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Antibacterial Properties of Amalgam and Composite Resin Materials Used as Cores under Crowns

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Abstract - *The Aim of this Study was to compare the bacterial growth in the bulk of both amalgam and fluoridated composite resin materials used as cores under crowns at core's surface (in the superficial area of the bulk) and depth levels. With 24 lower premolars, 12 of them were restored with metal posts and amalgam cores (group 1). The rest were restored with glass Fiber-reinforced Composite (FRC) posts and fluoridated composite resin cores (group 2). All specimens were covered with aluminium crowns cemented with resin cement, and then they were soaked in natural saliva for three months. Excoriations abraded from the superficial and the depth areas of the core materials were cultured under aerobic conditions on blood agar plates. After incubation for 2 days, colonies formed on the plates were identified, and the CFU mg^{-1} counts were recorded accordingly. Statistical analysis was performed using an independent sample T test. The mean values of CFU mg^{-1}counts in group 2 excoriations (surface 39.75, and depth 9.75) were higher than the group 1 excoriations (surface 1.67, and depth 0.42). This study supports the use of amalgam for building up cores due to its antibacterial properties. Composite resin, however, enhanced sizable bacterial growth despite the presence of fluoride.*

KEY WORDS: Bacterial growth, Dental amalgam, Fluoridated Composite resin.

INTRODUCTION

One of the most important factors for a successful post & core restoration is to prevent coronal bacterial microleakage. This depends on the quality and perfection of the restoration. Another important factor is that the used material should have antibacterial activity [1].

Prefabricated posts & cores technique has been commonly used in clinical practice. Moreover, both amalgam and composite resin are the most common materials used to build cores with this technique. Many (in vitro & in vivo) studies, that tested bacterial accumulation on the surface of the mentioned materials, have shown that amalgam has profound and lasting antibacterial effect [2]. This is not applicable to composite resin which has been shown to enhance bacterial growth [3]. This may explain the clinical observation of significant accumulation of vital biofilm on composites compared to amalgams [4]. Some other studies, however, had different outcomes for example Si-su *et al* [5] demonstrated no significant differences in the composition of the microflora between the two materials. It was suggested that fluoride addition to composite resin inhibits bacterial growth and secondary caries formation around restorations. The study of Torii *et al* [6] demonstrated this fact. While other studies such as Imazato *et al* [7] affirmed that fluoride addition does not prevent bacterial accumulation on composite resin surface.

Although all the previous studies investigated bacterial accumulation on materials outer surface, none of these studies, however, had estimated bacterial potentiality to colonize the bulk of the material at the surface, or to penetrate in to the depth. In addition, none had studied the material's antibacterial properties under crowns, which are the purposes of this study.

The aim of study was to compare the bacterial growth in the bulk of both amalgam and fluoridated composite resin materials used as cores with prefabricated posts. This was investigated under crowns, at the superficial core level and deep core level just near the post.

METHODS

This in vitro randomised controlled trial was performed in two stages. In the first stage, 24 post and core restorations were applied to 24 extracted and endodontically treated one root lower premolars. 12 of them (group 1) were randomly selected and then restored with metal posts [Anthogyr Europosts© (2237 Avenue André Lasquin- 74700 Sallanches – France)], and amalgam cores [ANA 2000© (Nordiska Dental AB Box 1082, 262 21 Ängelholm, Sweden)]. The other 12 (group 2) were restored with Fiber-Reinforced Composite posts FRC Postec® Plus© (Ivoclar Vivadent AG Bendererstrasse 2, FL-9494 Schaan, Liechtenstein), and dual-curing fluoridated composite resin for core build-ups MultiCore flow© (Ivoclar Vivadent AG Bendererstrasse 2, FL-9494 Schaan, Liechtenstein), according to manufacturer instructions. [The composition of composite: the monomer matrix consists of Bis-GMA, urethane dimethacrylate and triethylene glycol dimethacrylate (29 wt %). The inorganic fillers are barium glass, ytterbiumtrifluoride, Ba-Al-fluorosilicate glass and highly dispersed silicon dioxide (70 wt %). Additional contents are catalysts, stabilizers and pigments (1 wt %)]. The particle size ranges from 0.04 to 25 µm.Ytterbiumtrifluoride (base 16.4 wt %) (catalyste 16.2 wt%). Polymerisation shrinkage: 2-3 vol%. Water absorption 7 days (25 µg/mm^3). Thermal expansion coefficient 50-60 ppm °C $^{-1}$.

