DENTAL SCIENCES

In Vitro Study of Antibacterial Properties of Dental Restorative Materials towards *Streptococcus sobrinus* and *Lactobacillus salivarius*

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ABSTRACT

Introduction: **Dental restorative materials may not be able to provide a perfect seal from microorganism from penetrating the cavity walls, thus lead to formation of secondary caries. Therefore, presence of antibacterial properties in dental restorative materials can give a great significance in preventing formation of secondary caries.**

Objectives: **1) To evaluate the antibacterial effects of dental restorative materials Silverfil (amalgam), RMGIC (Ketac Nano), Composite (Amaris) and GIC (Inofil Molar AC-VOCO) against *Streptococcus sobrinus* and *Lactobacillus salivarius*, 2) To compare the antibacterial properties of each materials against tested bacteria.**

Materials and method: **Agar Diffusion Test (ADT) was used in this study. Dental materials were tested on Brain Heart Infusion Agar. Zone of inhibition exhibited by the materials were measured with digital caliper and data was analyzed by Kruskal Wallis and Man-Whitney Test.**

Result: **Silverfil shows the greatest antibacterial properties on *Streptococcus sobrinus* 22.41 mm (1.10) and *Lactobacillus salivarius* 11.99 mm (2.00) with a significant difference ($P < 0.05$) followed by Ketac Nano and Ionofil Molar. Amaris exhibit an antibacterial activity in *Lactobacillus salivarius* 8.86 mm (1.37) but none in *Streptococcus sobrinus*. *Streptococcus sobrinus* was more sensitive towards Silverfil, Ketac Molar and Ionofil Molar with a significant difference $P < 0.05$ except for Amaris which is more sentisitive toward *Lactobacillus salivarius***

Conclusion: **All materials exhibit antibacterial properties toward *Lactobacillus salivarius* and *Streptotococcus sobrinus* except for Amaris which did not show any antibacterial properties towards *Streptococcus sobrinus*.**

KEY WORDS

antibacterial properties, dental restorative materials, *Streptococcus sobrinus*, *Lactobacillus salivarius*

INTRODUCTION

Dental caries is a multifactorial disease caused by demineralization and remineralization imbalance on tooth surface due to acidic action of bacteria by-products. Among these oral bacteria, *Streptococcus mutans* is considered to be one of the major dental caries etiologic agents and their prevalence had been reported in many epidemiology studies (Hamada and Slade, 1980; Whiley and Beighton, 1998). Furthermore, these bacteria showed some selectivity of site in the tooth surface for them to adhere, such Mutan streptococcus is a predominant organism that invaded all tooth surfaces while *Lactobacilli* is commonly found on dentinal deep caries lesion (Ngo and S.Gaffney, 2005) and *Streptococcus sobrinus* had been closely related with high caries activity in oral cavity. Cariogenic bacteria also will exert its effect toward restored cavity thus causing development of secondary caries. Bacteria activity on this site may be due to improper caries removal during cavity preparation and also due to marginal leakage of restoration.

According to (Tobias *et al*., 1988), there is no current dental material that is able to provide a perfect seal within a cavity wall and there will always be a presence of micro space at the interface that is susceptible to microorganism penetration and migration. Thus a possibility of possessing antibacterial properties in dental restorative materials is of great significance since the longevity of dental restorations maybe improved. Therefore many studies had been done to evaluate the antibacterial of three main restorative materials which is amalgam, composite and glass ionomer cements. The antibacterial properties of amalgam is found to be due to its metal component such as mercury and copper (Morrier *et al*., 1998) while glass ionomer cements which produce fluoride and its acidity in pH is one of the important factor contributing toward the antibacterial properties of these materials (DeSchepper and White, 1989). However many studies on composite resin were conducted and showed no significant antibacterial effects (Karanika-Kouma *et al*., 2001; Vermeersch *et al*., 2005). Thus, many studies had done to enhance its antibacterial activity by incorporating or adding antibacterial element such as bactericide immobilized filler (Ebi *et al*., 2001) or antibacterial filler (Syafiuddin *et al*., 1997) to its composition which is by far succeeded and still maintained its physical properties.

