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RESEARCH ARTICLE

EVALUATION OF ANTIMICROBIAL AND SHEAR BOND STRENGTH OF ORTHODONTIC PRIMERS CONTAINING THREE ANTIMICROBIAL AGENTS – "AN INVITRO STUDY"

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ABSTRACT

Background: Orthodontic treatment make oral hygiene maintenance difficult, there by creating a favourable environment for bacterial accumulation. Acid produced by the oral bacteria can demineralise the tooth leading to white spot lesions. Antimicrobial agents are recommended to reduce the bacterial growth. The aim of this study to compare the antimicrobial properties of primer containing Chlorhexidine, Cetyl Pyridinium Chloride, Triclosan and conventional primer using planktonic and biofilm model of Streptococcus mutans and to compare physical properties of primers by analyzing shear bond strength and bond failure pattern. **Methodology:** Three antimicrobial agents were incorporated respectively to Transbond XT primer. MIC and MBC values of newly prepared primers were determined. Antibacterial activity of four primers (TX, TX-CHX, TX-CPC and TX-Triclosan) against growth of Streptococcus mutans in both planktonic and biofilm model was analysed by performing growth and biofilm assay. These antimicrobial agents added primers were used for bonding brackets on to the tooth. The shear bond strength of conventional and newly prepared primers were tested using universal testing mechine. **Result**: TX-CHX had stronger antimicrobial activity against S. mutans in the planktonic and biofilm phases than TX, TX-CPC and TX-Triclosan. The antimicrobial activity of TX-CHX was maintained after thermocycling. Chlorhexidine incorporated with XT primer shows better Shear bond strength than Triclosan and Cetyl pyridenium chloride incorporated primers but less than XT primer. **Conclusion:** TX-CHX has better antibacterial property than TX-CPC, TX-Triclosan and TX. Antibacterial agents added primers has sufficient bond strength to withstand orthodontic force. TX-CHX has better shear bond strength than TX-CPC and TX-Triclosan but less than XTprimer

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INTRODUCTION

Fixed orthodontic appliances provide retentive areas for plaque which results in enamel demineralization commonly at the bracket tooth interface. Excessive adhesives around the bracket act as a potential plaque retentive areas and provide site for adhesion and growth of bacteria. The surface of brackets, bands and wires make tooth cleansing difficult and thereby leading to bacterial colonization. Streptococcus mutans (S mutans) and Lactobacillus are present in higher concentration in the oral cavity and has higher adhesion capacity around orthodontic brackets.

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The antimicrobial primers are helpful to minimize biofilm formation and demineralization at the bracket tooth interface. The demineralization of enamel can be regulated by inhibiting the development of biofilms (Marsh, 2006). By using commercial antimicrobial mouthwashes the pathogens that cause dental caries can be effectively reduced. Antibacterial mouthwash containing Chlorhexidine (CHX) and Cetyl pyridinium chloride (CPC) are commonly used oral hygiene products that have antiseptic properties. Chlorhexidine is a powerful antiseptic agent of hydrophilic bisguanide (Emilson, 1994). It decreases plaque formation and inhibits the growth of microorganisms, including S mutans and Lactobacillus species. It attaches to bacterial cell walls, mucosa and salivary pellicle, giving it a high degree of substantivity (Law, 2007). At low concentrations (<1%), chlorhexidine is bacteriostatic and at high concentrations (>1%) it is bactericidal. Chlorhexidine has

