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Hybrid layer morphology on sound and caries-affected primary dentin

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Key words: primary teeth, adhesive systems, hybrid layer, caries-affected dentin, sound dentin

Abstracts:

Introduction: Resin composite is the most suitable esthetic alternative option for primary teeth restoration. Dentists encounter with caries-affected dentin (CAD) nor sound (SD), in every day practice. Caries-affected dentin is uninfected, only partially demineralized and remineralizible under physiological conditions. Morphological features of CAD can affect hybrid layer formation.

The aim of this in vitro study was to evaluate morphology of hybrid layer produced by different adhesive systems on sound and caries-affected primary dentine.

Materials and methods: Forty sound and caries-affected extracted primary molars were used in this study. Teeth from each substrate (SD and CAD) were randomly allocated to 4 groups, according to adhesive systems (Optibonf FL (Kerr), Optibond SoloPlus (Kerr), Single Bond Universal in etch&rinse and self-etch mode (3M)). Hybrid layer morphology and homogeneity, presents of resin tags and gaps were analyzed in each specimen by FE-SEM (JSM-6700F JEOL, Japan). Statistical analysis was conducted with Kruskal-Wallis test ($p<0,05$).

Results: All adhesives formed clearly visible hybrid layer with numerous resin tags on CAD of primary teeth, even self-etch mode. The resin tags were shorter irregular hybrid layer was porous. The porous zones were visualized beneath the hybrid layer in CAD and etch&rinse adhesive systems. Dentin tubules were often obliterated with rhomboid crystals which wasn't removed by acid. Some specimens of caries-affected dentin had microcavities, cracks and porosity under hybrid layer. There were no significant differences in hybrid layer thickness between SD and CAD for each adhesive system.

Conclusions: Etch&rinse adhesive systems produce pronounced hybridize complex on sound and caries-affected primary dentin. Dentin substrate and adhesive system type affects hybrid layer formation.

Introduction.

Resin composite is the most suitable esthetic alternative option for primary teeth restoration [1]. Self-etch or etch&rince adhesive approach can be used is this case [2]. Nowadays, universal adhesive systems became more popular due to technical friendly [3]. Also, lots of investigations are focused on sound primary or permanent dentin. Despite this, dentists encounter with caries-affected dentin (CAD) nor sound (SD), in every day practice. Caries-affected dentin is uninfected, only partially demineralized and remineralizible under physiological conditions. It should be preserved during minimal-invasive caries tissues removing [4]. Caries-affected dentine characterized by less mineral content, reversibly decreasing of collagen cross-links, minerals depositions in dentin tubules, high water content. Also, CAD is softer then SD [5,6,7,8,9]. This features are important for long-term durable bonding.

The previous studies demonstrate that adhesive systems produce thicker hybrid layer not only on primary dentin compare to permanent, but on CAD too [10, 11]. Bond strength is lower to CAD, with cohesive failure type predominantly [12]. Additionally, more researches were conducted on artificially-induced caries-affected dentin model nor natural CAD of primary teeth. It is very important to evaluate adhesive systems behavior on natural caries-affected primary dentin [3, 13].

The aim of this in vitro study was to evaluate morphology of hybrid layer produced by different adhesive systems on sound and caries-affected primary dentine. The null hypothesis was "there are no significant differences in the hybrid layer morphology on sound and caries-affected dentin".

Materials and methods:

Forty sound (SD) and caries-affected (CAD) extracted for orthodontic reasons primary molars were used in this study. The teeth were stored in 0,5% chloramine solution at 4^0C no more than one month. Occlusal third was removed with slow-speed water-cooled diamond saw. In CAD substrate the caries tissue was remover with round carbide bur in low-speed handpiece. In SD substrate the standard smear layer was created by wet sanding with 600-grit SiC paper. Teeth from each substrate (SD and CAD) were randomly allocated to 4 groups, according to adhesives (Optibond FL (Kerr), Optibond SoloPlus (Kerr), Single Bond Universal in etch&rinse and self-etch mode (3M)) (Table 1). For etch&rinse adhesives dentin was etched with 37% phosphoric acid per 15 s (according to manufacturer's recommendations), rinsed with water for 30 s and wet-dried with air. The tested adhesives were applied according to manufacturer's recommendations and light-cured for 20 s. Bonded dentin surfaces were built-up with 2 mm of resin composite Filtek Universal (3M) and cured for 20 s. Then, they were stored in distillated water for 24 h at 37^0C. Next, restored teeth were cross-sectioned perpendicularly to resin-dentine interface with slow-speed water-cooled diamond saw. Prepared specimens were wet-polished with decreasing abrasiveness SiC paper (600, 1000, 1200, 1500, 2000 and 2500-grit) and ultrasonically cleaned for 10 min. Polished surfaces were etched with 37% phosphoric acid for 15 s, rinsed with distillated water and deproteinized with 2,5% sodium hypochlorite per 15 min followed by rinsing in distillated water. Then, the specimens were air-dried and dehydrated in ascending ethanol concentrations (50%, 75%, 95% and 100%) for 20 min in each solution. Mounted specimens were platinum-sputtered under high vacuum (JFC-1600 JEOL, Japan)) and evaluated by FE-SEM (JSM-6700F JEOL, Japan)) in second electron mode, at 10 kV accelerating voltage, under 500-40000x magnifications. Hybrid layer morphology and homogeneity, presents of resin tags and gaps were analyzed in each specimen. Statistical analysis was conducted with Kruskal-Wallis test and Mann-Whitney U test post hoc ($p<0,05$).