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All specimens were covered with aluminium crowns cemented with resin luting cement Multilink© (Ivoclar Vivadent AG Bendererstrasse 2, FL-9494 Schaan, Liechtenstein) (Table.1). Subsequently, roots were coated with dental wax 2mm far from the edge of the crown. The aim of this procedure was to prevent the bacterial microleakage from the root apex and to enable this microleakage only from crown margins. Antiseptic conditions were considered in all procedures of this stage.

Every restored tooth was then soaked in 0.5 cm^3 of natural saliva and 1cm^3 of 5% dextrose solution within a sterile glass tube and incubated at 37 °C for three months. Bacterial refreshment was performed by removing old saliva and washing the tooth with sterile water and adding new saliva from the same volunteer with dextrose solution. This refreshment was applied every two days for all teeth.

In the second stage (bacterial analysis), teeth were removed from saliva and washed with sterile water and this was followed by removing aluminium crowns using sterile scissors. The residual luting cement was completely removed, and teeth were thoroughly washed. Surface excoriations were abraded from the superficial area of the bulk of the core materials by a sterile blade (Fig 1). After thoroughly rewashing, the cores were broken using a sterile pair of forceps to reach the centre of cores (Fig 2). Using the above technique, excoriations were abraded from the depth of core materials just near the posts (Fig 3). All excoriations were immediately cultured on blood agar plates. A metal loop was heated and then it was immersed in a portion of agar far from the inoculating space to achieve cooling as well as wetting of the loop. Subsequently, this loop has been passed on the excoriations (Fig 4). The mounted measure from the excoriations has been weighed and it was equivalent to 1mg for all excoriations (Fig 5). This was subsequently smeared on the specific space of blood agar (Fig 6) and incubated in aerobic conditions at 37 °C for 2 days. Colonies formed on the plates were initially identified with the assistance of amplifier lens, and the CFU mg^{-1} counts were recorded accordingly.

According to shapes and size of different colonies, two types of colonies were seen in different amounts. Subculturing on fresh blood agar plates was therefore performed to harvest pure cultures of the pathogens. Further identification of bacteria was conducted by microscopic checking after Gram's staining.

Statistical analysis

In order to assess the effect of the material used in core build-ups on the CFU mg^{-1} counts, statistical analysis was performed on the dependent samples using an independent sample T test. A confidence level of 95% was adopted for assessing the *P* value of the mean values of CFU\mg counts between the two groups (1&2) according to the excoriation's level

RESULTS

Primary and sub-culturing qualitative results

Two types of colonies were seen in different averages on the primary plates of blood agar (Fig7, 8). The dominant type was small flat transparent colonies (Fig 9). These gave in sub-culturing a huge number of accumulating pure colonies resembling gelatinous cover in line with the shape of pure cultures of *Streptococcus mutans* (Fig 11). The rare type was big high colonies (Fig 10), which gave in sub-culturing clustering of large numbers of pure golden-yellow colonies comporting with the shape of pure cultures of *Staphylococci* (Fig 11).

Light microscopic checking results

Gram stained smears from the dominant type of colonies were examined with the oil immersion objective (×100) using the (×10) eyepiece. Gram-positive cocci in chains were seen mimicking *Streptococci* (Fig 12.). Smears from the rare type showed gram-positive cocci arranged in characteristic grape-like clusters, akin to *Staphylococci* (Fig 13).

Table 1. *Materials used in the study together with their manufacturer.*

Manufacturer	*Chemistry*	*Product*
Anthogyr Euro-posts© (2237 Avenue André Lasquin- 74700 Sallanches – France)	Stainless steel	Metal post
Ivoclar Vivadent AG Bendererstrasse 2, FL-9494 Schaan, Liechtenstein	Glass fibres, ytterbium trifluoride, the polymer matrix is composed of aromatic and aliphatic dimethacrylates	Fiber-Reinforced Composite post FRC Postec® Plus
Nordiska Dental AB Box 1082, 262 21 Ängelholm, Sweden	High copper admixed alloy. Sn 30.8% - Cu 26.1%. Ag 43.1%	Amalgam ANA 2000
Ivoclar Vivadent AG Bendererstrasse 2, FL-9494 Schaan, Liechtenstein	The matrix: Bis-GMA, urethane dimethacrylate and triethylene glycol dimethacrylate (29 wt %). The inorganic fillers are barium glass, ytterbiumtrifluoride, Ba-Al-fluorosilicate glass and highly dispersed silicon dioxide (70 wt %). catalysts, stabilizers and pigments (1 wt %). The particle size ranges from 0.04 to 25 µm.	Composite resin for core build-ups MultiCore flow
Ivoclar Vivadent AG Bendererstrasse 2, FL-9494 Schaan, Liechtenstein	The matrix: ethoxylated Bis-GMA, UDMA, Bis-GMA, HEMA. The inorganic fillers are barium glass, ytterbiumtrifluoride, spheroid mixed oxide(39.7 vol %). The particle size range is 0.25-3.0 µm.	Resin luting cement Multilink

