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## Electric device improves bonds of simplified etch-and-rinse adhesives

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### ABSTRACT

**Objectives.** This study investigated the effects of an electric field produced by a new device for the application of etch-and-rinse adhesives on demineralized dentin surfaces.

**Methods.** Three simplified etch-and-rinse adhesives (Single Bond, Prime&Bond NT and One-Step) were applied with the electric device and compared with controls prepared with disposable sponges. Specimens were processed for microtensile bond strength test and nanoleakage investigation using high resolution SEM.

**Results.** Microtensile testing revealed higher bond strengths ( $p < 0.05$ ) for all adhesives tested when electricity was used. Adhesive interfaces prepared with electric impulses exhibited very homogenous hybrid layers with minimal nanoleakage compared with the controls.

**Significance.** The use of electricity produced by a new electronic device during the application of dentin adhesives may increase adhesive adaptation to the dentin substrate and improve dentin hybridization due to the substrate modifications induced by an electric field on the demineralized dentin organic matrix.

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## 1. Introduction

Despite the recent developments in adhesive dentistry to reduce the number of working steps and to simplify the clinical procedure, the new simplified adhesives do not produce better results in *in vitro* tests [1] or improve clinical efficacy [2]. Ironically, the most user-friendly simplified adhesives, the so-called self-etching one-step adhesives, exhibited the lowest bond strengths and the least predictable clinical performances over time when compared with the multi-step etch-and-rinse and self-etch systems [2,3].

Previous reports have shown that one of the major disadvantages of the etch-and-rinse systems is incomplete infiltra-

tion of the exposed dentin matrix due to the collapse of the collagen fibrils after removal of the mineral phase [4–6]. A layer of disrupted collagen fibrils may interfere with adhesive penetration and the formation of the hybrid layer. Incompletely infiltrated voids within these hybrid layers may be revealed with a tracer such as silver nitrate using transmission (TEM) or scanning electron microscopy (SEM). Tracer infiltration in the absence of a physical interfacial gap has been referred to as nanoleakage [4], which may occur in interfaces bonded with either etch-and-rinse or self-etch adhesives. Field emission-SEM (FE-SEM) and TEM studies showed that entrapment of water within adhesive interfaces may create additional voids or tracks that could be revealed by these tracers [7–9].

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There is a general consensus that the quality of dentin hybridization is more important than the actual thickness of the hybrid layers in establishing the long-term seal of bonded restorations [1]. The existence of incompletely infiltrated collagen fibrils within the hybrid layers and additional entrapped water within the polymerized adhesives may expedite the degradation of resin–dentin bonds, resulting in clinical and visibly detectable microleakage [10–12].

All dentin adhesives are currently applied mechanically to tooth structures using either disposable sponges or brushes. Recently, a technique has been introduced that utilizes an electric field to enhance resin infiltration into the demineralized collagen matrices of acid-etched dentin [13]. This electrical device (ElectroBond, Seti, Rome, Italy) is incorporated in a handpiece to which a small application sponge is attached [14]. It creates an electric potential difference between the etched tooth substrate and the adhesive-filled sponge that produces a constant electric flow during adhesives application. The purpose of this *in vitro* study was to examine the quality of dentin hybridization achieved with the use of this electrical application technique for the bonding of three etch-and-rinse adhesives to crown dentin. The null hypothesis tested was that there is no difference in the quality of dentin hybridization achieved between a conventional mechanical adhesive application technique and the use of an electric impulse assisted adhesive application technique.

## 2. Materials and methods

Thirty non-carious human third molars were used in this study after informed consent had been obtained for their use for research, under a protocol approved by the Human Assurance Committee of the University of Bologna, Italy. The teeth were stored in a 0.5% chloramine T solution at 4 °C and used within 1 month after extraction. Occlusal enamel and root dentin were removed perpendicular to the long axis of each tooth by a low speed diamond saw under water irrigation (Micromet, Remet, Bologna, Italy). A standardized smear layer was created on the exposed coronal dentin with 180-grit wet silicon carbide paper. Each tooth was longitudinally sectioned into two halves (experimental and control halves) to create two similar bonding substrates. The specimens were etched for 15 s with 35% phosphoric acid gel (3M EPSE, St. Paul, MN, USA) and rinsed for 15 s. Excess water was removed using lint-free tissues, in accordance with the wet bonding technique.