Table 1. Materials, composition and application mode

Adhesive system (manufacturer)	Main components	pH	Application mode
Optibond Fl (Kerr) (OBFL)	**Primer:** HEMA, GPDM, PAMM, ethanol, water, CQ **Adhesive:** Bis-GMA, HEMA, GPDM, CQ, glycerol,	1,9 6,9	Dentin etch for 15 s, rinse and wet-dry, apply Primer for 15 s, air dry 10 s, apply

	dimethacrylate resins, barium aluminoborosilicate glass, solicon dioxide, sodium hexafluorsilicate (filled 48%wt)		Adhesive for 15 s, air dry for 5 s and light-cure for 20 s
Optibond Solo Plus (Kerr) (OBSP)	Bis-GMA, HEMA, GDP, GPDM, CQ, water, barium aluminoborosilicate glass, solicon dioxide, sodium hexafluorsilicate (filled 15%wt)	2,2	Dentin etch for 15 s, rinse and wet-dry, apply adhesive for 15 s, air dry for 5 s and light-cure for 20 s
Single Bond Universal (3M) (SBU)	MDP phosphate monomer, dimethacrylate resins, HEMA, Vitrebond copolymer, filler, ethanol, initiators and silane	2.7	**Etch&rinse mode:** Dentin etch for 15 s, rinse and wet-dry, apply adhesive for 15 s, air dry for 5 s and light-cure for 20 s **Self-etch mode:** apply adhesive for 15 s, air dry for 5 s and light-cure for 20 s

Results:

SEM morphology of hybrid layer in dentin varied according to evaluated adhesives systems and dentin substrate (SD or CAD). Hybrid layer and resin tags were observed in each case (Fig 1.). All etch & rinse adhesives produced uniform hybrid layer with numerous resin tags on sound dentine. When dentin was etched with phosphoric acid for 15 s the smear layer and plugs were completely removed and dentin tubules orifices were opened and enlarged. OBFL formed significantly thick hybrid layer (4,15 **µm**) ($p<0,05$) witch was closely adapted to dentin surface. Numerous cone-shape resin tags with lateral branches were observed in dentin tubules. In case of OBSP submicron hybrid layer was defined (1,04 **µm**) and was visible only under high magnification (Table 2). All dentin tubules were completely sealed with cone-shape resin tags with numerous lateral branches. For OBFL and OBSP internal triangular hybridization of dentin tubules walls were typical. SBU produced substantial and continuous hybrid layer (3,68 **µm**) in etch&rinse mode. Resin tags were dense and numerous with unit lateral branches. SBU formed unclearly visible hybrid layer (2,85 **µm**) in self-etch mode. Hybridized area was wide deboned along dentin or composite interface. Unit short tape cylindrical resin tags were revealed. Majority of dentin tubules orifices were blocked by resin impregnated smear plugs. There was no significant difference in etch&rinse and self-etch mode of hybrid layer thickness for SBU.