Figure 1. *Excoriations abraded from the surface of the core materials using a sterile blade*

Figure 2. *Breaking the core using a sterile pair of forceps*

Figure 3. *Excoriations abraded from the depth of core materials just near the posts*

Figure 4. The loop is passing over the surface of the agar

Figure 5. *The mounted measure from the excoriations*

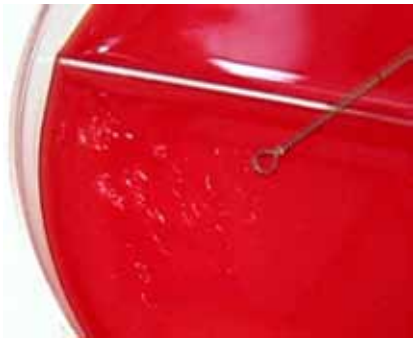

Figure 6. *Smearing the mounted measure on the specific space of blood agar*

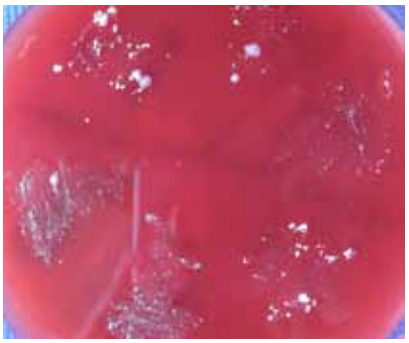

Figure 7. *Samples of colonies growing on blood agar from amalgam surface & depth excoriations after 2 days incubation*

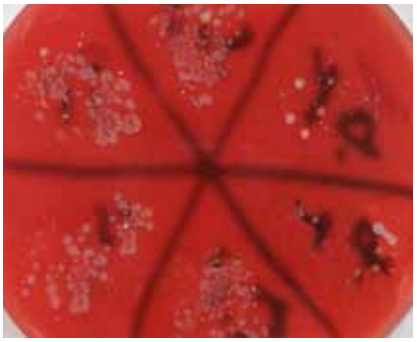

Figure 8. *Samples of colonies growing on blood agar from fluoridate composite resin surface & depth excoriations after 2 days incubation*

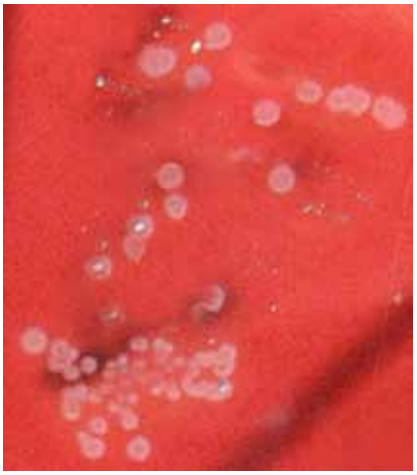

Figure 9. *A sample of the dominant small flat colonies growing on blood agar after 2days incubation*

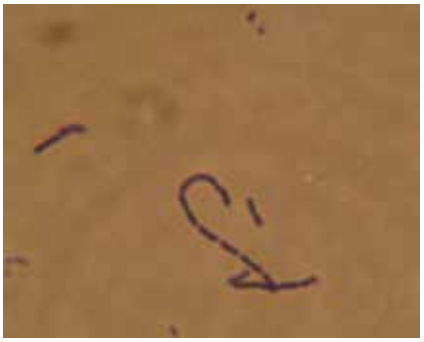

Figure12. *A sample of gram stained smears from the dominant type showing gram-positive cocci in chains.*

