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Original research

In vitro antibacterial activity of self-etch bio-active dental adhesives after artificial aging

Purpose
The aims to evaluate the antibacterial effect of different bioactive component containing dental adhesives before and after artificial aging.

Materials and Methods
Two bio-active adhesives; Clearfil Protect Bond and FL Bond II, two non-bioactive adhesives, Clearfil SE Bond and Clearfil S3 Bond were used for this study. Antibacterial activities of the fresh and aged samples against *Streptococcus mutans* were investigated with Direct Contact Test. Data were analyzed with Kruskal Wallis and Mann Whitney U multiple comparison tests.

Results
For fresh samples FL Bond II and Clearfil Protect Bond exhibit similar antibacterial effect but Clearfil Protect Bond showed significantly higher antibacterial effect after aging the samples ($p < 0.05$).

Conclusions
The incorporation of bio-active antibacterial components into adhesive systems may be considered as a fundamental component in inhibiting residual *Streptococcus mutans* when considering the antibacterial effect of fresh samples of bio-active adhesives.

Keywords: *Bio-active adhesives; antibacterial activity; S.mutans; Direct Contact Test; artificial aging*

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Introduction

Today in the treatment of dental caries, resin-based composites are widely preferred. Nevertheless, microleakage and the tendency for plaque accumulation is a downside of the resin composite material, which occurs due to polymerization shrinkage and is generally followed by secondary caries (1). Although the continuous improvement in composite restorative materials and dentin adhesive systems, it is not yet possible to completely prevent microleakage (2-4).

Therefore Ultimate novelty in adhesive materials should focus on special features rather than altering present technologies. With this being accomplished, bio-active adhesive materials could promote prognosis of restorative treatments (5).

In order to gain bio-active properties such as antibacterial, matrix metalloproteinase inhibitor, remineralization and anti-plaque effects; quaternary ammonium compounds based resin monomers, silver and calcium phosphate nanoparticles, ion-releasing glass fillers and growth factors such as 4-META/MMA (4-methacryloxyethyl trimellitate anhydride/methacrylate) are added into the resin materials (5,6).

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12-methacryloyloxydodecylpyridinium bromide (MDPB) is a polymerizable and bactericide, quaternary ammonium compound. When the MDPB incorporated resin is cured the antibacterial component is immobilized and not released from the material after polymerization. These component is damaged in contact with bacteria (7).

Another method for generating antibacterial bio-active adhesive was combined silver (Ag) and amorphous calcium phosphate (NACP) nanoparticles to dental adhesives (5). In vitro investigations revealed that Ag and NACP nanoparticles containing adhesives have remineralization and antibacterial features. These effects might be of great benefit to improve bond strength and prevent to secondary caries (8,9).

In recent years, a great number of surface pre-reacted glass fillers (S-PRG) containing dental materials were introduced by Shofu Inc. (5). These fillers are established by initiating an acid-base reaction between fluoroboroaluminosilicate glass and aqueous polyacrylic acid. Researchers reported that these bio-active fillers induce remineralization and show antibacterial effects (10,11) through the release of ions such as fluoride, strontium, sodium, boron, aluminum and silicon (12,13).

While presently marketed dental adhesives have satisfactory clinical performance, it is suggested that adhesive materials containing bio-active components could contribute to better outcomes. Antibacterial bio-active components are still in the experimental stage and only MDPB and S-PRG are available on the market.

The aim in this study is to investigate the antibacterial effect of different bio-active component containing dental adhesives.

Materials and methods

Adhesives

Two bio-active and two non-bio-active adhesive systems were used in this study: Protect Bond (MDPB containing adhesive resin), FL Bond II (S-PRG containing adhesive resin), Clearfil SE Bond and Clearfil S3 Bond. Adhesive systems were evaluated in this study are shown in Table 1.

Direct Contact Test (DTC)

Streptococcus mutans (ATCC 25175) was used in this study. The DCT is based on the bacteria counting method in 24-well microliter plates (24-well, flat-bottom Cellstar, Copenhagen, Denmark). Each tested adhesive resins were 20µL adsorbed to sterile paper disks (Oxoid, Hants, UK), adapted to the sidewall of the plate and cured with LED light curing unit (SDI Radii Plus, SDI Limited, Australia), according to manufacturer's instructions.

