

DETERMINATION OF ANTIBACTERIAL EFFICACY OF MDPB CONTAINING PRIMER AGAINST CONVENTIONAL PRIMER

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ABSTRACT:

Background: Fixed orthodontic appliances create stagnation areas for plaque and make tooth cleaning difficult. The irregular surfaces of brackets limit the naturally occurring self-cleansing mechanisms of the oral musculature and saliva thereby encouraging plaque accumulation and the colonization of aciduric bacteria over time resulting in active White Spot Lesions (WSL's). A various regimen including fluoride toothpaste, gels, varnishes, mouth rinses- antimicrobials, xylitol gum, diet counselling and casein derivate are available. WSL's can become noticeable around the brackets within 1 month of bracket placement, although the formation of regular caries usually takes at least 6 months.

Methodology: Streptococcus strains were developed and inoculated in BHI broth (Brain Heart Infusion) and 0.5 dilutions of Primers (P) – Primer 1 (conventional) and Primer 2 (MDPB) were added and serial dilutions were made of P1 and P2. An overnight culture of the primers was incubated for 24 hours. The culture was streaked on the Agar Plate and Colonies were counted (MBC).

Results: The culture plate containing MDPB did not show Bacterial Colonization. The antibacterial and fluoride releasing property of MDPB containing primer (un-polymerised) when locally applied on etched enamel will show a promising result in preventing the occurrence of WSL's during and after the course of fixed orthodontic treatment.

Conclusion: The study assessed the bacterial activity of the primer containing MDPB and determined the colony forming unit and thus it can be concluded that the antibacterial monomer containing primer, effectively reduces the occurrence of white spot lesion by preventing bacterial colonization even at the minimal concentration.

Keywords: white spot lesion, orthodontic bonding, MDPB primer, Transbond XT primer

INTRODUCTION:

Nearly 50% of orthodontic patients exhibit clinically visible white spot lesions during treatment that lasts approximately 2 years, with smooth surface lesions increasing up to 50% in prevalence during treatment [1]. These white spot lesions are due to demineralization of the enamel by organic acids produced by cariogenic bacteria. Preventing these lesions during treatment

is an important concern for the orthodontist because the lesions are un-esthetic, unhealthy, and potentially irreversible.

Conventional etching advocated by Buonocore^[2] with 37% phosphoric acid creates a roughness on the tooth surface which increases the bond strength but irreversibly damages the tooth thereby ultimately leading to enamel loss. Initial carious - "white spot" lesions, which implies that there is a subsurface area with most of

the mineral loss beneath a relatively intact enamel surface. It has been generally accepted that the combined application of fluoride regimens {dentifrice (1100 ppm fluoride) and a mouth rinse (0.05% sodium fluoride)}, oral hygiene instructions, and dietary control can contribute greatly to the inhibition of demineralization during fixed-appliance treatment.^[3] These methods, however, rely on patient compliance. In-office topical fluoride treatments have also been suggested to minimize the need for compliance. However, demineralization lesions of significant depth (75 µm) can develop in 4 weeks, a shorter time than many orthodontic appointment intervals of 6 to 10 weeks. Since the demineralisation area is focused only around the bracket attachment, it has been suggested that the combined use of antimicrobials and fluoride enhances the cariostatic effect.

A new antibacterial and fluoride-releasing self-etching adhesive have been developed by Imazato et al by incorporating -12-methacryloyloxydodecylpyridinium bromide (MDPB) monomer, that has strong bactericidal activity against oral bacteria. The present in vitro study is to compare and evaluate the antibacterial property of the conventional primer (Transbond XT) and the MDPB containing primer by Macro broth dilution method.

The objective of the study:

1. To evaluate the antibacterial efficacy of – Transbond XT (3M Unitek, Calif.)

2. To evaluate the antibacterial efficacy of – Clearfil Protect Bond (Kuraray Medical, Okayama, Japan)

To compare the antibacterial effects of the primer of a commercially available Transbond XT and Clearfil Protect Bond; which contains MDPB.

MATERIALS AND METHODS:

1. Conventional – Transbond XT primer.
2. Clearfil Protect Bond (Kuraray, Okayama Japan)- is a single-bottle self-etching/priming solution with the antibacterial monomer MDPB. It also contains 2-hydroxyethylmethacrylate (HEMA), water, and an acidic adhesion-promoting monomer 10-methacryloyloxydecyl dihydrogen phosphate (MDP)

Methodology:

From a pure culture of Streptococcus mutans, 3 to 4 colonies were picked and suspended in 4 ml saline tubes and mixed. The Suspension was adjusted to 0.5 Mcforland (DensiCHEK plus, Fig 1). The QC (quality check) passed BHI broth (Hi-Media, M 210-500g) was taken into several test-tubes, based on dilution required (10,20,30,40 and 50 microliters) and serial dilution of organism suspension was inoculated into BHI broth such as;10µl, 20 µl, 40µl, 80 µl, 160µl and 320µl per ml suspension to which serial concentration of primers was added to the BHI broth.

The primers were divided into two groups, Group A being Clearfil Protect Bond (Kuraray Medical, Okayama, Japan) and Group B being the control Transbond XT (3M Unitek, Calif.). After 2 hours of inoculation, 10µl of diluent was inoculated onto Blood agar plates and a control organism was inoculated without primer. All these plates were anaerobically incubated using a gas pack and after 48 hours (Fig 2), the plates were observed for Colony-Count to detect the efficacy of primer against the organism.