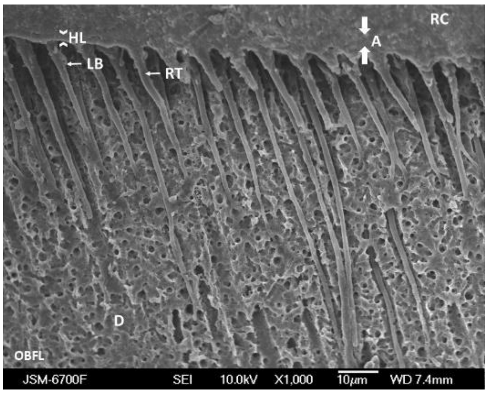
Table 2. Hybrid layer thickness (µm) means and standard deviations on sound and caries-affected primary dentin

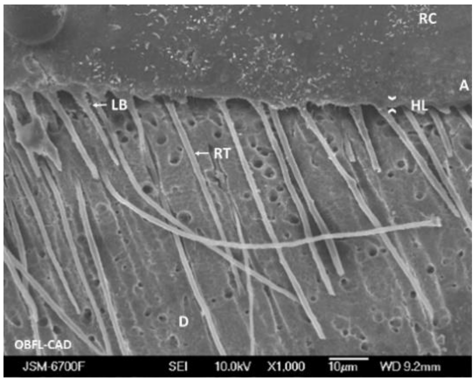
Adhesive system	Sound dentin [b]	Caries-affected dentin [b]
Optibond Fl (Kerr)	4,15±0,64	3,31±0,23
Optibond Solo Plus (Kerr)	1,04±0,21	1,44±0,31
Single Bond Universal (3M) etch&rinse mode	3,68±0,63	3,52±0,42
Single Bond Universal (3M) self-etch mode	2,85±0,81	1,87±0,42

[a] significant different between substrate

[b] significant different between adhesive system

Resin-dentin interface morphology for caries-affected primary dentin had some differences for evaluated adhesive systems. All adhesives formed clearly visible hybrid layer with numerous resin tags, even self-etch mode of SBU. But, the resin tags were shorter irregular and easily broken during specimen preparation, hybrid layer was porous. Also, the porous zones were visualized beneath the hybrid layer in caries-affected dentin and etch&rinse adhesive systems. Dentin tubules were often obliterated with rhomboid crystals which wasn't removed by acid. Some specimens of caries-affected dentin had microcavities, cracks and porosity under hybrid layer. Cohesive type of debonding was the most typical. There were no significant differences in hybrid layer thickness between SD and CAD for each adhesive system.