The specimens were randomly assigned ( $N=10$ ) to three simplified etch-and-rinse adhesives: Single Bond (3M ESPE), Prime&Bond NT (Dentsply DeTrey, Konstanz, Germany) and One-Step (Bisco Inc., Schaumburg, IL, USA). For experimental halves of each tooth, the adhesive was applied using ElectroBond (Seti, Rome, Italy) that delivered a direct current (dc) between the acid-etched dentin (working as cathode) and the adhesive-filled sponge (working as anode) [14]. As specimens showed different electrical resistances to dc, the tested ElectroBond automatically induced an electric flow over 20  $\mu$ A throughout the adhesive interface during the application procedure. To permit electricity conduction under *in vitro* bonding conditions, the tooth to be bonded was fitted into a copper ring that was wired to the electric application device and bond-

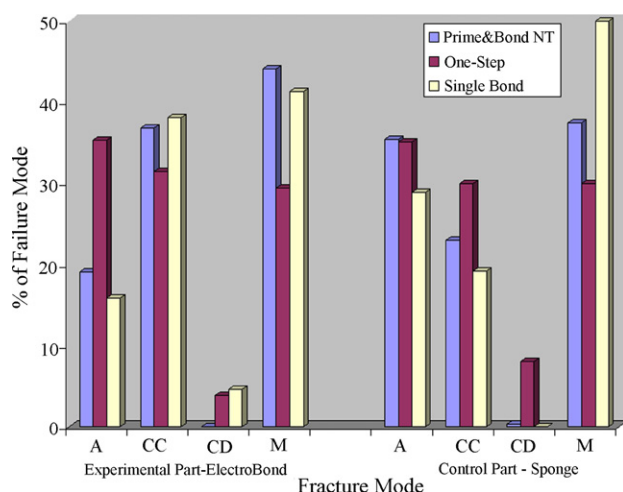
ing was performed by holding the copper ring with ungloved fingers. For the control halves of each tooth the selected adhesive was applied in the same manner, but with the electric current switched off. A single blind study design was used, in which the operator performing the bonding procedure was not aware of the operating state of the electrical device (i.e. switched-on mode or switch-off mode). The electric applicator was used with a brushing motion, continuously supplying energy (experimental halves). To ensure consistency in all the experimental groups, two adhesive coats were applied for 10 s each, irrespective of the adhesive selected, and air-dried for 5 s to evaporate the respective solvent. Each adhesive was light-cured for 20 s with a quartz–tungsten–halogen light-curing unit (Curing Light 2500, 3M ESPE). A 2-mm thick layer of microhybrid resin composite (Z250, 3M ESPE) was subsequently placed over the bonded dentin surface and polymerized for 20 s.

### 2.1. Microtensile bond strength evaluation

The pulp chamber of each tooth half was bonded with Single Bond in accordance with the manufacturer's instruction and filled with resin composite (Z250). Sticks with surface areas of approximately 0.9 mm<sup>2</sup> were created from each specimen using a low speed saw under water irrigation. The dimension of each stick was individually measured with a digital caliper to the nearest 0.01 mm and the calculated area was recorded for subsequent bond strength calculation. The specimens were observed using a stereomicroscope to avoid the inclusion of sticks containing residual enamel during bond testing. The sticks were stored in deionized water for 24 h, attached to a modified jig for microtensile testing and stressed under tension until failure with a universal testing machine at a crosshead speed of 1 mm/min. The failure modes were evaluated at 50 $\times$  (Stemi 2000-C, Carl Zeiss Jena GmbH, Germany) and classified as cohesive (C), adhesive (A), or mixed (M) failures. The number of prematurely debonded sticks per group during specimen preparation was also recorded. As values were not normally distributed (Kolmogorov–Smirnov test), a Mann–Whitney test was used to compare the data with statistical significance set at  $\alpha=0.05$ .

### 2.2. FE-SEM nanoleakage evaluation

Bonded sticks from the center of each bonded tooth half were employed for nanoleakage evaluation. The specimens were covered with nail varnish, leaving 1 mm free at the interface. They were immersed immediately in a 50 wt.% ammoniacal AgNO<sub>3</sub> solution that was prepared according to the method described by Tay et al. [7]. After immersion in the tracer solution for 24 h, the specimens were photodeveloped to reduce the diamine silver ions ([Ag(NH<sub>3</sub>)<sub>2</sub>]<sup>+</sup>) into metallic silver grains. The silver-impregnated sticks were polished with 1200-grit silicon carbide papers to remove the surface silver deposits and expose the bonded interfaces. They were dehydrated and dried in accordance with the technique reported by Suppa et al. [9]. Dehydrated, uncoated specimens were examined using the in-lens mode of a FE-SEM (JSM 890, JEOL, Tokyo, Japan) at 7 kV and 1  $\times$  10<sup>-12</sup> A. Images were obtained with both secondary electron (SE) and back-scattered electron (BS) signals.