long term retention in the oral cavity and slow release into saliva at bacteriostatic concentration preventing bacterial adherence to the tooth surface. Cetyl pyridinium chloride is a quaternary ammonium agent with antiseptic property having a high affinity for Mutans streptococci. Cetyl pyridinium chloride has a high binding affinity for negatively charged cell walls as that of chlorhexidine. It induces disruption of the cell membrane, leakage of cytoplasmic elements, inhibition of metabolism and proliferation of bacterial cells. Cetyl pyridinium chloride prevents cellular aggregation and plaque maturation in dental biofilms (Ayad, 2011; Williams, 2011). Cetyl pyridinium chloride has limited ability to adhere to oral surfaces and hence has limited substantivity (Williams, 2011). Triclosan is an antimicrobial agent which can disrupt cell membrane, inhibit Streptococcus mutans and glycolysis in dental plaque biofilm (Tuan-Nghia Phan, 2006). The purpose of this study is to compare the antimicrobial properties of each experimental primer containing (Transbond XT (TX), TX-Chlorhexidine, TX-Cetyl Pyridinium Chloride and TX-Triclosan) using planktonic and biofilm models of Streptococcus mutans and to compare physical properties of primers by analyzing shear bond strength and bond failure pattern.

MATERIALS AND METHODS

Three antibacterial agents Chlorhexidine (CHX)(0.12%), Triclosan(0.03%) and Cetyl pyridinium chloride (CPC)(0.1%) were incorporated respectively into Transbond XT primer (TX) to form antibacterial primers, TX-CHX, TX-Triclosan and TX-CPC and stirred for 12 hrs in room temperature. Transbond XT primer was alone used as control.

Minimum Inhibitory Concentration (MIC) determination: Streptococcus mutans was grown in Brain Heart Infusion (BHI) medium at 37^0C in 5%CO_2 aerobic atmosphere. 6.5 x 10^7 colony forming units/mL of S mutans growth was prepared and diluted in BHI broth and compared with 0.5 Macferland standard. It was then added to polysterine 96 well plate. To measure MIC primers where added to each well to final dilutions of 1/2,1/4,1/8,1/16,1/32,1/64,1/128,1/256,1/512 and 1/1024. Ampicillin was used as positive control. After incubating for 24 hrs at room temperature, the lowest primer concentration that inhibited visible growth was considered as MIC.

Minimum Bactericidal Concentration (MBC) determination: 100µl of bacterial culture from each well at MIC value was diluted 10 through 1000 fold and plated to BHI agar plate for bacterial strain. Agar plate was incubated at 37^0C for 48 hrs and the number of colonies were counted. The concentration that killed 99.9% of bacteria was considered as MBC value.

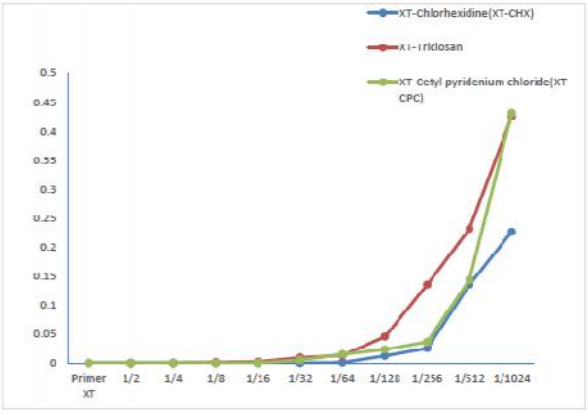
Growth assay (planktonic study): It was performed using 96 well plate to analyze the effect of primers on planktonic growth of Streptococcus mutans. Before assaying the primers, its minimum inhibitory concentration were applied to bottom of cell culture plate and cured for 20 sec from top and bottom each using Light cure(Woodpecker). To compare the growth rate, BHI broth was inoculated with 1:100 dilution of over night culture of S. mutans and bacterial suspension were incubated at 37^0C in well with primer. Optical density at 600 nm was recorded to monitor the bacterial growth using a microplate reader. To assess water aging in mouth these plates were thermocycled in water .The thermocycling machine was set at 20 sec dips to 5 sec of transfer time .Immersion temperature range from 5^0C to 55^0C.