For group 1, 20 µL bacterial suspension ($1{,}5x10^8$ CFU/ml) was placed on each sample and placed on plates incubated at 37°C, in a vertical position, for one hour. Wall of uncoated wells were contaminated with bacterial suspensions and used as control. During incubation, evaporation of suspension liquid ensured direct contact between bacteria and the tested material. Then, 3ml of tryptic soy broth (TSB) broth was added to each of the wells and gently mixed for two minutes. The bacterial suspension of each well was transferred and serially diluted in TSB. Culturing aliquots 0,1/l onto tryptic soy agar

Table 1: *Adhesive composition and application procedure. (Abbreviations: MDP: 10-Methacryloyloxydecyl dihydrogen phosphate, MDPB: 12-Methacryloyloxydodecyl pyridinium bromide, Bis-GMA: bisphenol A glycidyl methacrylate, HEMA: Hydroxyethylmethacrylate, S-PRG: surface pre-reacted glass ionomer, UDMA: urethane dimethacrylate, TEGDMA: Triethylene glycol dimethacrylate).*

Adhesive System	Material Type	Composition		Manufacturer
Clearfil Protect Bond, Bioactive dental adhesive	Two component self-etch adhesive	Primer	MDP, MDPB, HEMA,Hydrophilic dimethacrylate, Water	Kuraray Medical Inc., Okuyama, Japan
		Bond	MDP, Bis-GMA, HEMA,Hydrophobic dimethacrylate,dl-Camphorquinone,N,N-Diethanol-p-toluidine,Silanated colloidal silica, Surface treated sodium fluoride	
FL Bond II, Bioactive dental adhesive	Two component self-etch adhesive	Primer	Water, Ethanol,Carboxylic acid monomer, Phosphoric acid monomer and initiator	Shofu Inc., Kyoto, Japan
		Bond	S-PRG filler based on fluoroboroalimoslicte glass, UDMA, TEGDMA, 2-HEMA, initiator	
Clearfil SE Bond	Two component self-etch adhesive	Primer	MDP, HEMA, Hydrophilic dimethacrylate, Water	Kuraray Medical Inc., Okuyama, Japan
		Bond	MDP, Bis-GMA, HEMA,Hydrophobic dimethacrylate,dl-Camphorquinone,N,N-Diethanol-p-toluidine,Silanated colloidal silica	
Clearfil S3 Bond	One component self-etch adhesive		MDP, HEMA, Bis-GMA, Hydrophilic aliphatic dimethacrylate, Hydrophobic aliphatic methacrylate, Colloidal silica, dl-Camphorquinone, Accelerators, Initiators, Water	Kuraray Medical Inc., Okuyama, Japan

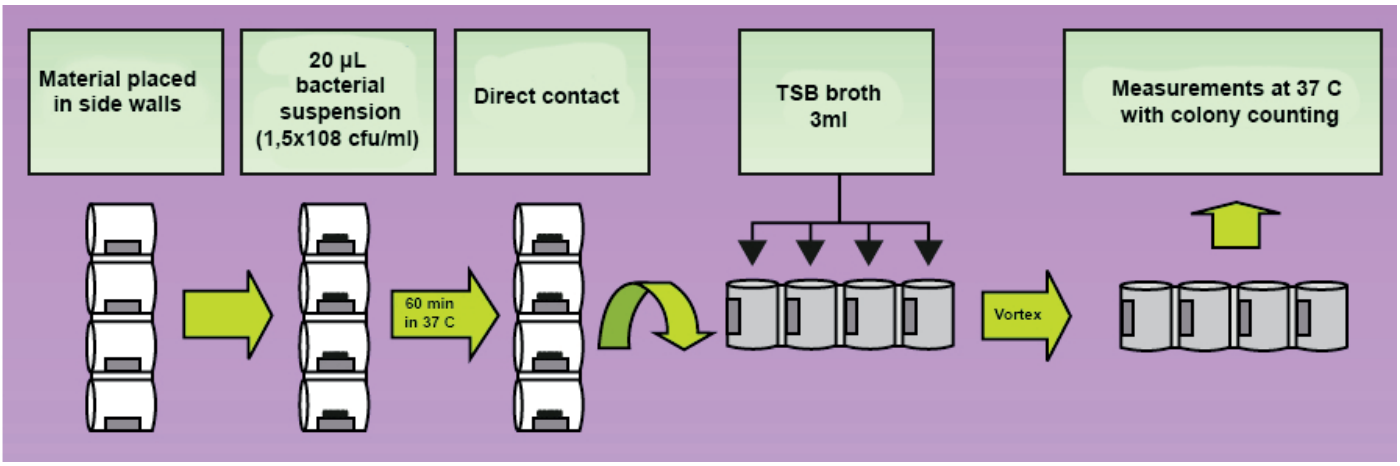

Figure 1. *Schematic drawing of direct contact test performed.*

after serially diluted range from 10^{-1} to 10^{-3} detected surviving bacteria. After 24 hours incubation at 37°C, colonies were counted, and Cfu/ml was calculated (Figure 1).

For group 2, after the polymerization of the adhesives, 3ml of sterile distillated water was added to each of the wells. At the end of the aging period (7 days) samples were removed from the distillated water. The antibacterial activity of fresh and aged samples was tested at the same time as described above. All experiments were performed in triplicate.