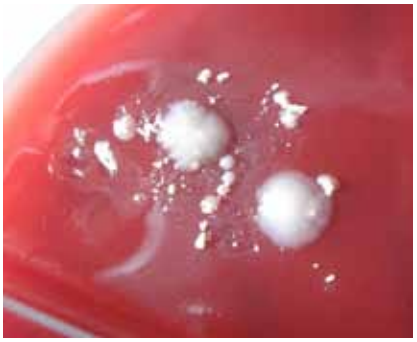

Figure 10. *A sample of the rare big high colonies growing on blood agar after 2days incubation*

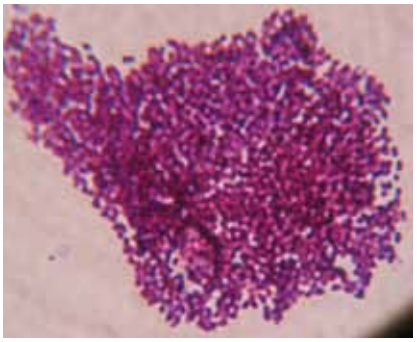

Figure13. *A sample of gram stained smears from the rare type showing gram-positive cocci arranged in characteristic grape-like clusters.*

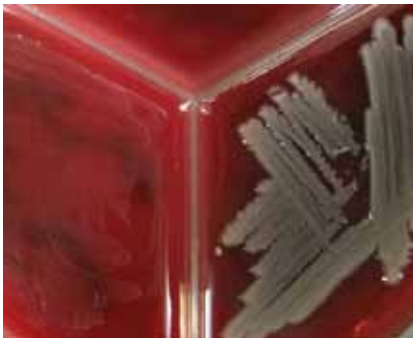

Figure 11. *On the right, pure golden-yellow colonies in sub-culturing of rare type which comport with Staphylococci pure cultures. On the left, pure colonies gelatinous cover in sub-culturing of dominant type which comport with S.mutans pure cultures are evident.*

Primary culturing quantitative results

Representative colonies were counted using a colony counter, the results were expressed in CFU mg^{-1}. Independent Sample T test was applied to check any significant differences in mean values of CFU\mg between the two groups (1&2) according to the studied excoriation's level variable (Table 2).

The table above shows that *P*-values were much lower than 0.05 for all studied level subgroups (Core Surface Level subgroup, Core Depth Level subgroup, Both Core Surface & Core Depth Levels). We can, therefore, conclude, at 95% of confidence level, that there are significant differences in bacterial colonies No variable's mean values between Metal Posts and Amalgam Cores Group and FRC Posts and Fluoridated Composite Resin Cores Group. Negative algebraic sign of mean differences indicate that the Bacterial Colonies No (CFU\mg) in Metal Posts and Amalgam Cores Group were lower than those of FRC Posts and Fluoridated Composite Resin Cores Group.

DISCUSSION

Bacterial microleakage prevention and bacterial growth inhibition are essential terms for successful post & core restorations under crowns. In this study the blood agar

non-selective medium was used as a culturing method with some modifications to suit the aim of the study that was investigating bacterial colonization within the superficial area of the bulk of materials and its possible penetration to the depth. The specimens, therefore, were taken as excoriations from the bulk of materials not from the growing biofilm on the top surface. Quantitatively, differences in this study between bacterial colonization in composite resin and amalgam were statistically significant. Despite the rich containment of fluoride, composite resin enhanced bacterial growth under crowns compared to amalgam which showed strong antibacterial effects. This result is in agreement with results of in vitro studies [2,3,4,7]. In addition, it seems that composite cores have facilitated the penetration of bacteria through the bulk until reaching its depth.

As for amalgam, the results can be interpreted by the fact that some of corrosion products which are released from this material due to aging and environmental interactions deposit in microgaps between the restoration and dental hard tissues or in dentin tubules covered by amalgam therefore may reduce the bacterial microleakage. Moreover, these corrosion products (mercury, silver, copper, zinc) are detected on the surface of the material, in adhering plaque, and in adjacent soft tissues. It has been demonstrated that these products are responsible of time dependent antibacterial effects of amalgam[8].