Bio Film study: Primers with chemical agent at MIC values were applied to the plates and light cured. To assess biofilm formation Streptococcus mutans were grown in semi defined biofilm medium with 18mM glucose and 2mM sucrose as carbohydrate source. Overnight culture of Streptococcus mutans was transfered to pre warmed BHI and grown at 37^0C in 5%CO_2 aerobic atmosphere then the culture was diluted 100 fold in pre warmed biofilm medium. Each primer containing well was coated with artificial saliva and biofilm assays were performed. Plates were incubated at 37^0C for 2hrs and then washed twice with phosphate buffer saline [pH7.2]. After air drying for 30 min, wells were inoculated with 150µl of cell suspension. Then all plates were incubated at 37^0C in 5%CO_2 atmosphere for 24 hrs. The culture media was decanted and plates were washed twice with sterile distilled water to remove planktonic and loosely bond cells. Bacteria was stained with 0.1% crystal violet for 15 minutes and rinsed twice with water, Bound dye was extracted from stained cells using 200 microliter of 99% ethanol. Biofilm formation was quantified, measuring absorbance of solution at 600nm using spectrometer.

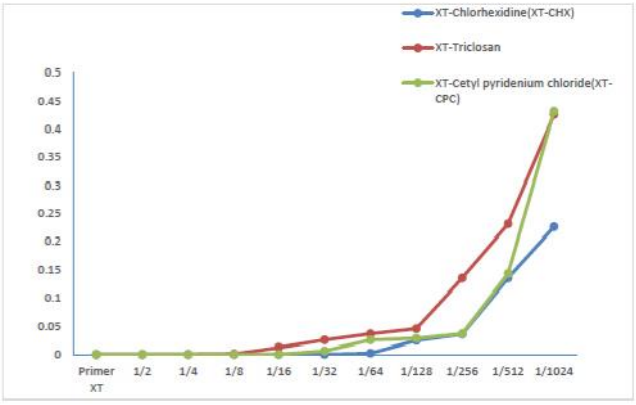
Shear Bond strength: Human premolar were prepared by cutting the crown portion of the tooth and then fixed in acrylic. Each tooth surface was etched with 32% phosphoric acid gel then primers (TX, TX-Chlorhexidine, TX-Cetyl PyridInium Chloride, TX-Triclosan) were applied respectively to each samples and then light cured for 10 seconds. Then brackets were positioned using bracket positioner and then light cured using Led (woodpecker). Thermocycling was done on each sample's. Shear bond strength was tested using universal testing machine (Instron 4465, Canton, Mass). 1mm/minute was the cross head speed at which the loading jig hits the specimen. After obtaining the results they were compared. The debonded surface was examined under stereomicroscope with 10X magnification to check the bond failure pattern and adhesive remaining on the tooth is scored using adhesive reminant index.

RESULTS

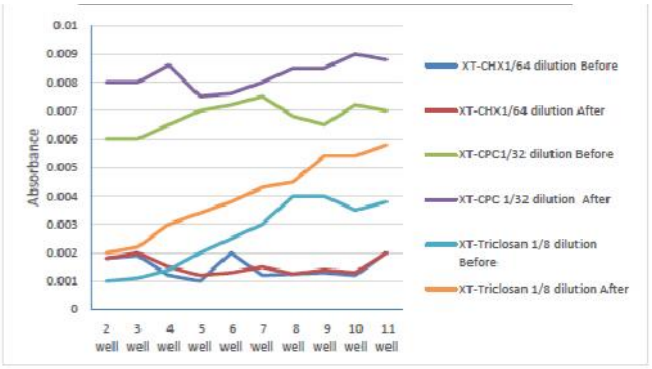
The MIC value of TX-CHX was 19µg/ml, while those of TX-CPC and TX-Triclosan were 31µg/ml and 37µg/ml respectively. The MBC value of TX-CHX was 37µg/ml, while those of TX-CPC and TX-Triclosan were 62µg/ml and 75µg/ml dilution respectively. This indicates that TX-CHX had more antimicrobial property than TX-CPC, TX-Triclosan and TX (Graph 1 and 2). Growth assay results showed that application of TX-CHX to the plates completely inhibited bacterial growth. Thermo cycling did not significantly influence the growth inhibition effects of TX-CHX on S. mutans (Graph 3). There were significant differences in biofilm development by Streptococcus mutans according to primer type (Graph 4).