Statistical Analysis: Chi square test was used to evaluate variables in the two category of primers and since 4 cells have expected count less than 5, Fisher's exact test was considered

RESULTS:

Visual examination of the sub-cultures in the agar plates (Fig 4) revealed no growth of the organisms in any concentration with respect to GROUP A - showing significant bactericidal activity as shown in figure 4. Group B showed significant bactericidal activity in 40 µl and 50 µl concentration (Table 1)

Group B, Transbond XT showed significant effects of Antibacterial property in higher concentration (> 40µl) (Table 1)

Group A, Clearfil Protect Bond showed significant antibacterial activity from minimal till the highest concentration. Upon evaluation of the variables of both the categories it was proved to be statistically

not significant($p=0.083$), although clinical findings proved positive otherwise. (Table 2)

DISCUSSION:

The antibacterial monomer MDPB is a derivative of quaternary ammonium, synthesized by combining dodecylpyridinium bromide with a methacryloyl group. Quaternary ammonium is known to have a wide spectrum of antibacterial activity and is used for various formulations to control bacteria in the dentistry field.¹ Before being polymerized, MDPB acts as the free antimicrobial agent and has been reported to show strong antibacterial activity against oral microorganisms.^[2]

The adverse effect of the placement of fixed appliances is the enhanced risk of dental caries, which is caused by the increase of *Streptococcus mutans* due to a low resting pH value in the plaque and impeded oral hygiene with increased retentive sites and retention of food particles. In the presence of fermentable carbohydrates, demineralisation of the enamel around the bracket is an extremely rapid process. Nearly 50% to 70% of patients have white spot lesion (WSL) after fixed appliance therapy. These lesions appear to be very resistant to remineralization.^[3] To determine the minimum bactericidal concentration (MBC) of antimicrobials, standard broth dilution method according to NCCLS guidelines is frequently used.^[4,5] In this method, bacterial viability is

determined based on the ability to form colonies on the agar plates after incubation for at least 24 hours. MDPB, a derivative of quaternary ammonium, is considered to show inhibition of bacteria in the same way as other quaternary ammonium; it has high affinity for negatively charged bacterial cells by a nitrogen atom on pyridinium ring and binds to the cell surface, and subsequently disturbs the charge balance, disrupting the cell membrane and leading to the cell death.

These actions of MDPB may occur within a short period at high concentrations. Against this background, a rapid assessment of the killing effects of MDPB is useful in elucidating the detailed antibacterial characteristics of this monomer.

Imazato et al in his study reported the achievement of an antibacterial adhesive system by incorporation of the new monomer 12-methacryloyl-oxydodecylpyridinium bromide (MDPB) that has strong bactericidal activity against oral bacteria.^[6,7] Satoshi Imazato 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).^[8]

The present study we used micro-broth dilution test and the accuracy of these tests are comparable to agar dilution, the gold standard of susceptibility testing. Provided the advantages being the availability of

plates, ease of testing and storing the plates. The distribution of antibacterial growth in Primer A and B (Table 2) clinically revealed nil growth in the antibacterial monomer containing primer A whereas, primer B had significant growth up to 30 μ l. Although Fishers Exact test was statistically insignificant, owing to the decreased sample size which is one of the drawbacks of the study, it can be clinically validated that efficacy of antibacterial containing primer is positively against *Streptococcus mutans* and also conventional primer effective in concentrations higher than 40 μ l. The primer containing MDPB effectively inhibits bacterial colonization compared to the conventional primer without having to increase its concentration as the latter. These results suggest that MDPB can be effectively incorporated in dental resin-based materials to provide bactericidal activity against oral bacteria.

A study by Tancan Uysal^[9] conducted an in-vivo study which solely evaluates the effect of antibacterial monomer-containing adhesive on enamel demineralisation around orthodontic brackets. The results showed that the antibacterial monomer-containing adhesive was significantly more efficient than the conventional adhesive system, reducing enamel demineralization in almost all evaluations. Considering the clinical situation that the commercial self-etching primer containing MDPB (Clearfil Protect Bond primer) applied to the tooth surface is polymerized after 20 seconds, it is

further needed to evaluate the killing activity of MDPB within a shorter time period.

CONCLUSION:

The antibacterial monomer containing primer, effectively reduces the occurrence

of white spot lesion by preventing bacterial colonization even at the minimal concentration. Further investigations regarding the efficacy in-vivo must also be performed in order to investigate the bactericidal activity under oral conditions.

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TABLES:

Table 1: The below table describes bacterial growth in the respective primer in their respective dilutions.

Vol/micro-litre	Bacterial growth PRIMER A	Bacterial growth PRIMER B
10 µl	Nil	Present
20 µl	Nil	Present
30 µl	Nil	Present
40 µl	Nil	Absent
50 µl	Nil	Absent

Table 2: Distribution of antibacterial growth in Primer A and Primer B

Primer	Growth		Total	Chi square	P value
	Absent	Present			
Primer A	5(100)	0(0.0)	5	4.286	0.083*
Primer B	2(40)	3(60)	5		
Total	7	3	10		

*Fischer's Exact Test, statistically Not Significant.

FIGURES:



Figure 1: Densi Chek Plus



Figure 3: Bacterial growth comparison in Primer A and B



Figure 2: Incubator



Figure 4: Streaking of sample in blood agar