JJBS

Jordan Journal of Biological Sciences

Volume 1, Number 3, September. 2008
ISSN 1995-6673
Pages 100 - 104

The Effect of Crown Restorations on The Types and Counts of Cariogenic Bacteria

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Abstract

This study investigated the effect of crown restorations on the numbers and types of cariogenic bacteria. Plaque samples were collected from thirty eight individuals who had crown restoration (crown or fixed partial denture) and an adjacent normal tooth by using sterile curettes. The bacterial counts (Colony Forming Unit/ ml) were obtained by the cultivation of plaque samples on certain selective media that were used for the cultivation of *Streptococcus mutans*, *Lactobacillus* species, and *Actinomyces* species. The number of *Lactobacillus* species were higher in the samples obtained from crown restorations than the samples obtained from natural teeth (P=.02). Also, it was found that the metal-acrylic crown restorations have higher number of lactobacillus species compared to the metal-ceramic (P =.003) and the crowns with subgingival margin has the highest counts of Streptococcus (P=.001) and Actinomyces species (P= .032). Moreover, the number of the cariogenic bacteria was found to be significantly associated with the periodontal conditions of the person and the age of crown restorations. It can be concluded that high counts of cariogenic bacteria was found to be associated with; crowns, metal acrylic crowns, placing the crown margin subgingivally, as well as the age of the crown restoration.

الملخص

لقد تم دراسة تأثير التركيبات السنية (التيجان والجسور) على أعداد وأنواع البكتيريا المسببة للتسوس، الدراسة شملت 38 شخص، حيت أن كل شخص لديه تلبيسة سنية (تاج أو جسر) وسن طبيعي مجاور للتلبيسة، تم جمع عينات البلاك (الصفائح الجرثومية) بواسطة أداة المجرفة (curette) . وتم احتساب أعداد المستعمرات البكتيرية بزراعة عينات البلاك على الأوساط الغذائية الاختيارية المناسبة لنمو كل من الأنواع والأجناس البكتيرية التالية: الستريتوكوكس ميوتانس، اللاكتوباسيلاس ، والأكتينومايسيس. حيث وجد أن أعداد اللاكتوباسيلس كانت أعلى في العينات المأخوذة من أسطح التركيبات السنية (التلابيس السنية) منها من أسطح الأسنان الطبيعية المجاورة، وأعداد اللاكتوباسيلاس أيضا كانت أعلى في العينات المأخوذة من التلابيس السنية المصنوعة من الأكريل بالمقارنة مع أنواع التركيبات السنية الأخرى. ولقد تم ايجاد أن أعداد الستربتوكوكس و الأكتينومايسس كانت أعلى في التركيبات السنية ذات الحافة تحت اللثة. ولقد وجد أيضاً أن العدد الكلي للثلاثة أنواع من البكتيريا اللاهوائية الاختيارية له علاقة إرتباط معنوي (significant) مع وضع اللثة للشخص وأيضا مع العمر الزمني للتركيبة السنية.

Keywords: Streptococcus mutans, *Lactobacillus* species, *Actinomyces* species, Crown restorations, Cariogenic bacteria .

1. Introduction

The placement of crown restoration maintains the morphology and the function of the tooth for a long period of time. Crowns and fixed partial dentures (FPDs) can be made from various types of material combinations; all ceramic, full cast metal, metal- ceramic and metal- acrylic. However, certain studies have reported that the margin of dental restorations stimulates bacterial recolonization and the acid production from cariogenic bacteria could attack the tooth restoration margin interface (Savarino et al., 2002; Mjor, 1985).

The bacterial community of dental plaque is subjected to physiological and compositional shifts as a result of environmental stresses generated by the placement of dental restoration and this is could lead to serious complications that result in the failure of the restoration (Mjor, 1997). Furthermore, many studies have found that there is variation in the effect of the various types of the restorations on the growth of certain bacteria in dental plaque according to its material combinations (Beyth et al., 2007; Satou et al., 1988).

Inspite of the widespread use of crown and fixed partial denture (FPD) restorations made from different types of materials, there are no studies to evaluate their effect on host tissues. The indirect effect of different types of crown and fixed partial denture (FPD) restorations on host tissues can be determined by the detection of the changes of

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bacterial counts in dental plaque. Comparison of the bacterial counts between the control site (natural tooth) and the experimental site (crown or retainer), is critical in the determination of whether different material types of crown and fixed partial denture (FPD) restorations have an effect on the bacterial composition in dental plaque or not. Therefore, this study aimed to investigate the effect of the various types of crowns and fixed partial dentures (FPDs) on the counts of Streptococcus mutans, lactobacillus species, and Actinomyces species in dental plaque.

