MDPB-Containing Self-Etch Dental Adhesive: Antibacterial Activity and Effect of Different Dentinal Surface Preparation

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Abstract:

Objective: To evaluate the effect of MDBP-containing self-etch (SE) adhesive and different dentin surface pretreatments on antibacterial activity **Materials and Methods**: A total of forty disc shaped specimens fabricated (8 mm in diameter and 1.5 mm thickness) were divided into four main groups according to type of the surface treatment method; deionized water(control), CHX, Papin enzyme and Riboflavin. Each group was sub-divided into 2subgroups (n=5) according to the type of adhesive: classic or MDPB containing SE adhesives. Bonded specimens were stored in deionized water for 24 hr. (n=6). The plates contained dices shaped specimen was incubated at 37^{o} C for 24. The diameter of inhibition zones were measured with sliding callipers and the data were statistically analyzed using Mann-Whitney U-test and multiple comparisons Kruskal Wallis H-test and level of significance were tested. **Result:** The diameter of inhibition zones evaluated that Clearfil SE protect has antibacterial activity and pre-surface treatment with CHX and Papin enzyme increased antibactrial activity with both types of adhesives. **Conclusions:** MDPB containing SE adhesives have antibacterial activity. Application of CHX prior to adhesive application improves antibacterial activity compared to Papin and Riboflavin.

Introduction:

Improvements in dental adhesive technology extensively influenced modern restorative dentistry. Nowadays, the surgical approach of 'extension for prevention' proposed by GV Black. In 1917 is no longer justifiable, and has been replaced by the concept of 'minimally- invasive dentistry.[1] This modern approach focuses on the achievement of a more conservative cavity design, basically providing sufficient access for the complete removal of the carious tissue. in order to blend all the adhesive components into one single solution, one-step adhesives were made more acidic and hydrophilic. Unfortunately, these properties induce a wide variety of seemingly unrelated problems that may jeopardize the effectiveness and stability of adhesion to the dental substrate. Incidentally, one of the factors that may interfere with the bonding effectiveness of adhesives is the technique used for caries removal and cavity preparation.[2] Several tools are on the market today to effectively remove carious tissue, thereby respecting the current trend of minimum intervention. Despite their promising performance, such techniques modify the tooth substrate in different aspects, possibly affecting bonding effectiveness. Altogether, we may conclude that not only the adhesive formulation, but also substrate nature must be taken into account to achieve a stable bonding interface.

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DOI: 10.21608/mjd.2022.148496.1056

One interesting cross linker for use in dentistry is riboflavin (RF).[3] It is a natural hydrosoluble vitamin from the group B (B2). It has been initially used as a therapeutic agent for cartilage reconstruction in head and neck surgery. When RF is applied on the tissue surface and activated either by ultra violet A (UVA) or ultra violet B (UVB).[4] it acts as a photo sensitizer that will stimulate the formation of reactive oxygen and Therefore, of covalent cross-links through oxidation. RF has been reported to improve dentin bonding as well, by increasing mechanical properties of the collagen layer.[5] Dentin collagenolytic and gelatinolytic activities can be reduced or suppressed by protease inhibitors.[1] indicating that matrix metalloproteases (MMP) inhibition could be beneficial in the preservation of hybrid layers. Based on this findings, the classical 2% CHX cleaning solution, normally used as a cavity disinfectant has been also employed to preserve the hybrid layer integrity.[6,7] The rationale behind this new use of CHX relies on its broad inhibitory effect on numerous mmps. Lysosomal cysteine proteinases of the papain enzyme family are traditionally believed to degrade proteins that have entered the lysosomal system.[8] Nevertheless, the role of cysteine cathepsins is not limited to protein degradation within lysosomes. Moreover, cysteine cathepsins can degrade type I collagen, laminin, fibronectin and proteoglycans extracellularly, and they are involved in several diseases related to extracellular matrix degradation.[9] Cysteine proteinases can also activate the tartrate-resistant acid phosphatase, an important enzyme involved in dentin resorption. The rationale behind use the cathepsin B is degradation property of mmps.

In addition, oral bacteria which are inadvertently left

under restorations or invade through microgaps between restoration and tooth induce not only secondary caries but also damage to the pulp. Therefore, to maintain oral health.[10] it is important to provide dental restorative materials with antibacterial activity and to prevent harmful effects caused by oral bacteria.[11]to immobilize an antibacterial component in dental resin-based material, a new monomer 12-methacryloyloxydodecylpyridinium bromide (MDPB) has been synthesize MDPB is a compound of the antibacterial agent quaternary ammonium and a methacryloyl group, and the antibacterial agent is covalently bound to polymer matrix by copolymerization of MDPB with other monomers when the material is cured. [12]Considering the previous studies.[7,8,15,17] the effect of dentine treatment with different materials and addition of MDPB to SE on antibacterial activity will be evaluated. The hypothesis of this study were that the antibacterial activity of MDPB containing SE adhesive/dentin interface will not be significantly influenced by dentin pre-treatment with Chlorohexdine , Riboflavin or Papin enzyme

Materials and methods:

The materials used in this study included two different self-etch adhesives; Tetric N bond universal (Ivoclar, Vivadent, Liechtenstein), clearfil SE protect bond (Kuraray Medical Inc. Tokyo, Japan), and CHX 2% con, Papin enzyme, Riboflavin.

