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In vitro antibacterial effects of the dentin primer of Clearfil Protect Bond

Satoshi Imazato[a,*], Akiko Kuramoto[a], Yusuke Takahashi[a], Shigeyuki Ebisu[a], Mathilde C. Peters[b]

[a]*Department of Restorative Dentistry and Endodontology, Osaka University Graduate School of Dentistry, 1-8 Yamadaoka, Suita, Osaka 565-0871, Japan*
[b]*Cariology, Restorative Sciences and Endodontics, School of Dentistry, The University of Michigan, 1011 N., D2361 Ann Arbor, MI, USA*

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Summary *Objectives*. This study aimed to investigate the antibacterial effects of the dentin primer of a commercially available self-etching adhesive system, Clearfil Protect Bond, which contains antibacterial monomer 12-methacryloyloxydodecylpyridinium bromide (MDPB).
Methods. Inhibitory effects against *Streptococcus mutans*, *Lactobacillus casei*, or *Actinomyces naeslundii* were examined by an agar-disc diffusion method using the Clearfil Protect Bond primer containing 5% MDPB and an acidic adhesion-promoting monomer MDP, the primer only with MDP, and the primer with 1% cetylpyridinium chloride. The minimum inhibitory/bactericidal concentrations (MIC/MBC) of each primer for the three bacterial species were determined by serial microdilution assays. For testing the bactericidal effects seen in dentin, the primer was applied to demineralized dentin blocks in which *S. mutans* had been impregnated, and numbers of viable bacteria were counted.
Results. For all three bacteria, the sizes of the inhibition zones produced by Clearfil Protect Bond primer were significantly greater than for the other primers ($p<0.05$, ANOVA and Scheffe's *F*-test). The MIC/MBC values of Clearfil Protect Bond primer were less than those of the primer without MDPB, and comparable to those of the primer containing cetylpyridinium chloride. No bacterial recovery was obtained after application of Clearfil Protect Bond primer to the bacteria-impregnated dentin, although the primer without MDPB showed some bactericidal effect.
Significance. Clearfil Protect Bond primer has strong antibacterial activity based upon MDPB against *S. mutans*, *L. casei* and *A. naeslundii*, and the capability to disinfect cavities containing residual bacteria.

* Corresponding author. Tel.: +81 6 6879 2928; fax: +81 6 6879 2929.
E-mail address: imazato@dent.osaka-u.ac.jp (S. Imazato).

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Introduction

One problem regarding the treatment of caries in clinical situations is the lack of a comprehensive and precise diagnosis of the extent of dentinal caries. Although there are some subjective and objective methods to diagnose carious lesions and to differentiate between 'infected' and 'affected' dentin, clinical criteria that reflect the existence of cariogenic bacteria or their virulence in dentin have not been established. Although advocated for almost a century, traditional complete caries removal failed to render caries-free cavities [1]. A classic study showed that after traditional complete caries removal carious dentin was left in 72% of cavities, which were deemed to be caries free [2]. More recently, after complete removal of soft caries at the dentino-enamel junction samples of the stained but hard dentin still showed a low level of infection [3].

The available evidence no longer supports the concept of complete removal of carious dentin during cavity preparation [4]. In many cases, a surgical approach can be replaced by a minimally invasive tissue-saving approach [5]. By saving more affected tissue it can be expected that minimally excavated lesions inadvertently will harbor more residual bacteria [6,7]. With some pioneer bacteria to be found in the remineralizable affected layer after minimally invasive caries removal, the subsequent use of materials that have an antibacterial or bactericidal effect provide an adjunct treatment contributing to suppression of residual infection and increasing the survival of the restored tooth.

Etching of the dentinal surface with an acidic solution, such as phosphoric acid, during the bonding procedures may be effective to reduce the number of residual bacteria in a cavity [8]. However, the cleansing effect by the acid followed by water rinsing is limited and should not be regarded as reliable [9]. With self-etching/priming systems in which the smear layer is not washed away, residual bacteria can be anticipated. Therefore, adhesive systems that possess antibacterial activity may be useful for eliminating harmful effects caused by bacteria, and contribute to better prognoses for minimal restorative treatments of dental caries.

We have reported the achievement of an antibacterial adhesive system by incorporation of the new monomer 12-methacryloyloxydodecylpyridinium bromide (MDPB), that has strong bactericidal activity against oral bacteria [10,11], into the primer of two-step self-etching/priming system Clearfil Liner Bond 2 [12]. The primer incorporating MDPB has been shown to be promising for inactivating residual bacteria in cavities in in vitro and in vivo studies [12–14]. Based on the results obtained for this experimental material, a new single-bottled 5% MDPB-containing primer was developed, and the adhesive system employing this primer was commercialized as Clearfil Protect Bond.

This study assessed the intrinsic antibacterial activity of the dentin primer of the Clearfil Protect Bond system using an agar disc-diffusion test and by determining the minimum inhibitory/bactericidal concentrations (MIC/MBC). The hypothesis that the Protect Bond primer shows cavity disinfecting effects was also examined by in vitro tests using dentin blocks containing bacteria.

Materials and methods

Primers

The materials used are listed in Table 1. Clearfil Protect Bond primer (PB; Kuraray Medical, Tokyo, Japan) is a single-bottle self-etching/priming solution with the antibacterial monomer MDPB incorporated at 5 (w/w)%. It also contains 2-hydroxyethylmethacrylate (HEMA), water, and an acidic adhesion-promoting monomer 10-methacryloyloxydecyldihydrogen phosphate (MDP). To investigate the net contribution of MDPB to antibacterial activity, a PB-based primer without MDPB [PB(−/+)] and one with neither MDPB nor MDP [PB(−/−)] were prepared and examined. A primer prepared by incorporation of 1 (w/w)% cetylpyridinium chloride (CPC; Sigma Chemical, St Louis, USA) into PB(−/−) was also included as a positive control. The bactericide CPC is

Table 1 Materials used in this study.

	Code	Inclusion of MDPB[a]/MDP[b]
Clearfil Protect Bond primer	PB	+/+
Primer without MDPB	PB(−/+)	−/+
Primer without MDPB and MDP	PB(−/−)	−/−
Primer containing 1% CPC[c]	CPC-primer	−/−

[a] 12-methacryloyloxydodecylpyridinium bromide.
[b] 10-methacryloyloxydecyldihydrogen phosphate.
[c] Cetylpyridinium chloride (CPC) was added at 1% to PB(−/−).