International Medical Journal Vol. 21, No. 1, pp. 70 - 72 , February 2014

SHORT COMMUNICATIONS

The Potential of Antibacterial Effects of Dental Restorative Materials towards *Streptococcus mutans*: *In Vitro* Study

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ABSTRACT

Background: **One of the major causes of failure in restoration of carious tooth is the reoccurrence of dental caries around or under the restoration. Preferably, restoration with antibacterial properties will have a great advantage in controlling the progression of dental caries.**

Objectives: **The objectives of this study were: (a) to evaluate the antibacterial effects of dental restorative materials (conventional amalgam, Silverfil, Fuji II LC, Fuji IX, Composite resin) against *Streptococcus mutans*; and (b) to compare the antibacterial properties of each materials against *Streptococcus mutans*.**

Materials and methods: **Ten pellets of each material (conventional amalgam, Silverfil, Fuji II LC, Fuji IX, Composite resin) were prepared and placed on the top of blood agar inoculated with *Streptococcus mutans*. Zone of inhibition was measured and statistical analysis done using SPSS 12.0.1 Kruskal-Wallis test and Mann-Whitney test.**

Results: **Silverfil, conventional amalgam, Fuji IX, Fuji II LC and composite resin has significant potential of antibacterial effect against *Streptococcus mutans* with the $p < 0.05$. Silverfil had the most significant antibacterial effect and followed by conventional amalgam, Fuji IX, Fuji II LC and composite resin.**

Conclusion: **All restorative materials tested (conventional amalgam, Silverfil, Fuji II LC, Fuji IX, composite resin) had antibacterial effect against *Streptococcus mutans* and composite resin had the least antibacterial activity.**

KEY WORDS

antibacterial effects, dental restorative materials, *Streptococcus mutans*, amalgam, silverfil, composite resin

INTRODUCTION

Dental caries is the lesion on the tooth structure resulting from the progression of demineralization of the tooth by acid formed by the interaction of plaque micro-organisms and fermentable carbohydrates. The principal causative agents are population of streptococci species by which Streptococcus mutans is the most important causative agent (Balakrishnan *et al*., 2000). Other bacteria including lactobaccili and other strains of streptococci are only weakly cariogenic or are none cariogenic (Cawson and Odell, 2002). Numerous numbers of failures of dental restoration are due to reoccurrence of dental caries adjacent or underneath the restoration, dental materials with some cariostatic or bactericidal effect is potentially a great benefit to prevent occurrence and progression of caries (Edwina *et al*., 2003).

Recently, concerns have been raised regarding the therapeutic effects exhibits by dental restorative materials. A lot of studies had been done to investigate the potential antibacterial effects of common use restorative materials (Clemens et al., 2002; Foley and Blackwell, 2003; Morrier *et al*., 1998; Qvist *et al*., 1997; Van Dijken *et al*., 1997).

Morrier (Morrier *et al*., 1998) claimed that mercury and copper contained in amalgam had the antibacterial properties and they believe mercury had greater antibacterial effects than copper. Currently, Silverfil was introduced by a Malaysian local company and the aim of this new amalgam material was to solve problems associated with conventional amalgam that contain free excess mercury (Mohamad *et al*., 2013). This material contains of pure silver with mercury acting as the solvent. Silver ion will bind to the mercury and it is believed that there will be no free mercury that can be leach out from the amalgam once it set (Manavalan, 2004). We believe that there is no record to date of any studies on the potential antibacterial properties of Silverfil amalgam. Study by Clemens (Clemens *et al*., 2002), showed that the hybrid resin composite did not show any antibacterial effect on Streptococcus mutans. However, all other tested materials (glass ionomer cement, resin modified GIC and zinc oxide eugenol) showed antibacterial properties against *Streptococcus mutans*.

The aims of this study were to evaluate the antibacterial effects of dental restorative materials (conventional amalgam, Silverfil, Fuji II LC, Fuji IX, and Composite Resin) against *Streptococcus mutans* and to compare the antibacterial properties of each tested material against *Streptococcus mutans*.

Received on July 25, 2012 and accepted on April 8, 2013
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Table 1. Diameter of the inhibition zone on the blood agar for each tested material after 48 hours

Materials n = 10	*S Mutans* median (IQR) in mm	X^2 statistic[a] (df)	*p* value[a]
Amalgam	5.0 (0.35)		
Silverfil	11.65 (0.53)		
Fuji IX	1.3 (0.40)		
Fuji II LC	0.9 (0.45)	66.47 (6)	< 0.001
Composite Resin	0.2 (0.23)		
Negative control	0.05 (0.20)		
Positive control	24.85 (0.65)		

[a] Kruskal Wallis test

Table 2-1. Comparison of amalgam [a] with other tested material

Material (n = 10)	Zone of Inhibition (mm) Median (IQR)	*Z* Statistic[b]	*P* Value[b]
Silverfil	11.65 (0.53)	-3.79	< 0.001
Fuji IX	1.3 (0.40)	-3.80	< 0.001
Fuji II LC	0.9 (0.45)	-3.79	< 0.001
Composite resin	0.2 (0.23)	-3.80	< 0.001
Negative Control	0.05 (0.20)	-3.82	< 0.001
Positive Control	24.85 (0.65)	-3.79	< 0.001