Preparation of control groups:

Six paper disks (8 mm diameter and 1.5 mm thickness) were used to be coated with the tested materials. These disks were prepared as follow: filter paper was punched using a hole punch to make small circular paper disks. These disks were wrapped in aluminium foil and sterilized in the hot air oven at 160° C for 30 min. specimens were prepared and categorized into six groups: (MDP contain adhesive, MDPB containing self-etch adhesive, CHX, Papin enzyme, Riboflavin, phosphoric acid gel)**.**

Preparation of experimental adhesives specimen: Forty disc shaped specimen fabricated (8mm in diameter and 1.5mm thickness) from classic(Tetric N ceram) and MDPB containing self-etch adhesives(Clearfil SE protect), pre surface treatment materials mixed with these adhesives after light-cured for 20 s with a LED light-curing unit delivering 600 mW/cm2. Each plate contained 6 specimes.

Determination of antibacterial activity: The antimicrobial activity was evaluated by the Agar Diffusion Test. This test was conducted in the Medical Diagnostic and Infection Control Unit (MDICU) at the Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University. Five sterile Petri dishes (15 cm diameter and 5 mm thickness) were filled with molten brain heart inffusion medium and left to cool. When the medium became gel, microbial colonies (*Streptococcus mutans* ATCC15157) were evenly distribute over the medium surface using a sterile disposable swab and were cultured at 37° C for 24 hours in an aerobic atmosphere.[10]Paper disks containing the tested material were seated on the sterile petri dish at equal distances from each other by applying firm pressure to the disks with a sterile forceps against the medium surface, 6 disks per dish. The five plates were incubated at 37° C for 24 h. The diameters of inhibition zones were measured with sliding calipers and calculated as follows:

$$\text{Size of inhibition zone} = \frac{\text{diameter halo} - \text{diameter specimen}}{2}$$ [13]

The results were recorded in terms of the average diameter of inhibition zone (mm), then analyized using IBM-SPSS software (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0, Armonk, NY: IBM Corp.

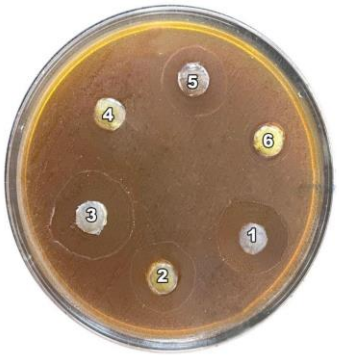

Figure 1: the inhibitory zones of the tested groups against Streptococcus mutans; 1.MDPB+CHX disc, 2.MDP+CHX disc, 3.MDPB+Papin enzyme disc 4.MDP+Papin enzyme disc, 5.MDPB+Riboflavin disc, and 6.MDP+Riboflavin.

Results:

There was a significant difference in antibacterial activity among the tested group including MDP (P = 0.009) and between tested groups including MDPB (P=0.793). Kruskal-Wallis H statistical test showed no statistical significant difference between groups due to small sample size which were used to compare non-normally distributed quantitative data between more than two groups. Considering the diameter of inhibition zones, this study showed that MDPB containing SE adhesives have antibacterial activity, the Inhibition zones in MDPB +PAPIN>MDPB+CHX> MDPB +Riboflavin as shown in Table1. This table shows higher inhibition zone and total disc in MDPB + papin> MDPB + CHX > MDPB alone > MDPB + riboflavin but the differences did not achieve statistical significance.

Discussion:

The purpose of this vitro study was to evaluate the effect MDBP containing SE adhesive and different dentin surface pre-treatments with (CHX, Papin, or Riboflavin) on antibacterial activity compared to MDP .It was hypothesized that The anti-bacterial activity of MDPB containing SE adhesive/dentin interface were not be significantly influenced by dentin pre-surface treatment with Chlorohexidine, Riboflavin or Papain

enzyme.According to the result of our study : the antibacterial activity of MDPB containing SE adhesive/dentin was significantly influenced by dentin pre-treatment with Chlorohexdine , Riboflavin or Papin enzyme, so the hypothesis was rejected. In the present study, for antimicrobial activity evaluation, agar-disc diffusion test method was used because it is a simple direct inhibition test and it has been most frequently used.[10] Moreover, S. mutans strains were tested because they are essential in initiation of dental caries due to their capability of colonization on the tooth surfaces, synthesization of insoluble polysaccharides (glucans), and fermentation of sucrose to form lactic acid that demineralized the tooth structure.[11] Clearfil SE protect has MDPB monomer in Its composition.

