm^2. The bonded enamel specimens from Groups 1–6 were built up with resin composite (Filtek™ Z250 Universal Restorative composite resin, 3 M ESPE, St. Paul, MN, USA) through custom-made brass cylindrical tubes (Fig. 2). The brass cylindrical tubes were stabilized on the bonded enamel specimens using a custom-made jig (Fig. 2).

The height of the jig was 150 mm, which gave the operator ample room to manipulate the substrate and specimens with no interference with the arms "c" and "d", as they were locked to the top end of the shaft when not in use. This was achieved by assembling a linear bearing shaft "b" with two pairs of ball bushing pillow blocks on a platform "a". Each pair was interconnected with an L-shaped connecting aluminium plate to form arms "c" and "d". Arm "c" was used as illustrated in Fig. 2 (close-up view), where the flanges hold on the wings of the brass tube, stabilizing it against the substrate. The beveled edges of the inner diameter of the brass tube helped to achieve a tight seal with any leakages noticeable during compaction. The arm "d" was mounted with a plunger "e", the diameter of which corresponded to approximately the inner diameter of the brass tube, to facilitate even distribution of the compaction forces. A Teflon tape was used on the plunger tip during compaction to prevent the pullback effects of the composite from affecting the bond strength.

2.5. Bond strength test

The bonded enamel specimens, stored in deionized water at 37 °C for 24 h, were subjected to microshear bond strength testing (MSBS). The brass cylindrical tubes were not removed prior to bond strength