[a] Amalgam Median (IQR) = 5.0(0.35) mm, [b] Mann-Whitney test

Table 2-2. Comparison of Silverfil [a] with other tested material

Material (n = 10)	Zone of Inhibition (mm) Median (IQR)	*Z* Statistic[b]	*P* Value[b]
Fuji IX	1.3 (0.40)	-3.79	< 0.001
Fuji II LC	0.9 (0.45)	-3.79	< 0.001
Composite resin	0.2 (0.23)	-3.79	< 0.001
Negative Control	0.05 (0.20)	-3.82	< 0.001
Positive Control	24.85 (0.65)	-3.78	< 0.001

[a] Silverfil Median (IQR) = 11.65(0.53) mm [b] Mann-Whitney test

Table 2-3. Comparison of Fuji IX GIC [a] with other tested material

Material (n = 10)	Zone of Inhibition (mm) Median (IQR)	*Z* Statistic[b]	P Value[b]
Fuji II LC	0.9 (0.45)	-3.20	0.001
Composite resin	0.2 (0.23)	-3.80	< 0.001
Negative Control	0.05 (0.20)	-3.82	< 0.001
Positive Control	24.85 (0.65)	-3.79	< 0.001

[a] Fuji IX GIC Median (IQR) = 1.3(0.40) mm [b] Mann-Whitney test

Table 2-4. Comparison of Fuji II GIC [a] with other tested material

Material (n = 10)	Zone of Inhibition (mm) Median (IQR)	*Z* Statistic[b]	*P* Value[b]
Composite resin	0.2 (0.23)	-3.72	< 0.001
Negative Control	0.05 (0.20)	-3.82	< 0.001
Positive Control	24.85 (0.65)	-3.79	< 0.001

[a] Fuji II LC GIC Median (IQR) = 0.9(0.45) mm [b] Mann-Whitney test

Table 2-5. Comparison of composite resin [a] with other tested material

Material (n = 10)	Zone of Inhibition (mm) Median (IQR)	*Z* Statistic[b]	*P* Value[b]
Negative Control	0.05 (0.20)	-2.14	0.032
Positive Control	24.85 (0.65)	-3.80	< 0.001

[a] Composite resin Median (IQR) = 0.2(0.23) mm [b] Mann-Whitney test

MATERIALS AND METHODS

Materials that were used for this study were Conventional Amalgam (*dp* nuAlloy ®), Silverfil (Dunia Perwira Manufacturing Sdn.Bhd), Fuji IX Fast (GC Dental Corp., Tokyo, Japan -GIC), Fuji II LC (GC Dental Corp., Tokyo, Japan -RMGIC), Composite resin (Coltène® Synergy® Nano Formula), Penicillin streptomycin (In Vitrogen Corporation. Cat No. 15070-063 Lot No. 1166430) as positive control, and plain sterile paper discs as negative control. All the materials were collected from HUSM Dental Clinic. Manual caliper (Boley Gauge) with measurement range from 0 mm to 100 mm was used for the measurement.

Data Collection Procedure

Ten pellets of specimens (4.0 mm in diameter and 3.0 mm in thickness) were prepared from each material. The specimens prepared according to the manufacturers' specifications, and all pellets were allowed to set for 24 hours in a humid environment before use. For negative and positive control, ten sterile paper discs in the same size (4 cm x 3 cm) were prepared. For negative control, the plain sterile paper discs were used, and for positive control, 5 ml of Penstrep were concentrated on the paper discs. *Streptococcus mutans* (ATCC 35668) is cultured in the Brain Heart Infusion (BHI) broth for 24 hours and incubated at 37℃. Using a sterile cotton bud, the solution in the BHI that contain *Streptococcus mutans* was spread out carefully over the surface of the blood agar, avoiding its margin. It is done to ensure uniform bacterial distribution and growth. All of the steps mentioned above will be done under lamina flow. Then, five pellets of the same materials were placed on the each plate of blood agar.

Each material was placed on the top of the each agar plates. All materials were repeated for ten times. The plates are inverted and incubated at 37℃. Observations were made after 48 hours. Following that, the inhibition zone diameter around the pellet was measured by used manual caliper (Boley gauge) and photographed. This was measured by only one operator supervised by laboratory expertise. The greatest inhibitory zone around the discs was measured.

Statistical Method

Zone of inhibition of each material were analyzed. The data was analyzed by use the SPSS 12.0.1 program, and differences among median values was analyzed using Kruskal-Wallis test and Mann-Whitney test was run for multiple comparisons. Non parametric analysis was used because the sample size for each material in this study was less than 30. A $p < 0.05$ was considered statistically significant.