Quaternary ammonium compounds are known to have a broad spectrum of antibacterial activity and MDPB a derivative of alkylpyridinium halide, is considered to be able to inactivate various anaerobes based on the antibacterial mechanism of quaternary ammonium.[12] According to bactrial growth inhibition test result of this study of control groups was that there were statistically significant higher inhibition zone and total disc in Clearfil SE protect containing MDPB monomer >Tetric N Universal containing MDP monomer ,CHX > papin > riboflavin. phosphoric acid gel 37% was used in the present study as the positive control, because the antibacterial activity of phosphoric acid gel, especially for S. mutans bacteria, is well established. The results of the present study also showed that addition of CHX produced a significant improvement in antibacterial activity among the different groups but no statically significant difference due to small sample size. Imazato et al.[11] who reported that the incorporation of CHX is considered to be an agent-releasing material, the MMP inhibition effect, and the physical properties of the resin, decreased over time. However; the antibacterial monomer MDPB is a non-agent releasing material because the immobilized MDPB does not leach out from the cured resin.[14] This mechanism overcomes the disadvantage of agent-releasing materials. Despite the positive findings reported in in vitro studies about the incorporation of CHX as pre-surface treatment material in bacterial growth inhibition, Osorio et al.[15] who found that CHX pre-treatment of etch-and-rinse adhesive- bonded acid etched dentin failed to prevent hybrid layer degradation for up to 9 months after the initial inhibition of endogenous collagenolytic activities in dentin. Chlorhexidine digluconate in solution may produce digluconate anions, which may result in gradual precipitation in the presence of other monovalent and Divalent cations derived from body fluids. Moreover,

Table 1: Effect of MDPB groups on antibacterial activity

Parameter	MDPB alone	MDPB + CHX	MDPB + papin	MDPB + riboflavin	H [3]	P value
Inhibition zone	2.25 (2 – 2.5)	2.75 (1.5 – 4.25)	3.75 (1.25 – 5)	1.5 (1 – 3.5)	1.035	0.793
Total disc	12.5 (12 – 13)	13.5 (11 – 16.5)	15.5(10.5– 18)	11 (10 – 15)	1.035	0.793

Notes: Data is median (Q1 – Q3). Test of significance is Kruskal-Wallis H-test.

as CHX only binds electrostatically to demineralized dentin collagen, it may slowly diffuse out of a collagen matrix via a competitive desorption mechanism in the presence of other cations. This present study also showed that the addition addition of papin as surface treatment material before bond application has produced a significant improvement in antibacterial activity among the different groups but no statically signficant difference due to small sample size. These result in agreement with fawzy et al.[6] who found that both self-etching adhesives used after application of papin showed antimicrobial potential, although clearfil protect bond proved more effective against lactobacillus casei because in a micromorphological study, it was observed that the papain chemomechanical agent formed an amorphous layer similar to the smear layer and few exposed dentinal tubules. In comparison, a rotatory instrument produced a smooth and regular dentinal surface, with a typical smear layer and exposed dentinal tubules, in spite of a similar tag formation when a conventional total etching adhesive system was used. But zenlido et al.[16] found that apacaries gel can effectively inhibit s. Mutans strain atcc25175. Apacaries is capable of s. Mutans inhibition better than both mangosteen extract or papain separately because apacaries gel is a novel dental material and composed of a mixture of polyphenol from mangosteen extracts and papain in a gel preparation and papain activity can hydrolyse the proteins in the outer portion of gram-negative bacteria and, as a result, perturb the membrane permeability. The inhibition zone of mangosteen extract and papain mixture in gel preparation was larger than the zones for the separate components, indicating that papain and mangosteen have a synergistic effect on s. Mutans. Statistical significance implies that the difference seen in the sample also exists in the population. Clinical significance implies that the difference between treatments in effectiveness is clinically important, and it is possible that clinical practice will change if such a difference is seen. Statistical significance is used to inform clinical significance. However, clinical significance and statistical significance are often confused.[17] this present study also showed that the addition of riboflavin after activation with UVA as surface treatment material before bond application has produced no statically signficance difference and little laboratory difference, ahgilan et al.[3] found that antibacterial effect of riboflavin is demonstrated through a study found that riboflavin alone at a concentration of 50 µl inhibits the growth of the laboratory cultured gram-positive bacteria .daood et

al.[5] demonstrated that the negatively charged bacterial cell wall comes in contact with the positive antimicrobial arms, incorporating inside the cell membrane, disrupting the osmotic balance and eventual breakage of the cellular wall. There is formed free volume in the cell membrane increasing the intracellular pressure.[9] in addition; the pathogenic bacteria may also have been inactivated as a process of by-products of RF after light activation. The limitations of this study were that bacterial growth inhibition test was done against s.mutans only and the small sample size. This study has clinical relevance as self-etch adhesives with mdpb and the treatment of dentin surface with CHX, Papin or Riboflavin would improve the durability of such restorations by providing appropriate antibacterial activity.

