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Hybridization quality in cervical cementum and superficial dentin using current adhesives

Yang Yuan[a,b,*], *Yasushi Shimada*[a], *Shizuko Ichinose*[c], *Junji Tagami*[a,b]

[a] *Department of Cariology and Operative Dentistry, Tokyo Medical and Dental University, Tokyo, Japan*
[b] *Center of Excellence Program, FRMDRTB at TMDU, Tokyo, Japan*
[c] *Instrumental Analysis Research Center, Tokyo Medical and Dental University, Tokyo, Japan*

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ABSTRACT

Objectives. The aim of this research was to determine the hybridization quality of adhesives to gingival cementum and close superficial dentin using both total-etch and self-etch, one-step and two-step adhesives in vitro.

Methods. Five adhesive systems were used and evaluated in this study; three kinds of two-step adhesives (total-etch—Single Bond and self-etch—Clearfil SE Bond and Clearfil Protect Bond) and two one-step adhesives (Clearfil S^3 Bond, G Bond). Fifteen extracted intact human third molars were used in this study. A diagonal cut which was approximately 45° to the long axis of the roots, with the initiating point located 2 mm below the buccal enamel–cementum junction and ascending towards the pulp chamber was prepared on each tooth. Flat cervical cementum and dentin surfaces were ground with wet 600-grit silicon carbide paper, and bonded with one of the adhesives and finished by applying a flowable resin composite. After 24 h storage at 37 °C in water, the bonded assemblies were sectioned into approximately 1 mm thick slabs. Two central slabs from each tooth were chosen. One slab was totally demineralized in 0.5 M EDTA and the other was not demineralized and immersed into 50% (w/v) solution of ammoniacal silver nitrate for 24 h, and successively exposed to photodeveloping solution for 8 h. The specimens were then processed for TEM observation. Both the stained demineralized silver unchallenged and unstained non-demineralized silver challenged resin–cervical cementum/proximal superficial dentin interface were observed and evaluated under a transmission electron microscope.

Results. The nanoleakage pathway and extent vary among the different adhesives used and also between the resin–cementum interface and resin–dentin interface. Two-step self-etch adhesives showed better hybridization quality both in cementum and proximal superficial dentin as compared to those of two-step total-etch adhesive and one-step self-etch adhesives.

Significance. Two-step self-etch adhesives may provide a better sealing in cervical cementum and the proximal superficial dentin region.

* *Corresponding author at*: 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Japan. Tel.: +81 3 5803 5483; fax: +81 3 5803 0195.
E-mail address: yuanyang.ope@gmail.com (Y. Yuan).

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1. Introduction

Recently, with the improvement of adhesive techniques and development of new resin materials, the frequency of marginal leakage and cavity wall gap formation has decreased dramatically. However, evidence of insufficient marginal sealing of bonded restorations could still be found in various microleakage studies [1,2]. Elderly people are at high risk to have sub-gingival and root caries. The margins located below the cementum–enamel junction are cervically limited by a layer which is different from bulk dentin [3], and proximal superficial dentin which lacks dentinal tubules [4]. Cementum in the cervical area belongs to acellular extrinsic fibre cementum. Inserted by Sharpey's fibres, the overall degree of mineralization is about 45–60%. The composition of cementum contains about 50% organic matrix and type I collagen is the predominant organic component [5]. The cervical margin located in cementum–dentin was still thought to be the most unpredictable area of an adhesive resin restoration [6]. Failure is seen primarily in adhesive resin restorations when the cervical margins are below the cementum–enamel junction in the case of class V cavities.