Graph 1. MIC of three antmicrobial agent incorporated to XT primer



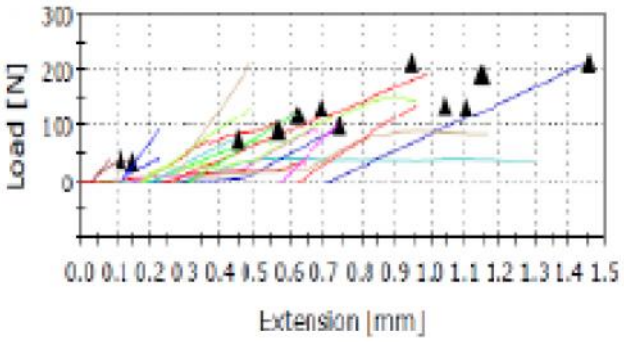
Graph 2. MBC of three antmicrobial agent incorporated to XT Primer



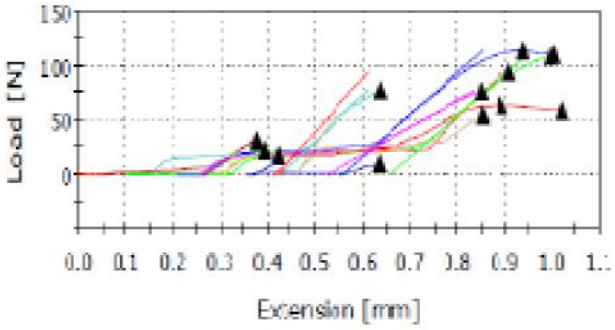
Graph 3. Growth of streptococcus mutants before and after thermocyclin

Graph 4. Growth of streptococcus mutants before and after thermocycling

TX-Triclosan, TX-CPC, and TX-CHX significantly inhibited the biofilm formation of S. mutans, compared to the control, but inhibition patterns were different from each other. There were no significant differences in biofilm inhibition between TX-Triclosan and TX-CPC, but TX-CHX completely inhibited biofilm formation of S. mutans. Thermocycling did not significantly influence the effects of primers on the biofilm formation of S. mutans. This indicates that TX-CHX has stronger antimicrobial activities against S. mutans than does TX-Triclosan, TX-CPC and TX, irrespective of thermocycling. No growth was observed in Ampicillin. Comparison of bond strength of brackets were done using ANOVA test and significant difference in bond strength was obtained. These results indicate that incorporation of CHX, CPC and Triclosan into the primers adversely affect the physical properties of the original primer that is reduction in shear bond strength. But TX-Chlorhexidine had shown increase in bond strength than Cetyl pyridenium chloride and triclosan. (Graph 5,6,7,8).



Graph 5. Shear bond strength of XT-Triclosan



Graph 6. Shear bond strength of XT-Cetyl phrideninum chloride (XTCPC)

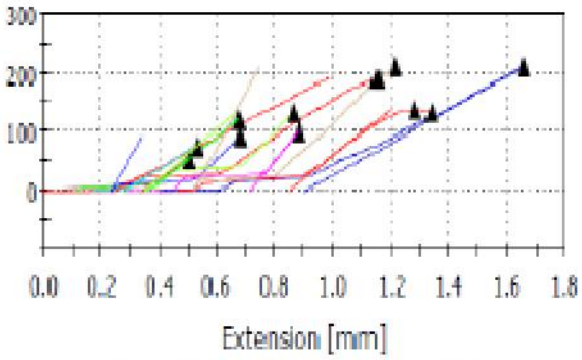
DISCUSSION

Fixed orthodontic appliances provide suitable conditions for the colonization of oral microorganisms.[13] Increase in plaque retention increases the risk of enamel demineralization adjacent to the appliances. Streptococcus mutans were considered as main pathogens associated with enamel demineralization because they induce mineral loss as they strongly adhere to the tooth surface and produce acid from the fermentation of carbohydrates making the local pH low (Ahn, 2008). Various approaches using antibacterial agents have been investigated to prevent enamel demineralization as a consequence of bacterial colonization and biofilm growth. One such method was to use mouthwash containing antibacterial agent but it depends on patient cooperation. So a better method was to incorporate antimicrobial agents on to the primer so that