2. Material and Methods

2.1. Subjects and Plaque Sampling

The current study composed of 38 subjects (9 men {23.7%}, and 29 women {76.3%}) ranging in age from 19 to 76 years (a mean age: 42.26 years). Each subject had a crown or a fixed partial denture (FPD) restoration and a natural tooth in the closet proximity to the crown site. Four samples were obtained from each participant at two sites, test and control: supragingivally and subgingivally for each. Samples were taken from 14 crowns and 24 fixed partial dentures (FPDs), made from metal- ceramic (26 cases), metal- acrylic (9 cases), metal only (only 1 case), and all ceramic (2 cases). The sample included 3 cases with diabetes, 6 with hypertension, and only 1 case with diabetes and hypertension. The study protocol was approved by the "Committee of the Search on Human", Jordan University of Science and Technology. The subjects were patients of the Dental Teaching Center at Jordan University of Science and Technology, and all of them provided their informed consent. The pregnant cases were excluded (4 cases out of 64), individuals who were having scaling and or taking antibiotics one month before sampling were excluded (16 cases out of 64), in addition to these, 6 cases out of 64 were excluded because of their missing data. All the thirty eight volunteers were non-smokers except for four.

The subjects were sampled at two sites, including natural teeth and teeth with crown restorations. Dental plaque samples were collected from the buccal side by using sterile curettes (Gracey Curettes). For both the tooth with crown restoration and the tooth without a restoration (control), the plaque sample was taken first from supragingival then the subgingival. The plaque samples were suspended in 1 ml of sterile phosphate buffer saline (PBS) (0.12 M NaCl, 0.01 M Na_2HPO_4, 5mM KH_2PO_4 [pH 7.5]). The samples were transported on icebox to the laboratory until processed within 24 hours. There were 76 plaque samples obtained from crown sites (experimental sites), and 76 samples from natural sites (control sites). The number of the cariogenic bacteria was obtained per ml of the plaque (CFU/ml) by cultivation on the proper selective media for each cariogenic bacteria.

2.2. Isolation and Enumeration of Bacteria From Plaque Samples

Plaque samples were dispersed by vortexing for 30 seconds with glass beads (diameter 4 mm), and diluted into different decimal serial dilutions in phosphate buffer saline (pH 7.5). Colony forming unit per ml (CFU/ml) was determined for each plaque sample by plating the appropriate dilutions on the following selective media (all media were purchased from Himedia Laboratories Pvt. Limited, Bombay, India): Mitis salivarius agar (MSA) supplemented with 0.2 U/ml bacitracin (Fluka; BioChemika, Buchs, Switzerland) and 5% sucrose (MSBS), Rogosa SL agar, and Cadmium Flouride Acriflavine Tellurite (CFAT) medium supplemented with 5% human blood were used for the cultivation of Streptococcus mutans, Lactobacillus species, and Actinomyces species respectively. The plates were incubated at 37°C for three days in an anaerobic jar with CO_2 gas generating kit (Oxoid Ltd, Cambridge, UK). A total of 152 plaque samples obtained from 38 persons were cultivated on (MSBS), Rogosa, and (CFAT) media. The resulting colonies were repeatedly subcultured for further analysis and detection.

2.3. Characterization of Cariogenic Bacteria in Plaque Samples

Morphological characterizations of bacterial isolates on the three selective media were performed according to the color, size, colony characteristics (margin, form, and elevation) and gram staining was done as a basic microbiological test for the identification. The Streptococcus isolates where further characterized using the following biochemical tests (based on Bergey's Manual of Systematic Bacteriology) (Sneath et al., 1986): Catalase activity, Fermentation of sugars: mannitol, sorbitol, raffinose, and Voges-Proskauer (VP) test. For the identification of the bacterial isolates that were grown on both Rogosa and (CFAT) media, the RapID ANA II biochemical kit (Remel, Lenexa, KS) was used according to the manufacture's instructions.

2.4. Statistical Analysis

Statistical analysis of the data was conducted using SPSS software (Statistical Package for the Social Science, version 11.5; SPSS Inc., Chicago, IL). All the bacterial count distributions were noticeably positively skewed; therefore, the nonparametric tests were used for the analysis. Mann Whitney U test and Kruskal-Wallis tests were used to analyze the data of the bacterial counts. Chi square test χ^2 was used to study the association between the numbers of bacteria with the age of the crown and the periodontal inflammation around the tooth. The level of significance was considered at $\alpha = .05$.