Nowadays, more highly developed restorative materials are found in markets with an improvement on their properties. In this study, Ketac Nano (3M ESPE-RMGIC), Amaris (VOCO) a newly developed nano composites and Silverfil (amalgam) are used in which its antibacterial properties still need to be investigated. Therefore the aims of this study are to evaluate and to compare the antibacterial properties of the restorative materials towards the oral bacteria which are *Streptococcus sobrinus* and *Lactobacillus salivarius.*

MATERIALS AND METHODS

Materials that have been used for this study were Silverfil -

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Table 1. Descriptive statistics of study samples

Variables	Material	n	Mean (mm) (SD)	Median (mm) (IQR)
Streptococcus sobrinus	Silverfil	10	22.41 (1.098)	22.48 (1.47)
	Amaris	10	0.00 (0.00)	0.00 (0.00)
	Ketac Nano	10	12.23 (1.701)	12.51 (2.58)
	Ionofil Molar	10	11.65 (1.99)	11.34 (3.96)
	Positive control	10	37.73 (6.24)	38.5 (8.71)
	Negative control	10	0.00 (0.00)	0.00 (0.00)
Lactobacillus	Silverfil	10	11.99 (2.00)	12.03 (2.77)
	Amaris	10	8.862 (1.37)	9.02 (2.11)
	Ketac Nano	10	9.79 (2.65)	9.18 (2.06)
	Ionofil Molar	10	1.72 (3.67)	0.00 (1.87)
	Positive control	10	10.29 (1.43)	10.11 (1.95)
	Negative Control	10	0.00 (0.00)	0.00 (0.00)

SD: standard deviation IQR: interquatile range

Table 3. Compare zone of inhibition of each material between *Streptococcus sobrinus* and *Lactobacillus salivarius*

Variables	*Streptococcus sobrinus* Mean (mm) (SD)	*Lactobacillus* Mean (mm) (SD)	Z Statistics	P Value *
Silverfil	22.41 (1.098)	11.99 (2.00)	-3.78	0
Amaris	0.00 (0.00)	8.862 (1.37)	-4.038	0
Ketac Nano	12.23 (1.701)	9.79 (2.65)	-2.647	
Ionofil Molar	11.65 (1.99)	1.72 (3.67)	-3.749	0
Positive control	37.73 (6.24)	10.29 (1.43)	-3.781	0
Negative control	0.00 (0.00)	0.00 (0.00)	0	0

* Mann-Whitney Test

Table 2. Comparing zone of inhibition between four dental restorative materials against each type of bacteria

Variables	Silverfil mean(mm) (SD)	Amaris mean(mm) (SD)	Ketac Nano mean(mm) (SD)	Ionofil Molar mean(mm) (SD)	Positive control mean(mm) (SD)	Negative control mean(mm) (SD)	X^2 statistic (df)	P Value*
Streptococcus sobrinus	22.41(1.098)	0.00(0.00)	12.23(1.701)	11.65(1.99)	37.73(6.24)	0.00(0.00)	56.23 (5)	0
Lactobacillus	11.99(2.00)	8.862(1.37)	9.79(2.65)	1.72(3.67)	10.29(1.43)	0.00(0.00)	43.45 (5)	0

* Kruskal-Wallis Test

Amalgam (Dunia Perwira Manufacturing Sdn Bhd), Ketac Nano - RMGIC (3M ESPE), Amaris - Composite resin (VOCO America Inc), Inofil Molar AC/Quick - GIC (VOCO Germany). For positive control Ampicillin was used and sterile paper dick used for negative control. All the materials were collected from HUSM Dental clinic. The bacteria used in this is *Streptococcus sobrinus* (AT33478) and *Lactobacillus salivarius* (AT 11741) obtained from Craniofacial Laboratory PPSG

Data collection procedures

Material preparation

The specimens prepared according to the manufacturers' specifications. For negative control, plain sterile paper discs were use and for positive control, 5 ml of Ampicillin were concentrated on the paper discs. All materials were prepared under lamina flow, *Lamina Floor* (Kendro, Germany) and all the instruments, glass mixing slab were sterilized and subsequently wiped with the 70 per cent ethanol.