Statistical analysis

The collected data from all groups were imported to Statistical Package for Social Sciences 16.0 for Windows software, (SPSS Inc., Chicago, IL, USA). The nonparametric Kruskal-Wallis one way analysis of variance by ranks and Mann-Whitney U tests were used for the multiple and pairwise comparisons, respectively. The confidence interval was set to 95% and $p < 0.05$ was considered statistically significant.

Results

The results of the antibacterial effects of the bio-active dental adhesives from DCT are presented in Figure 2. For fresh samples Clearfil Protect Bond and FL Bond II shows exhibited the most effective antibacterial activity against *S.mutans*. The difference between these two and the other groups including the control were significant ($p < 0.5$). When compared with fresh and aged samples, only Clearfil Protect Bond showed significant antibacterial effect after aging. Although fresh samples of FL Bond II exhibit similar antibacterial effects to Clearfil Protect Bond , when aged, antibacterial effects are similar to adhesive groups without bio-active component . There is no difference between the aged and fresh samples among the adhesive systems without the bio-active component.

Discussion

Over the last decade, composite resin materials have become highly preferable in restorative dentistry (14). Because of their superior aesthetic properties than dental amalgams and ability to be used with minimally invasive preparation techniques, moreover the potential for bonding

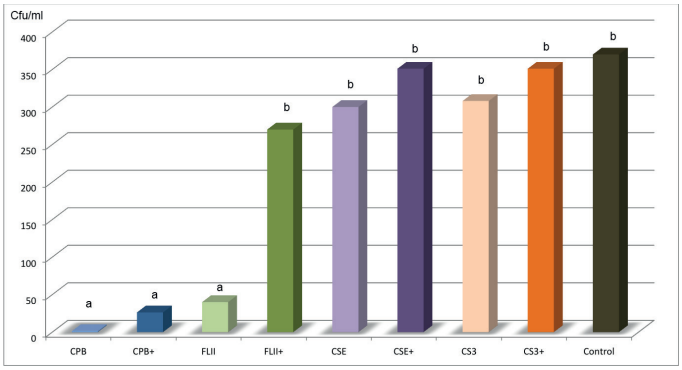

Figure 2. *Bacterial growth rate after direct contact with tested materials (Ab abbreviated 103). Identical letters indicate that mean values were not significantly different (p=0.05). (Abbreviations: PB: Clearfil Protect Bond, PB+: Clearfil Protect Bond aged, FLII: FL II Bond, FLII+: FL II Bond aged, CSE: Clearfil SE Bond, CSE+: Clearfil SE Bond aged, CS3: Clearfil S3 Bond, CS3+:Clearfil S3 Bond aged) .*

to dental hard tissues, composite materials are often used to restore decayed or traumatized teeth, and for the aesthetic restoration of discolored and malpositioned teeth, as a direct and indirect restorative material (15). Nevertheless, some studies have revealed shorter longevity and higher failure rates for amalgam compared to composite restorations (16-20). One of the major reasons for failure of composite restorations is secondary caries (15-17,19-22).

The etiology of the seconder caries is an infectious disease of bacterial origin, similar to primary caries (23), and consist mainly of *S. mutans*, *Lactobacilli* and *Actino-myces naeslundii* (24). The demineralization mechanism is the same as the primary caries, but the presence of restorative material creates some differences. The cariogenic attack in secondary occurs also from the tooth-restoration interface (15). Furthermore, other studies reported that the amount of plaque and caryogenesis was directly related to restorative material used (15,17-20, 24).

In general, composite resin materials are differed from amalgam and glass-ionomers, because they do not exhibit antibacterial properties (25). Studies have been reported that they show antibacterial properties due to metal ions in amalgam and fluorid-releasing capacity of the glass ionomer (26-28).

A number of experimental composites have been developed by combining antibacterial bio-active components to the resin or filler content of dental composites (29-33). Recent studies report that the mechanical properties of some of these materials are much the same to traditional composites (32,34), but lack information about a large part of their mechanical and aesthetic characteristics, furthermore antibacterial bio-active component containing composites are still in the experimental phase and other than fluoride-releasing composites are commercially unavailable.

At the present time, no suitable method or material has been developed to provide antibacterial activity to composite materials, on the other hand much has been done about the antibacterial activity of adhesive systems (5). If dentin adhesive systems can exhibit antibacterial effects during placement of the restoration, this may provide inactivation of residual bacteria. In addition, after the restoration was completed, the antibacterial activity of the adhesive systems may be effective to inactivated bacteria in the dental plaque.