On the other hand, the results for composite resin can be interpreted by many reasons which have been suggested to explain the effectiveness of this material on bacterial growth. The bacterial microleakage at restoration-tooth interface level may occur through gaps which may occur due to composite polymerization shrinkage, delayed expansion caused by high moisture sorption certainly in composites with an intended release of antimicrobial substances, or due to its high thermal expansion coefficient [9]. Adhesives can also hydrolytically degrade over time because of liquids coming in to contact with them via dentin tubules. The hybrid layer consisting of collagen and resin may degrade due to collagen degradation via endogenous matrix metalloproteinases (a group of endopeptidases). Degradation of adhesive and hybrid layer may result in decreased adhesion, and increased microleakage. Moreover, this degradation, as well as degradation of the whole composite restoration will result in releasing of monomers (such as Bis-GMA), and comonomers (such as TEGDMA) in higher amounts. It has been proved by many studies that the released comonomers enhance bacterial growth. Hansel *et al.* [10] suggested that the released EGDMA, TEGDMA from composite resin during its clinical aging, provoke bacterial growth due to stimulation of metabolic pathways to alterations of bacterial membrane fluidity. Busscher *et al.*[4] however, have reported that provoking bacterial growth was due to enhancing the glucosyltransferase activity in

Table 2. *Independent samples T test results.*

Studied Level	*Metal Posts and Amalgam Cores*			*FRC Posts and Fluoridated Composite Resin Cores*			*Mean Diff.*	*P-Value*	*Significant Diff.?*	*t Value*
	N	*Mean*	*St.D.*	*N*	*Mean*	*St.D.*				
Core Surface Level	12	1.67	1.30	12	39.75	4.18	-38.08	0.000**	YES	-30.128
Core Depth Level	12	0.42	0.67	12	9.75	2.30	-9.33	0.000**	YES	-13.492
Both Core Surface and Core Depth Levels	24	1.04	1.20	24	24.75	15.67	-23.71	0.000**	YES	-7.389

* Significant at (p=value<0.05). ** Significant at (p=value<0.01)

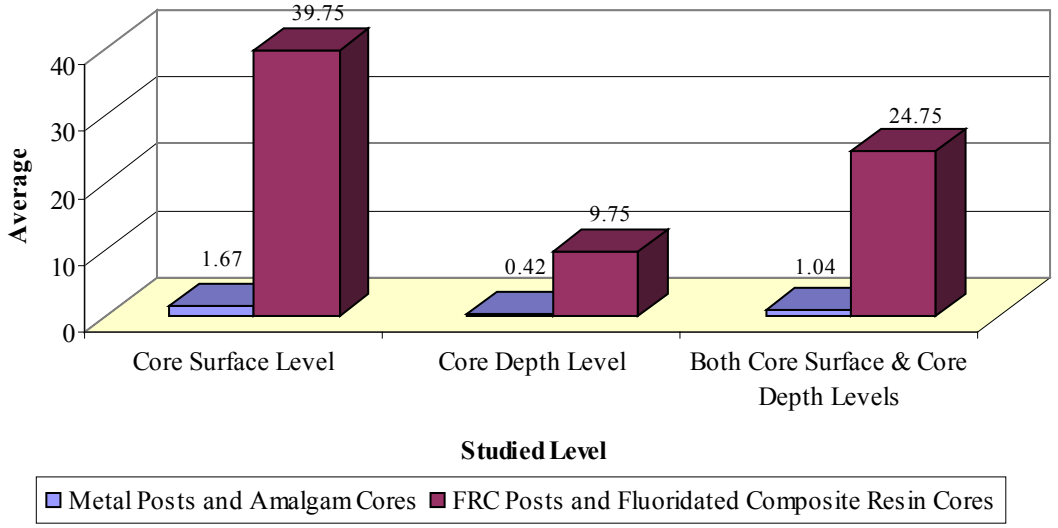

Chart 1. *Bacterial Colonies No mean (CFU/mg) according to used material & studied level variables.*

bacteria or modulating the expression levels of glucosyltransferase B involved in biofilm formation and yfiV as a putative transcription regulator gene in *S. mutans* by the triethyleneglycol, which is the ether portion of triethyleneglycol dimethacrylate (TGDMA). They also reported that bacteria during multiplication or metabolism produce hydrogen peroxide which forms free radicals. Polymerization of released methacrylate monomers is initiated by this radical formation which subsequently gives a chain reaction and production of resin polymer around bacterial cells in a vesicular structure. This vesicular structured polymer can be a scaffold for establishment of bacterial community. Furthermore, it may act as a barrier to protect the bacterial cell making bacteria more tolerant to chemical or physical attack. Moreover, biofilm formation on resin composite leads to a negative spiral of events, in which the colonizing organisms cause severe deterioration of the surface. This may promote biofilm formation and therewith more extensive deterioration of the surface.