Plate preparation

Both bacteria were cultured on a Blood Agar incubated for 48 hours in 37℃. Three to four wells of isolated colonies of the bacteria in the agar are transfer to tube containing 10mL Brain Heart Infusion broth and incubated for 24 hours in 37℃. After 24 hours, the turbidity of the bacterial suspension was measured by comparing with 0.5 McFarland standards. Using a sterile cotton swab dipped into the bacterial suspension, it is spread onto Brain Heart Infusion Agar surface and left for 10 minutes in room temperature to allow uniform bacteria growth. Previously, the agar has been divided into four wells by using a blunt end sterile pasteur pipette as a standard mould (4 mm x 4 mm). This method was adapted from previous study (Herrera M, 1999).

Disc diffusion analysis

The materials were dispended into their respective wells by using sterile plastic instrument on 10 agar plates. The plates are inverted and incubated at 37℃. The plates were incubated for 48 hours and the inhibition zone produced around the tested materials was measured by a Digital caliper, 0-25 mm (Mahr 40Ex).

Statistical analysis

Data was analyzed using SPSS version 12.0.1. For evaluation of the antibacterial effects in each dental restorative material against *Streptococcus sobrinus* and *Lactobacillus salivarius* respectively, non parametric test, Kruskal-Wallis Test has been used. This was fulfilled the objectives 1 and 2. On the other hand, for achieving objectives 3, Mann-Whitney test was used to compare zone of inhibition of each material between *Streptococcus sobrinus* and *Lactobacillus salivarius*, and P value < 0.05 was considered as statistically significant.

RESULTS

Table 1 shows the mean and median of the inhibition zones of the four materials toward *Streptococcus sobrinus* and *Lactobacillus*. In *Streptococcus sobrinus*, the median of the inhibition zones of the materials are Silverfil (22.41 mm), Amaris (0.00), Ketac Nano (12.23 mm) and Ionofil Molar (11.65 mm). As for *Lactobacillus salivarius*, median of the inhibition zones of the tested materials are Silverfil (11.99 mm), Amaris (8.86 mm), Ketac Molar (9.79 mm) and Ionofil Molar (1.72 mm). The table shows that Silverfil had the greatest antibacterial properties toward both tested organism compared to the other three materials.

Table 2 exhibit a comparison between the four tested materials toward each bacterium. In *Streptococcus sobrinus*, Silverfil showed the greatest inhibition zone of 22.41 mm (1.10) followed by Ketac Nano 12.23 mm(1.70) and Ionofil Molar 11.65 mm (1.99) with significant difference (P < 0.001) while Amaris does not show any inhibition zone. As for *Lactobacillus salivarius*, Silverfil also has the

largest inhibition zone of 11.99 mm (2.00) followed by Ketac Molar 9.79 mm (2.65), Amaris 8.86 mm (1.32) and Ionofil Molar 1.72 mm (3.67) with significant difference ($P < 0.001$)

Table 3 shows a comparison of two tested microorganism toward each tested materials. *Streptococcus sobrinus* is sensitive towards Silverfil, Ketac Molar and Ionofil Molar with a significant difference $P < 0.05$ while Amaris showed greater antibacterial properties towards *Lactobacillus salivarius* compared with *Streptococcus sobrinus* with a significant difference $P < 0.05$

DISCUSSION

In this study, agar diffusion method (ADT) is used as a method for assessing the antibacterial properties of dental materials as it the most frequently used method in previous studies (Beyth *et al*., 2007; Cal *et al*., 2006; Imazato *et al*., 2007) as agar diffusion test allow both solid and liquid material to be assayed (Meiers and Miller, 1996). However there is a dynamic and variables associated with ADT which are interrelated with each other to ascertain the successfulness of the test being done. It is namely a good contact, molecular weight, rate of diffusion, inoculum density, time, agar medium, temperature, measurement and the tested organism. On the other hand ADT also possess few disadvantages as it does not distinguish between bacteriostatic and bactericidal properties of the material tested (Tobias, 1988) and also it is depend on solubility and diffusion properties of the both tested materials and media (Lewinstein *et al*., 2005)