The current adhesives were mainly classified as total-etch adhesives, which employ separate inorganic acid to pre-treat dental substrate and self-etch adhesives, which contain adhesive promoting monomer in the primer agent to combine etching and priming into one step. Furthermore, the latest single step adhesives combined etching, priming and bonding into one bottle with the aim of shortening the application time and decrease technique sensitivity. So far, all marketed products permit various amounts of nanoleakage formation. Nanoleakage pathway was thought to be initiated at the interface between the adhesive-hybrid layer, the weakest point within the dentin-restoration junction [7,8], or within the hybrid layer which was not perfectly impregnated by bonding resin [9,10]. High quality bonding to cervical cementum and proximal superficial dentin is very important to prevent leakage (both microleakage and nanoleakage) and bacteria gaining access to the pulp. However, so far few researches concerning the most peripheral marginal seal have been reported using these current adhesives.

The aim of this research was to determine the hybridization quality of current adhesives to cervical cementum and proximal superficial dentin using both total-etch and self-etch, one-step and two-step adhesives in vitro. Stained demineralized silver unchallenged and non-demineralized silver challenged resin–cervical cementum/proximal superficial dentin interfaces were observed and evaluated under a transmission electron microscope to understand the possible nanoleakage pathway of this region.

2. Materials and methods

2.1. Tooth preparation

Fifteen extracted intact human third molars used in this study were collected after patient's informed consent was obtained under a protocol approved by the Institutional Review Board of the Tokyo Medical and Dental University. Before use, they were stored at 4 °C in isotonic saline saturated with thymol. A diagonal cut which was approximately 45° to the long axis of the root, with the initiating point located 2 mm below the buccal enamel–cementum junction was made using a slow-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under water lubrication. All the cervical cementum/dentin surfaces were finished with 600-grit SiC papers under running water to create a standardized smear layer.

2.2. Cementum/dentin bonding and specimen preparation

Five adhesives were evaluated: a two-step total-etch wet bonding adhesive (Single Bond—SB, 3 M ESPE, St. Paul, MN, USA), two two-step self-etch adhesives (Clearfil SE Bond—SE and Clearfil Protect Bond—PB, Kuraray, Osaka, Japan), and two one-bottle one-step self-etch adhesives (G Bond—GB, GC company, Tokyo, Japan and Clearfil S^3 Bond—TB, Kuraray, Osaka, Japan). Composition, batch number, application instructions, and manufacturers are listed in Table 1. The adhesives were applied to the cervical cementum and dentin surfaces and light-cured (600 mW/cm^2) prior to the placement of a light-cured low-viscosity flowable resin composite (Protect Liner, Lot #: 051120, Kuraray Medical Inc., Osaka, Japan) instead of resin composite to reduce damage to the diamond knife.

2.3. TEM observation of resin–cementum/superficial dentin interface

After storage in water for 24 h at 37 °C, the adhesive coated specimens were vertically sectioned, with a diamond saw (Isomet, Buehler Ltd., USA) under water lubrication through the adhesive layer and the underneath cementum/dentin, into approximately 1 mm thick slabs. These slabs were further trimmed to get approximately 5 mm × 1 mm × 3 mm sticks. Two central sticks were chosen from each of the bonded integrities, forming a total of six specimens per adhesive used. Successively, three out of six specimens were totally decalcified in 0.5 M EDTA solution for 3 weeks at room temperature. Demineralized, epoxy-resin-embedded, 90-nm thick sections were prepared following the TEM protocol described in detail [11,12]. The ultra-thin sections were stained with uranyl acetate for 20 min and lead citrate for 5 min and examined with a transmission electron microscope (H-7100, Hitachi Ltd.) at an operating voltage of 75 kV.

2.4. Nanoleakge evaluation

Three remaining specimens were used for nanoleakage evaluation. Bonded sticks were painted with two layers of fast-drying nail varnish applied up to within 1 mm of the adhesive layer. Tooth sticks were placed in the ammoniacal silver nitrate solution in total darkness for 24 h and exposed under a fluorescent lamp for 8 h following the tracer protocol previously reported [11]. They were processed for TEM observation in the same way as mentioned above without staining. Evaluation of nanoleakage formation in the cervical resin–cervical cementum and resin–proximal superficial dentin interface was carried out.