Many attempts have been made to produce dental adhesive systems that have bio-active component which could contribute to better prognosis of restorative treatments (5,9,35,36). The current study evaluated the inhibition of bacterial growth by DTC of two bio-active self-etch adhesive systems against *S. mutans*. Conventional Agar Diffusion Test (ADT) (37,38), tooth cavity model technique (39,40) dentin discs method (41) and bacterial penetration on the histological sections of extracted teeth (42,43) have been used in previous studies to investigate the antibacterial effect of dental adhesives. The DCT is a quantitative and reproducible technique that simulates the contact of the test microorganism with dental adhesives similar to microleakage. The technique also provides for better control of potential confounding factors than ADT (44-46). Tooth cavity model technique and dentin discs method involves a dentin substrate in methodology and according to the researchers they provides more reliable results about antibacterial activity of dental adhesives (39-41). However, these methods may not be suitable for investigating the antibacterial activity of dentin adhesives after aging process (42,43). The bacterial penetration test (42,43) provides information about the ability of the tested adhesives to prevent bacterial microleakege but does not indicate the ability to inhibit residual bacteria on the cavity. In this study, to evaluate the antibacterial activity of fresh and aged adhesive samples formed by direct contact with *S. mutans*, DTC method was used.

S. mutans was selected as the test microorganism because it is the primary pathogen responsible for the initiation of caries and the development of secondary caries (24). As in this study *S.mutas* has been used in many studies to examine the antibacterial effect of dental materials (10,39,40,47).

Among the fresh samples tested, Clearfil Protect Bond and FL Bond II exhibited antibacterial activity compared to the other self-etch adhesives and control ($p<0.05$). Furthermore, only the Clearfil Protect Bond's antibacterial activity persisted within the aged samples. The prolonged antibacterial effect of Clearfil Protect Bond is related to the antibacterial MDPB molecule. After curing, MDPB-containing resins inhibit the growth of bacteria in contact with the material, thereby act as a "contact inhibitor" (5). Studies have shown significant reduction of *S. mutans* number, when incubated in contact with the cured primer/adhesive surface containing MDPB (7,48).

In this study, fresh samples of FL Bond II also exhibited antibacterial activity compared to the other self-etch adhesives ($p < 0.05$) and this antibacterial effect is similar to the Clearfil Protect Bond ($p > 0.05$). However this antibacterial activity did not persist when it was aged.

Studies have shown that fluoride released by fluoride-releasing dental materials are intense during the first week of immersion in water; but reduce later on (49,50). Considering these studies, the reason for the decrease in antibacterial activity in aged FL Bond II samples might be related to loss of most of the fluoride concentration during the aging process.

Conclusion

This study highlights the incorporation of bio-active antibacterial component into adhesive systems which may become an essential factor in inhibiting residual *S.mutans* in the cavity.

Ethics committee approval: Not provided.

Informed consent: Not provided.

Peer review: Externally peer-reviewed.

Author contributions: GD, ME and GG participated in designing the study. GD, ME and HŞ participated in generating the data for the study. GD, ME and HŞ participated in gathering the data for the study. GD, ME and HŞ participated in the analysis of the data. GD wrote the majority of the original draft of the paper. GD, ME, HŞ and GG Participated in writing the paper. All authors approved the final version of this paper.

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Türkçe öz: *Self-etch bio-aktif dental adezivlerin yapay yaşlandirma sonrasi antibakteriyel etkilerinin in-vitro olarak incelenmesi. Amaç: Bu çalışmanın amacı; farklı bioaktif içeriklere sahip dental adezivlerin yapay yaşlandırma öncesinde ve sonrasında antibakteriyel etkilerinin değerlendirilmesidir. Gereç ve Yöntem: Çalışmada; iki adet biyo-aktif adeziv; Clearfil Protect Bond, FL Bond II ve iki adet biyo-aktif içeriğe sahip olmayan dental adeziv; Clearfil SE Bond ve Clearfil 3S Bond kullanılmıştır. Adeziv sistemlerin Streptococcus mutans'a karşı antibakteriyel etkinliklerinin değerlendirilmesinde direk temas testi kullanılmıştır. Veriler Kruskal Wallis ve Mann Whitney U çoklu karşılaştırma testleri kullanılarak analiz edilmiştir. Taze hazırlanmış örneklerde FL Bond II ve Clearfil Protect Bond benzer antibakteriyel etki göstermiştir ($p > 0.05$), ancak yaşlandırılmış örneklerde Clearfil Protect Bond'un antibakteriyel etkinliği anlamlı derecede yüksektir ($p < 0.05$). Sonuç: Taze hazırlanan bio-aktif adeziv örneklerinin göstermiş olduğu antibakteriyel etki dikkate alındığında, bio-aktif içeriğe sahip adezivler kavitedeki reziduel S.mutas eliminasyonu amacıyla kullanılabilir. Anahtar kelimeler: Bio-actif adezivler; antibakteriyel aktivite; S.mutans; Direkt Kontak Testi; yapay yaşlandırma*

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