In addition to unspecific adhesion phenomena, bacteria can also bind by means of specific adhesions (e.g. lectins) to special structures (e.g., sugar residues of glycoproteins), which are absorbed from saliva by the material. Furthermore, the presence of (-OH) in Bis-GMA, TEGDMA, helps in hydrogen bond formation which contribute with bacterial adhesion on composite resin surface [11].

The explanation of these results for fluoride: in this study the appended fluoride to composite resin failed in enhancing bacterial growth. This could be explained by the fact that the concentration of released fluoride is too low to be effective on bacteria as quantity dramatically decreases after a 24-h elution. Another reason is suggested to explain fluoride failure when added specifically to composite (contrary to amalgam or glass ionomer cement), that the fluoride-free dentin bonding system might adhere to the cavity wall so strongly that it, partially or completely, interfered with the diffusion of fluoride. In addition, composite resin enhances biofilm formation and bacterial accumulation, which in turn, affects the rate of ion-exchanging interactions (such as fluoride releasing), which drops sharply in the presence of this biofilm [12].

This study outcomes are at odds with other studies [5,6]. In the study by Torii *et al* [6] the authors concluded that fluoridated composite resin inhibits secondary caries. This disagreement may be due to the differences in methodology, as the same authors checked fluoride effectiveness on remineralisation of dental hard tissues in the presence of bacterial challenge, and did not checked its effectiveness on bacterial counts. Si-su *et al* [5] concluded that the kinds of restoration materials have no significant effects on the microflora of secondary caries. The disagreement here may be due to the use of samples collected from patients who had periodontal diseases. The microflora in saliva of these patients could be responsible for the dominant anaerobic bacteria of periodontal diseases which affected the results.

CONCLUSION

In this study composite resin used in core build up enhanced sizable bacterial growth (specifically *S.mutans*) under crowns, and promoted bacterial penetration to the depth of material, despite the presence of fluoride. In addition, our findings support the fact that amalgam cores have more antibacterial properties when compared to those made of composite. The results of this study, therefore, support the use of amalgam for building up cores in prefabricated post and core technique under crowns due to its antibacterial properties.

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REFERENCES

1. Imazato S. Bio-active restorative materials with antibacterial effects: new dimension of innovation in restorative dentistry. *Dent Mater J* 2009; **28**(1)**:**11-9.
2. Orstavik D. Antibacterial properties of and element release from some dental amalgams. *Acta Odontol Scand* 1985; **43**(4):231-9.
3. Splieth C, Bemhardt O, Heinrich A, Bemhardt H, Meyer G. Anaerobic microflora under Class I and Class II composite and amalgam restorations. *Quintessence Int* 2003; **34**(7):497-503.
4. Busscher HJ, Rinastiti M, Siswomihardjo W, van der Mei HC. Biofilm formation on dental restorative and implant materials. *J Dent Res* 2010; **89**(7):657-65.
5. Mo SS, Bao W, Lai GY, Wang J, Li MY. The microfloral analysis of secondary caries biofilm around Class I and Class II composite and amalgam fillings. *BMC Infect Dis* 2010; **10**:241.
6. Torii Y, Itota T, Okamoto M, Nakabo S, Nagamine M, Inoue K. Inhibition of artificial secondary caries in root by fluoride-releasing restorative materials. *Oper Dent* 2001; **26**(1):36-43.
7. Imazato S. Antibacterial properties of resin composites and dentin bonding systems. *Dent Mater* 2003; **19**(6):449-57.
8. Wang J, Liu Z. Influence of amalgam on the growth of mutans streptococcus: an in vivo study. *Chin J Dent Res* 2000; **3**(2):33-7.
9. Peutzfeldt A. Resin composites in dentistry: the monomer systems. *Eur J Oral Sci* 1997; **105**(2):97-116.
10. Hansel C, Leyhausen G, Mai UE, Geurtsen W. Effects of various resin composite (co)monomers and extracts on two caries-associated micro-organisms in vitro. *J Dent Res* 1998; **77**(1):60-7.
11. Satou N et al. Adhesion of *streptococci* to saliva coated and uncoated composite-based resins. *J Mat Sci: Materials in Medicine* 1996; **7:**749-752.
12. Hsu CY, Donly KJ, Drake DR, Wefel JS. Effects of aged fluoride-containing restorative materials on recurrent root caries. *J Dent Res* 1998; **77**(2):418-25.