RESULTS

Table 1 show the diameter of the inhibition zone on the blood agar for each tested material after 48 hours. It was shown that Silverfil, conventional amalgam, Fuji IX GIC, Fuji II LC GIC and composite resin has shown a significant potential of antibacterial effect against *Streptococcus mutans* with the $p < 0.05$. The median (IQR) of conventional amalgam were 5.0 (0.35) mm, Silverfil were 11.65 (0.53) mm, Fuji IX GIC were 1.3 (0.40) mm, Fuji II LC GIC were 0.9 (0.45) mm and median (IQR) zone of inhibition on the blood agar of composite resin were 0.2 (0.23) mm.

Table 2.1 to 2.5 shows the comparison of each material with each other. It has shown that Silverfil has the most significant antibacterial effect with the median (IQR) zone of inhibition on the blood agar 11.65 (0.53) mm, followed by conventional amalgam (5.0 (0.35) mm), Fuji IX GIC (1.3 (0.40) mm), Fuji II LC GIC (0.9 (0.45) mm) and the least was composite resin (0.2 (0.23) mm). (Silverfil > Conventional Amalgam > Fuji IX > Fuji II LC > Composite resin). Positive control had significantly showed its antibacterial effects with the median (IQR) of the zone of inhibition was 24.85 (0.65) mm. Negative control also showed a significant value (0.05 (0.20) mm).

DISCUSSION

In the present study, sterile paper disc that concentrated with 5 ml Penicillin streptomycin was selected as positive control because it contains 5000 units per ml Penicillin G Sodium and 5000 mg/ml streptomycin sulfate in 0.85 per cent saline that was well-known to have antibacterial potential. The positive control helps to determine the appearance of the zone of inhibition on the blood agar. Plain sterile paper disc was used for negative control. The negative control was important to look for any physical effects that take part in the zone of inhibition area. In this study, it shows that physical effects involved in the inhibition area with significant value.

The results obtained in this study shown variations of the potential of antibacterial effects against *Streptococcus mutans* associated with the type of material used. Silverfil had the most significant antibacterial effects compared to other tested material. Silverfil is made of pure silver with mercury acting as the solvent. The antibacterial effects of Silverfil against *Streptococcus mutans* in this study was probably due to the present of high silver ion content, mercury however did not have much role in this effect because the mercury contains was insufficient to react with all the silver ions and thus no free mercury could be leach out the surface of Silverfil filling once the material had set. Metal ions such as silver, silver-ziolite and copper also have been shown to have antibacterial effects in nature (Kawahara *et al*., 2000; Thibodeau *et al*., 1978). Conventional amalgam showed the next highest potential antibacterial effects against *Streptococcus mutans*. Morrier (Morrier *et al*., 1998) suggests that the antibacterial elements in amalgam would be due to mercury and copper, and the study also suggested that mercury had greater antibacterial properties than copper.

Fuji IX and Fuji II LC had shown some significant antibacterial effect. Study by Ostela (Ostela and Tenovuo, 1990) suggested that the fluoride ion and other heavy metal ions present in GIC may contribute to the antibacterial properties of these materials. Antibacterial properties of GIC could be attributed to acidity resulting from acidity from the polyalkenoic compound in the GIC and the fluoride ion release during the setting action (DeSchepper *et al*., 1989). Interestingly, previous study done by Van Dijken (Van Dijken *et al*., 1997), found that fluoride concentrations in GIC do not significantly cause bactericidal or bacteristatic activity after a month of placing the restoration. They believed that the antibacterial effects disappear after about a month of restoration been inserted, because the fluoride release reaches the steady state level. However study done by Botelho (Botelho, 2003) showed that there was no significant antibacterial effect with Fuji IX GIC. The possible reason for the different result obtained from the study by Botelho could be due to the difference in the method used compared with our study.

Composite resin had shown the least antibacterial potential against *Streptococcus mutans* in this study. Study done by Clemens (Clemens *et al*., 2002), revealed that fine hybrid resin composite (Tetric Ceram) had no antimicrobial activity against *Streptococcus mutans* but modified fine hybrid resin composite (Ariston pHc) had significant antibacterial effect. They believed that ion release from Ariston pHc contribute to the antibacterial activity. The underlying mechanism of the antibacterial activity observed in the tested composite resin (Coltène® Synergy® Nano Formula) in our study probably was due to the difference in the compositions and the possible diffusion of the free monomer from the materials.

This present study showed that new amalgam restoration, silverfil has the most potential of antibacterial effects against *Streptococcus mutans* compare with other materials. However, further scientific investigations are required to study the effectiveness against other microorganisms needed as well as on what other mechanism to optimism the effect, and mechanisms involved for these materials.

CONCLUSION

The potential of antibacterial effects of this study is determine according to the measurement of the zone of inhibition on the blood agar. Silverfil exhibited the most significant antibacterial activity and composite resin showed the least antibacterial effects against *Streptococcus mutans*. Comparing the different materials that were studied, the order of antibacterial effects against *Streptococcus Mutans* was: Silverfil > conventional amalgam > Fuji IX > Fuji II LC > Composite resin.

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