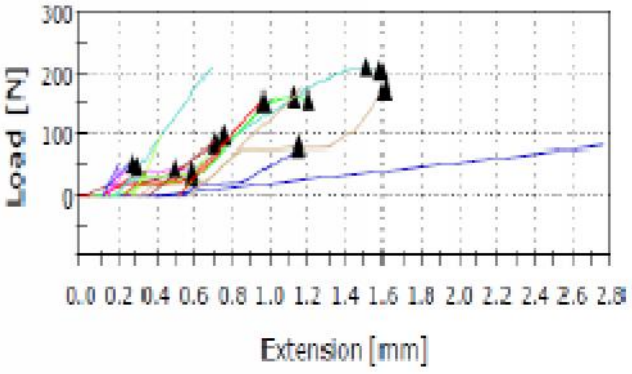
antimicrobial activity will be continued as the chemical leaches in to the oral cavity. The MIC and MBC tests are well-known in vitro sensitivity tests for antimicrobial agents against pathogenic bacteria, and low values indicate high potency for antimicrobial activity (Selma Elekdag-Turk, 2008). Adsorption and retention of CHX in natural and artificial intra-oral surfaces have been reported in studies (Joiner, 2006; Bonesvoll, 1974; Rolla, 1970) and this makes CHX clinically effective in the oral cavity. Intra-oral application of CHX has been reported to have no harmful effects on the mechanical properties of dental biomaterials (Ricci, 2010; Loguercico, 2009).

A sufficient concentration of antimicrobial agents in the primer was essential because a limited amount may reduce antimicrobial activity. Chlorhexidine was commonly used antibacterial agent for plaque control. It has antibacterial effect on gram positive and gram negative organisms and inhibit bacterial accumulation on tooth surface (Takeuchy, 2007; Zhang, 2006). Adsorption and retention of CHX in natural and artificial intraoral surface have been reported in studies (Joiner, 2006; Bonesvoll, 1974; Rolla, 1970) and makes clinically effective. Studies (Shin-Hye Chung, 2016; Juliana Rico Pires, 2007) showed that 0.12% of CHX 0.03% of triclosan and 0.1% of CPC was the ideal concentration for antimicrobial activity. Intra oral application of CHX has been reported to have no mechanical properties of dental biomaterials (Ricci, 2010; Loguercico, 2009). Triclosan is used in dermatological preparations (Andreas Rathke, 2010; William, 2007) Small amount was needed to produce powerful antibacterial effect (Juliana Rico Pires, 2007). The MIC and MBC results shows that the minimum inhibitory concentration of chlorhexidine, Triclosan and Cetyl pyridinium chloride that was required to inhibit the visible growth of Streptococcus mutans was 19µgm/ml, 37µgm/ml and 31µgm/ml respectively. The minimum concentration at which 99.9 % of bacteria killed was the MBC value. MBC values for Chlorhexidine,Triclosan and Cetyl pyridenium chloride was 37µgm/ml, 75µmg/ml and 62µgm/ml respectively. Hence for these particular MBC values, 99.9 % growth of S. mutans was completely inhibited.