**Fig. 1 – Percentages of the failure modes after microtensile test analyzed using stereomicroscope. Fracture were classified as: A, adhesive; CC, cohesive in composite; CD, cohesive in dentin; M, mixed. N = numbers of specimens.**

Additional silver-impregnated sticks were fixed and dehydrated as described previously and embedded in epoxy resin (Epon 812, Fluka, Switzerland). Undemineralized sections containing the resin–dentin interfaces were obtained with an ultramicrotome (Ultra-Cut S, Leica, Austria) and examined with the FE-SEM for comparison with the previously examined, unembedded bulk specimens.

### 3. Results

Significant increases in microtensile bond strength were observed for the three adhesives when they were bonded to dentin using the electric impulse assisted application technique (Table 1;  $p < 0.05$ ). Premature failures due to preparation procedures were not included in the statistical analysis (Table 1). No differences were observed in failure mode between the control and the electric impulse assisted application groups (Fig. 1).

FE-SEM images of the bonded specimens revealed the presence of electron-dense silver grains that had been confirmed by energy-dispersive X-ray analysis [9]. Unembedded bulk specimens and resin-embedded specimen sections exhibited similar leakage patterns using FE-SEM. Low magnification images revealed significant reductions in the number

and dimensions of silver clusters using the electric impulse assisted application technique when compared to the controls (Figs. 2 and 3). In particular, nanoleakage from the basal part of the hybrid layers created under an electric impulse was reduced even if silver deposits in adhesive and the superficial part of the hybrid layers still remained (Figs. 2a and c and 3a and c). Clusters of silver deposits could be also identified in proximity to the dentinal tubule orifices (Figs. 2c and 3d), which were probably created by the entrapment of water derived from outward fluid flux.

### 4. Discussion

Dentin consists of mineralized type I collagen fibrils embedded in a highly hydrophilic matrix of proteoglycans, other non-collagenous proteins and water [15]. Ideally, after acid-etching, the adhesive should completely infiltrate the delicate fibrillar network. Optimal resin infiltration is crucial to maximize bond strength [5,6] and bond durability [1], as unprotected collagen fibrils may be hydrolyzed over time [11,16,17].

The results of the present study showed that ElectroBond [14] was able to improve bonding efficacy, as shown by the increased microtensile bond strength when compared with the control application technique. The bond strength data were further supplemented by FE-SEM findings that revealed reduced nanoleakage in bonded interfaces that were created by adhesive application under an assisted electrical impulse.

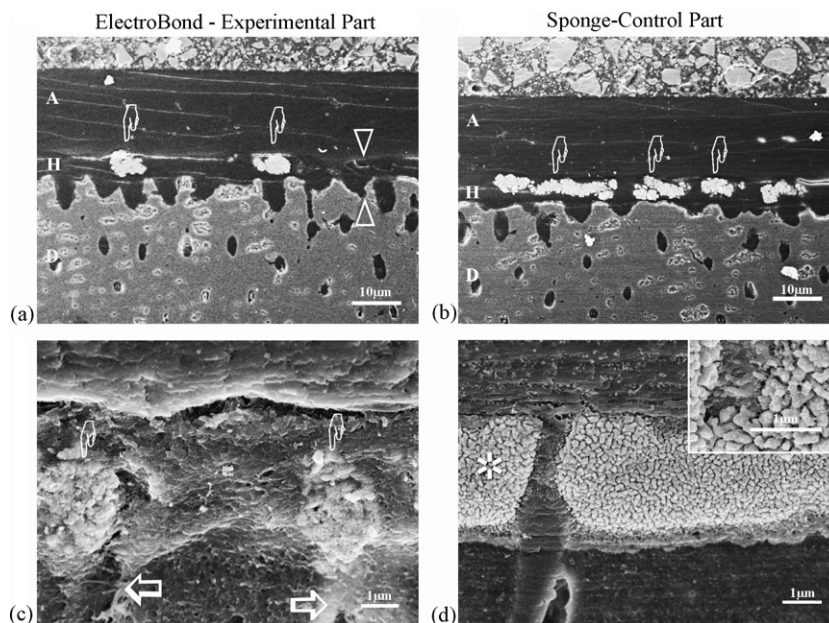
Interpretations of the infiltration phenomenon that accompanied the use of an assisted electric impulse may be achieved with different hypotheses: the difference in electric potential between the etched dentin and adhesive could have either enhanced the penetration of these adhesive monomers, or could have altered the wetting characteristics of the etched dentin surface, thereby improving the spreading of the adhesives. Low-frequency electric currents cause dielectric dispersion in tissues, which is associated with enhanced ionic diffusion and interfacial polarization, although the extent of such improvements is dependent upon the complexity of the substrate [18].