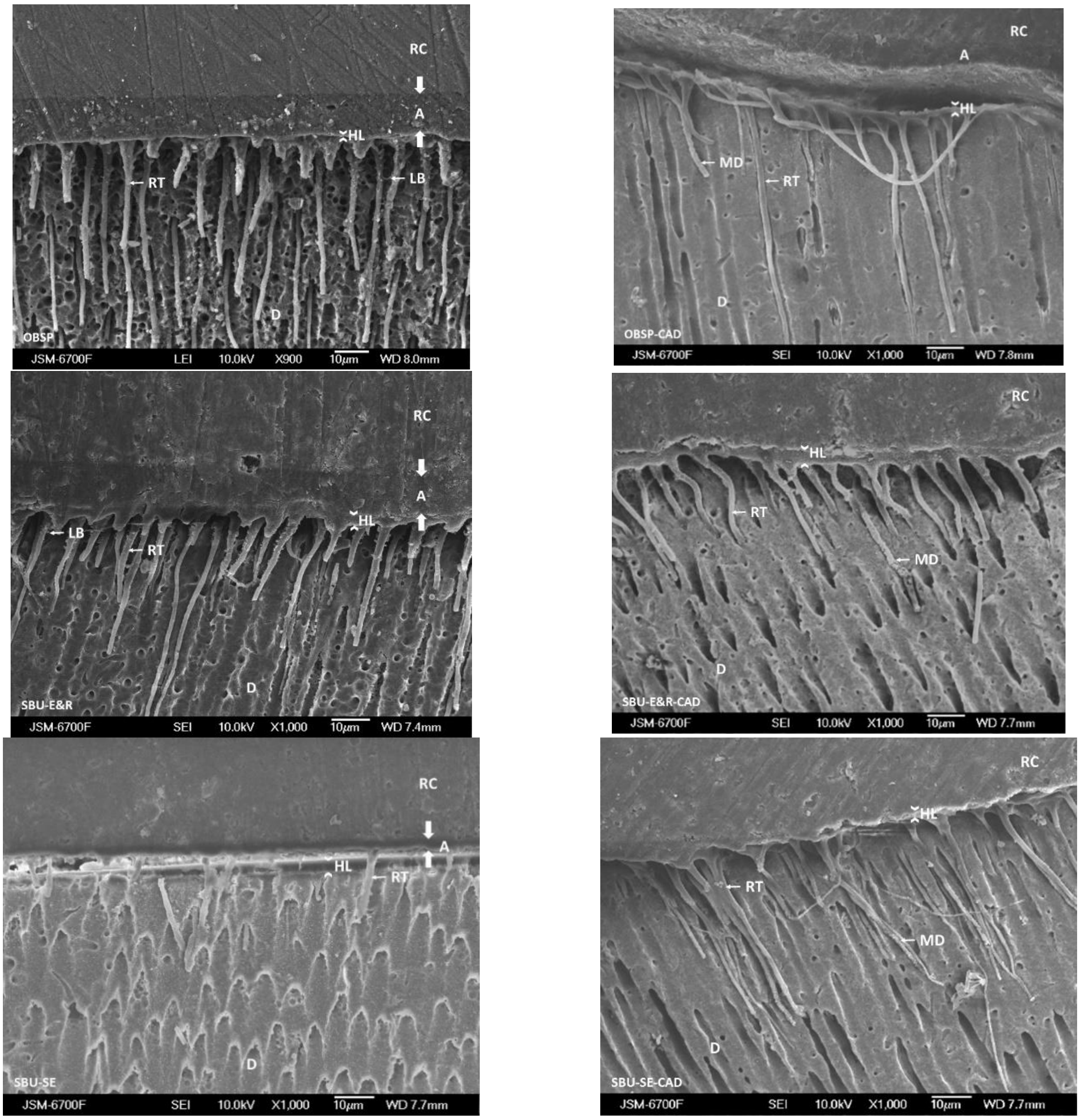


Fig 1. SEM microphotographs of resin-dentin interface of sound (left column) and caries-affected dentin (right column) (RC –resin composite, A – adhesive, HL – hybrid layer, RT – resin tag, LB – lateral branches, D – dentin, MD-mineral depositions).

Discussion:

Hybrid layer degradation due hydrolytic and enzymatic action with endogenous matrix metalloproteinase (MMP) is the main problem of resin-dentin interface [14].

Caries-affected dentin artificially-induced by pH-cycling model was used in previous studies [3,13]. The limitations of this model are that only superficial demineralization occur without alteration of collagen fibrils structure and mineral depositions in dentin tubules [15,16]. In the present study, natural caries-affected dentin of primary molars was used. It was obtained by complete caries excavating to firm dentin with caries marker control.

Since caries-affected dentin is less mineralized and more porous, acid etching produced deeper demineralization. This trends to formation of a substantial zone of unprotected collagen fibrils within the hybrid layer [17]. Additionally, a higher water content in deeper demineralized dentin would block penetration of resin monomers which is one the mechanisms of degradation process [18]. Presents of mineral deposits in CAD dentin tubules hinder compete resin penetration in dentin tubules [19]. These minerals cannot be dissolved by phosphoric acid, even in case of etching per 15 s. On the other hand, this deposits reduce dentin permeability and prevent hybrid layer degradation.

According to previous studies, hybrid layer thickness on CAD is higher compare to SD. We don't have significant differences in hybrid layer thickness between CAD and SD in our study. N. Gateva et al. (2012) reports that hybrid layer thickness for Optibond FL on sound primary and permanent dentin was 5,66 μm and 4,46 μm respectively [11]. This is comparably with our results.

Single Bond Universal is the universal adhesive system with middle acidity (pH 2,7) which contains 10-MDP and Vitrebond Comolymer. Self-etching mode leads to on-time demineralization and infiltration of dentin and excludes formation of uninfiltrated collagen fibrils layer [20]. Despite this, mild acidity of self-etch systems is not sufficiently effective in dissolution of smear layer and sound dentin with less hybrid layer and resin tags formation. In case of CAD, self-etch mode produces deeper demineralization with cylindrical resin tag formation.

Also, bond strength is lower to caries-affected dentin than to sound [20]. However, intrinsic weakness of caries-affected dentin may not be a clinical problem, if there is surrounding sound dentin and enamel that can provide high bong strength [5,10].

According to the results of our in vitro study, the null hypothesis was partially rejected. The study showed morphological differences in hybrid layer and resin tag formation on caries-affected dentin compare to sound dentin of primary teeth. But there were no significant differences in hybrid layer thickness.

Conclusions:

Etch&rinse adhesive systems produce pronounced hybridize complex on sound and caries-affected primary dentin. Dentin substrate and adhesive system type affects hybrid layer formation. Despite the result of this in vitro study, feather evaluating of optimal etching time and long-term bonding stability needed for definitive recommendations.

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