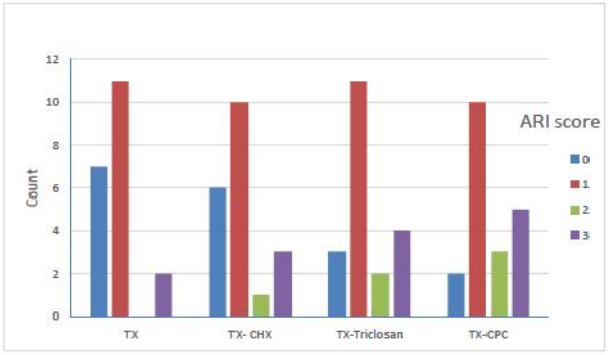
Planktonic growth of Streptococcus mutans was done in primer conditioned well plates. Plates were thermocycled and growth rates before and after thermocycling showed no difference. Results shows that Chlorhexidine has better property than others. Biofilm assays were done in plates conditioned with primer and coated with artificial saliva. The biofilm inhibitory effects of TX- Chlorhexidine had better antibacterial properties compared with TX and other antimicrobial primers such as TX-Cetyl pyridinium chloride and TX-Triclosan. No growth was seen in well with ampicillin. The inhibitory effect influenced by water aging was investigated. The growth and biofilm assays were done after thermocycling. Results showed that TX-CHX, TX-CPC and TX-Triclosan appear to have a major inhibitory effect on bacterial growth and biofilm development after thermocycling. There was a slight difference in the inhibitory effect between noncycled and thermocycled plates. Even after thermocycling the inhibitory effect was better for TX- Chlorhexidine compared to TX-Cetyl Pyridinium chloride and TX-Triclosan. The invading bacteria in marginal gaps directly contact the primer rather than the enamel surface. The primers containing antimicrobial agents, that is TX-CHX, TX –CPC, TX- CPC can have direct contact with bacteria invading the adhesive enamel margins and inhibit bacterial growth and biofilm formation at the interface.



Graph 7. Shear bond strength of XT



Graph 8. Shear bond strength of XT-Cholorhexidine (XT-CHX)



Graph 9. Frequency and Distribution of Adhesive Reminant Index (ARI) when different primers were used for bonding brackets

The shear bond strength of newly prepared primers (TX-Chlorhexidine, TX-Triclosan and TX-Cetyl pyridenium chloride) were examined by using Universal testing machine. Studies (William Brandley) had shown that the ideal bond strength was in the range of 6-8MPa.The normal bond strength of Transbond XT Primer was 15.51MPa[27]. The shear bond strength of samples bonded with TX-CHX was greater than TX-Triclosan, TX-CPC. Shear bond strength obtained from this study for TX was 14.68MPa, TX-CHX was 12.30MP a for TX CPC was 7.79MPa and for TX-Triclosan was 8.47MPa (Graph 5,6,7,8), that was less than the bond strength of XT primer alone. The composite bonded with primer added antimicrobial agents has sufficient bond strength to withstand the orthodontic force. Studies (Shin-Hye Chung, 2016) had

shown that thermo cycling did not affect on shear bond strength. From this study chlorhexidine added to the conventional primer had shown increased shear bond strength value and better inhibiting property against Streptococcus mutants than primers incorporated with triclosan and Cetyl pyridenium chloride. No significant difference in bond failure pattern was observed when different primers (TX,TX-CHX,TX-CPC and TX-Triclosan) were used. Amount of adhesive left on the tooth was scored for each tooth using adhesive reminant index and no significant difference was observed (Graph 9).

Conclusion

This study concludes that CHX incorporated primer provided stronger antimicrobial properties than the control, Cetyl pyridinium chloride and triclosan incorporated primers. The shear bond strength of different antimicrobial agent incorporated primer was assessed using Universal testing machine. The bond failure pattern was evaluated using scanning electron microscopic examination of tooth surface and it was the qualitative method.

Conclusions arrived at the end of study were as follows.

- Adhesive Remnant Index indicate no significant difference in bond failure pattern among TX,TX-Chlorhexidine, TX-Triclosan and TX-Cetyl pyridenium chloride.
- Chlorhexidine incorporated with XT primer shows better result than Triclosan and Cetyl pyridenium chloride incorporated primers but less than XT primer.

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