The use of electrical currents to facilitate the rate of permeation of ionized substances (such as different drugs, anesthetics, etc.) through dentin has been previously reported [19–21]. For example, delivery of drugs through intact and caries-affected dentin is enhanced with the use of iontophoresis [20]. Likewise, resin monomer infiltration through a partially demineralized collagen network may be accelerated via the use of electric currents. This should not be related

**Table 1 – Microtensile bond strength data obtained applying the adhesives on parts A (experimental) and B (control) of the same teeth with the experimental electric device or in accordance with the manufacturers' instructions (control)**

Adhesive	Electric impulse assisted adhesive application <sup>a</sup> (MPa)	Control, conventional sponge application <sup>a</sup> (MPa)
Prime&Bond NT	38.2 (4.0) [68] A	26.6 (5.2) [48] B
One-Step	38.8 (4.1) [51] A	28.7 (5.1) [57] B
Single Bond	44.2 (7.1) [63] A	29.4 (8.6) [52] B

<sup>a</sup> Values are mean, (S.D.) and [number of intact sticks tested]. Groups with the same letters are not statistically significant ( $p > 0.05$ ).



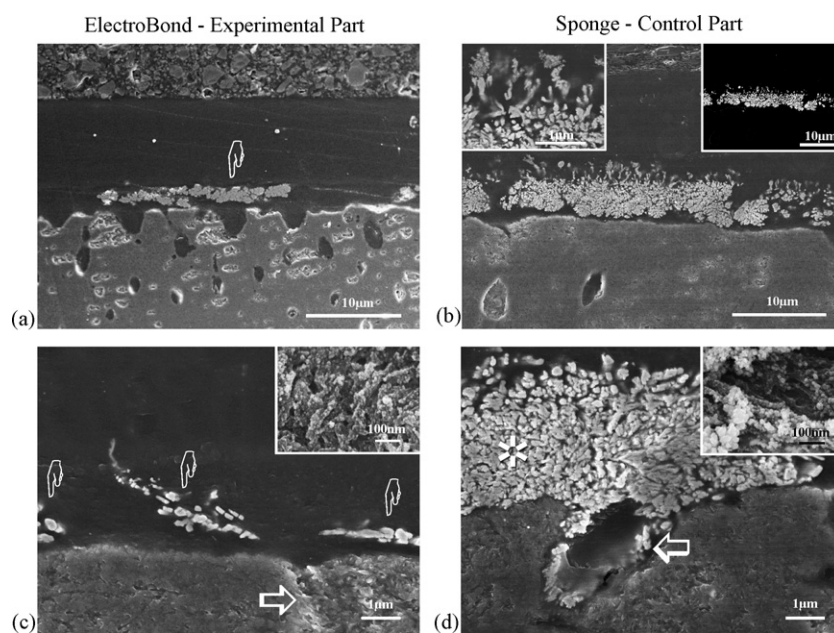
**Fig. 2** – FE-SEM micrographs of bonded interfaces in Single Bond that were created using the electric impulse assisted application technique (experimental) or conventional sponge application technique (control). (a) An epoxy resin-embedded experimental specimen section that was cut with diamond knife revealing dentin (D), hybrid layer (H) adhesive (A) and composite (C). Small clusters of silver grains with a diameter of 3–6  $\mu\text{m}$  were seen within the hybrid layer, created using the application (pointers). (b) An epoxy resin-embedded control specimen section revealing extensive nanoleakage within the hybrid layer (H); dentin (D), adhesive (A), composite (C). (c) A high magnification view of the silver grains that were found within the adhesive layer (pointers) in an unembedded experimental section. (d) An unembedded control specimen revealing extensive silver deposits (asterisk) within the entire thickness of the hybrid layer. The insert depicts a high magnification of these silver clusters. Similar regions with extensive nanoleakage were scattered along the interface and separated by regions with less nanoleakage.