Conclusion:

In light of the results of the current study, it seems reasonable to conclude that, MDPB containing self etch adhesives have antibacterial activity against s.mutans. CHX and Papin in conjunction of adhesives increase antibacterial activity, riboflavin in conjunction with adhesives have a little effect of antibecrial activity against s.mutans

References:

1. Imazato S, Russell RR, McCabe JF. Antibacterial activity of MDPB polymer incorporated in dental resin. J Dent 1995;23:177-181.
2. Breschi L, Lopes M, Gobbi P, Mazzotti G, Falconi M, Perdigao J. Dentin proteoglycans: an immunocytochemical FEISEM study. J Biomed Mater Res 2002;61:40-46.
3. Ahgilan A, Sabaratnam V, Periasamy VJIJoFP. Antimicrobial properties of vitamin B2. J Dent 2016;19:1173-1181.
4. Garnero P, Ferreras M, Karsdal MA, Nicamhlaoibh R, Risteli J, Borel O, et al. The type I collagen fragments ICTP and CTX reveal distinct enzymatic pathways of bone collagen degradation. J Bone Miner Res 2003;18:859-867.
5. Daood U, Malik AA, Ilyas MS, Ahmed A, Qasim SSB, Banavar SR, et al. Antimicrobial and self-crosslinking potential of experimentally developed dioctadecyldimethyl ammonium bromide and riboflavin dentin adhesive. J Biomed Mater Res A 2021;109:2392-2406.
6. Fawzy AS, Nitisusanta LI, Iqbal K, Daood U, Neo J. Riboflavin as a dentin crosslinking agent: ultraviolet A versus blue light. Dent Mater 2012;28:1284-1291.
7. Hashimoto M, Hirose N, Kitagawa H, Yamaguchi S, Imazato S. Improving the durability of resin-dentin bonds with an antibacterial monomer MDPB. Dent Mater J 2018;37:620-627.
8. Basting RT, Gonçalves FR, França FM, do Amaral FL, Flório FM. Antimicrobial Potential of Papain Chemomechanical Agent on Streptococcus Mutans and Lactobacillus Casei Followed by the Use of Self-Etching Adhesive Systems. J Clin Pediatr Dent 2016;40:62-68.
9. Daood U, Matinlinna JP, Pichika MR, Mak KK, Nagendrababu V, Fawzy AS. A quaternary ammonium silane antimicrobial triggers bacterial membrane and biofilm destruction. Sci Rep 2020;10:10970.
10. Ozer F, Karakaya S, Unlu N, Erganis O, Kav K, Imazato S. Comparison of antibacterial activity of two dentin bonding systems using agar well technique and tooth cavity model. J Dent. 2003;31:111-116.
11. Imazato S. Bio-active restorative materials with antibacterial effects: new dimension of innovation in restorative dentistry. Dent Mater J. 2009;28:11-19.
12. Ozaki K, Matsuo T, Nakae H, Noiri Y, Yoshiyama M, Ebisu S. A quantitative comparison of selected bacteria in human carious dentine by microscopic counts. Caries Res. 1994;28:137-145.
13. Poggio C, Arciola CR, Cepurnykh S, Chiesa M, Scribante A, Selan L, et al. In vitro antibacterial activity of different self-etch adhesives. Int J Artif Organs. 2012;35:847-853.
14. Cheng L, Weir MD, Zhang K, Arola DD, Zhou X, Xu HH. Dental primer and adhesive containing a new antibacterial quaternary ammonium monomer dimethylaminododecyl methacrylate. J Dent 2013;41:345-355.
15. Osorio R, Yamauti M, Osorio E, Ruiz-Requena ME, Pashley D, Tay F, et al. Effect of dentin etching and chlorhexidine application on metalloproteinase-mediated collagen degradation. Eur J Oral Sci. 2011;119:79-85.
16. Zenlido Sousa AB, Vidal CMP, Leme-Kraus AA, Pires-de-Souza FCP, Bedran-Russo AK. Experimental primers containing synthetic and natural compounds reduce enzymatic activity at the dentin-adhesive interface under cyclic loading. Dent Mater 2016;32:1248-1255.
17. Harris JD, Brand JC, Cote MP, Faucett SC, Dhawan A. Research Pearls: The Significance of Statistics and Perils of Pooling. Part 1: Clinical Versus Statistical Significance. Arthroscopy. 2017;33(6):1102-1112.