The results from this study showed that Silverfil amalgam (Mohamad *et al*. 2013) had the greatest antibacterial properties toward both microorganisms compare to other tested materials. The antibacterial properties maybe derived from element in amalgam which is mercury, silver and copper. A study done also suggested that mercury and copper contribute significantly to the antibacterial properties of amalgams, however, a high copper content does not necessarily relate to high antibacterial effectiveness. These elements could be useful in conferring antibacterial properties to amalgam although their effects on host cells must be investigated (Morrier *et al*., 1998). (Matsumura *et al*., 2003) had investigated bactericidal effect of silver ion, had proposed that silver ion inhibit general function in the cell and consequently damage the bacterial cell.

Meanwhile both Ketac Nano and Ionofil Molar both also showed the antibacterial properties towards *Streptococcus sobrinus* and *Lactobacillu salivarius*. Glass ionomer cement had special features of ability to release fluoride ions but there may be other possible mechanisms that contribute to the antibacterial properties. (DeSchepper E.D and White, 1989) on his study on antibacterial properties of GIC revealed that both fluoride ion and acidic pH from polyalkenoic compound of GIC. However study by (Yap *et al*., 1999) noted that there is no correlation between fluorides releasing potential of GIC with antibacterial properties. (Vermeersch *et al*., 2005) done a study on GIC, conventional composites and polyacid modified resin composites found that there is direct relationship between acidity and growth inhibition of *Streptococcus* in GIC. Further study should be done to investigate the exact amount of fluoride release in GIC and RMGIC which can exhibit an antibacterial effect.

Amaris shows different result toward *Streptococcus sobrinus* and *Lactobacillus salivarius* where Amaris exhibit no inhibition zone on *Streptococcus sobrinus* but exhibit a large inhibition zone toward *Lactobacillus salivarius*. However previous study by (Karanika-Kouma *et al*., 2001) had a different result on the antibacterial properties of three brands of composite resin toward *Streptococcus sobrinus* and *Lactobacillus salivarius*. Investigation showed that all the three brands of composite resin did not exhibit antibacterial effect. The possible factor that differentiates in this present study may be due to the different components presence in Amaris that may act differently toward both microorganisms and the difference in the method used.

Composite resin had a resin component and filler component which bind with a coupling agent. Currently composite resin with nano particles of filler component had been invented to achieve a better strength and esthetic, thus include Amaris (VOCO) a newly developed nano composites by VOCO. Adding antibacterial properties to composites resin involved in alteration of resin component and the filler component. It is then subsequently classified into two groups based on release profile of antibacterial component either agent releasing or non agent releasing (Imazato, 2003). A study by (Beyth *et al*., 2007), a quarternary ammonium polyethylamine nano particles were embedded in clinically used bonding, flowable and hybrid dental composite resin to enhances it antibacterial properties towards *Streptococcus mutans*. It is proven that quaternary ammonium polyethylamine nanoparticles immobilized in resin-based materials have a strong antibacterial activity upon contact without leach-out of the nanoparticles and without compromise in mechanical properties. Study by (Imazato *et al*., 2007) by incorporating antibacterial monomer, Methacryloyloxydodecylpyridinium bromide (MDPB) to composite resin and results show that in reduces plaque accumulation. There are many other studies on composite resin to enhance its antibacterial properties by changing its component mainly the filler and resin monomer such as silver-supported antibacterial component (Yoshida *et al*., 1999), antibacterial filler powder (Syafiuddin *et al*., 1997) and chlorhexidine (Jedrychowski *et al*., 1983).

CONCLUSION

Silverfil show the greatest antibacterial activity towards both microorganisms tested followed by Ketac Nano and Ionofil Molar. Amaris on the other hand is more sensitive toward *Lactobacillus salivarius* rather than *Streptococcus sobrinus* compared to other tested materials which are more sensitive towards *Streptococcus sobrinus*.

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