to an increase in the intrinsic dentin permeability but to a faster rate of impregnation as ionic monomers are moving across the dentin with increasing ion mobilities that are caused by the imposed electrical gradient [21]. Adhesive infiltration may be positively influenced by the application of electric currents, since polar resinous components such as polyelectrolyte (polyalkenoic acid copolymer), HEMA, PENTA and BPDM are present and may interact with the electric field generated by the device. Single Bond contains polyalkenoic acid polymer that is charged. One-Step contains biphenyl dimethacrylates (BPDM) that contains two ionisable carboxylic acid groups. Prime&Bond NT contains dipentaerythritol-pentaacrylate phosphate ester (PENTA), which is a phosphate-substituted methacrylate. Thus, iontophoresis may accelerate the movement of ions across dentin [19]. Iontophoresis may also remove excess water from the hybrid layer. Since it is likely that “water-saturated” demineralized dentin also contains salt ions from collagen and dentinal fluid, iontophoretic electric fields may induce electro-osmotic fluid movement. That is, the attraction of ions to the applied electrode osmotically obligates fluid to follow the ions. This may occur at the same time when the oppositely charged monomers are being driven into the hybrid layer. The use of electric currents may also influence the dentin substrate by increasing its wettability and rate of water substitution by the adhesive monomers, as the affinity between these molecules and water dipoles is modified.

We speculate that apart from the polarization effect occurred upon the polar functional groups of the adhesive monomers, these molecules may also be physically attracted by the electrical field, thus increasing the flow throughout the intricate layer of demineralized dentin.

Application of electricity may also transiently alter the biochemical characteristics of the dentin organic matrix, since collagen fibrils are polar in nature by virtue of the 3.7 Debye dipole moment present in the peptide unit of the triple helix [22]. Moreover, the dipoles of water molecules contribute to the polar nature of collagen [23], as they exist in a hydrated state during the process of wet bonding. Molecular modeling of collagen with adjacent charged residues predicted that side-chains participate in the formation of intra-chain and inter-helix ion pairs [24,25]. Moreover, proteoglycans associated with dentin collagen fibrils are characterized by lateral glycosaminoglycans chains with negatively charged residues that are responsible for the high water binding and tissue swelling behavior of this collagen–proteoglycan complex [26,27]. Indeed, the latter represents a polyelectrolyte hydrogel with a diffusion coefficient of ions that approximates to the diffusion coefficient of free, unbound water [28].

Since both collagen and proteoglycans are polar, the authors speculate that an electric impulse assisted adhesive application technique influences the three-dimensional arrangements of the demineralized dentin network, which in turn, may favorably influence adhesive infiltration. By



**Fig. 3 – FE-SEM micrographs obtained from epoxy-embedded specimens that were sectioned with a diamond blade. (a) Bonded interface obtained after the application of One-Step with the electrical device, revealing silver clusters within the hybrid layer (pointer). (b) Bonded interface obtained when One-Step was bonded to acid-etched dentin without the use of an electrical impulse (control). Extensive silver deposits were visible along the entire thickness of the hybrid layer, with an increasing gradient of silver deposits along the base of the hybrid layer. Left insert reveals high magnification features of the silver infiltration. Right insert shows a back-scattered image of the same area. (c) Bonded interface created with Prime&Bond NT that was applied using the electrical device, revealing minimal nanoleakage (pointers) within the hybrid layer. Insert illustrates high magnification features taken from an unembedded specimen, with silver grains identified along the exposed collagen fibrils. (d) Bonded interface created when Prime&Bond NT was applied to acid-etched dentin without the use of an electrical impulse (control). Extensive silver deposits (asterisk) could be seen within the base of the hybrid layer and around the dentinal tubules (open arrow). Insert reveals uninfiltreated collagen fibrils that were covered with silver grains.**

suspending this highly polar organic matrix in a water environment, such as with the use of a wet bonding technique, not only are the dipoles of individual molecules modified, but the quaternary protein structure may also be altered due to the existence of intra- and interfibrillar hydrogen bonds among the collagen fibrils [29] and the partially retained proteoglycans [27]. Moreover, collagen fibrils have also been demonstrated to possess piezoelectric capability that enable them to alter their three-dimensional arrangement [19,30,31] in relation to the amount of adsorbed water [32]. For these reasons, the application of an electric current to a demineralized, wet dentin surface may alter the dentin surface characteristics, inducing subtle orientation changes within the organic fibrillar network that favors adhesive infiltration.

In conclusion, this study represents a preliminary attempt to identify the improvements in microtensile bond strengths and reduction in nanoleakage that are associated with the use of an electric impulse assisted application technique for the bonding of simplified etch-and-rinse adhesives to wet, acid-etched dentin. The null hypothesis tested has to be rejected since the use of an electric impulse assisted adhesive application technique improved the quality of dentin hybridization and bond strength values when compared with a conventional mechanical adhesive application technique. Further studies are required to quantify the effect of electric

currents on adhesive infiltration, for instance, by correlating the voltage with the solubility parameter for hydrogen bonding of adhesive monomers with the use of a modeling approach in which all available adhesive constituents are known. When this additional information is available, this electric impulse assisted application technique may be considered ready for in vivo testing.

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