3. Results

The morphological and biochemical characterization revealed the presence of the following cariogenic bacterial species: Streptococcus mutans, Streptococcus salivarius, Streptococcus sanguis, Lactobacillus acidophilus, Actinomyces odontolyticus, Actinomyces meyeri, and Actinomyces israelii. The mean number of CFU per ml of plaque for each cariogenic bacteria showed considerable variability between teeth sites (natural tooth vs. crown restoration) and in relation to plaque position (supragingival plaque vs. subgingival). (Table 1)

Table 1: The mean values of bacterial counts (CFU/ml) for each type of bacteria in relation to plaque position and teeth site

Bacterial genera	Natural site		Crown site	
	supra[a]	sub[b]	supra[a]	sub[b]
Streptococcus	$(5.5\pm34)\times10^{12}$	$(7.0\pm37)\times10^{3}$	$(5.3\pm33)\times10^{12}$	$(1.5\pm9.1)\times10^{12}$
Lactobacillus	$(5.9\pm13)\times10^{3}$	$(1.1\pm4.2)\times10^{4}$	$(2.5\pm8.5)\times10^{4}$	$(2.3\pm13)\times10^{3}$
Actinomyces	$(2.7\pm9.3)\times10^{10}$	$(5.5\pm15)\times10^{8}$	$(7.3\pm18)\times10^{9}$	$(1.6\pm5.9)\times10^{10}$

[a]: supragingival plaque.

[b]: subgingival plaque.

Table 2: Frequency distribution of plaque samples with Lactobacillus species counts CFU/ml in terms of plaque positionand teeth site

T.site[b]	Plaque	CFU classes n (%)[a] 0.0	>0.0-10^3	>10^3-10^6	Total [c]
natural	supra[d]	34 (89.5)	3 (7.9)	1 (2.6)	38(100.0)
	sub[e]	33 (86.8)	3 (7.9)	2 (5.3)	38 (100.0)
crown	supra[d]	26 (68.4)	7 (18.4)	5 (13.2)	38 (100.0)
	sub[e]	32 (84.2)	3 (7.9)	3 (7.9)	38 (100.0)

[a] count of samples (percentage of samples).

[b]: tooth site.

[c]: Total: was estimated from all CFU classes within each group.

[d]: supragingival plaque.

[e] : subgingival plaque.

Analysis of the proportions of plaque samples in relation to CFU classes showed that Lactobacillus species have the largest frequencies of zero count with regard to plaque type and tooth site (Table 2). Table 3 shows that Actinomyces species were the predominance species being cultivated with the greatest proportions on the selective media. The total prevalence of Lactobacillus and Actinomyces in supragingival plaque was higher at crown sites than at natural sites (Tables 2, 3).

Table 3: Frequency distribution of plaque samples with Actinomyces species counts CFU/ml in terms of plaque position and teeth site

T. site	Plaque	CFU classes n (%) 0.0	>10^3-10^6	>10^6-10^9	>10^9-10^{12}	**Total**
natural	supra	19 (50.0)	14 (36.8)	0 (0.0)	5 (13.2)	38 (100)
	sub	25 (65.7)	5 (13.2)	3 (7.9)	5 (13.2)	38 (100)
crown	supra	11 (29.0)	14 (36.8)	5 (13.2)	8 (21.0)	38 (100)
	sub	19 (50.0)	3 (7.9)	13 (34.2)	3 (7.9)	38 (100)

All symbols and abbreviations are in Table 2.

While there were no large differences in the total prevalence of Streptococcus species in plaque samples between natural teeth and crowned teeth (Table 4).

The crown sites displayed significant increased in Lactobacillus counts in the supragingival plaque (P = .02). While there was no statistically significant difference between the natural tooth and crown tooth sites in the counts of *Streptococcus* and *Actinomyces* species (P > .05). Furthermore, the different types of crown material combinations revealed significant differences in Lactobacillus counts (Figure1), where metal-ceramic crowns have lower counts than the metal- acrylic (P= .003). Also the position of the crown margin displayed differences in bacterial counts (Figure2), where the subgingival margin has the highest counts of Streptococcus (P= .001) and Actinomyces species (P= .032). Significant differences in Lactobacillus counts were recorded according to the location of teeth in the oral cavity, where anterior teeth have lower counts than the posterior (P = .01). The count of the cariogenic bacteria was found to be significantly associated with the periodontal conditions (χ2 test, P=.002) and the age of the crown restoration (χ^2 test, P = .006).

Table 4: Frequency distribution of plaque samples with Streptococcus species counts CFU/ml in terms of plaque position and teeth site

T. site	Plaque	CFU classes n (%) 0.0	>0.0-10^3	>10^3-10^6	>10^6-10^9	>10^{12}	Total
natural	supra	25 (65.9)	6 (15.8)	6 (15.8)	0 (0.0)	1 (2.6)	38 (100)
	sub	27 (71.1)	6 (15.8)	5 (13.2)	0 (0.0)	0 (0.0)	38 (100)
Crown	supra	22 (57.9)	8 (21.1)	6 (15.8)	1 (2.6)	1 (2.6)	38 (100)
	sub	25 (65.8)	8 (21.1)	4 (10.5)	0 (0.0)	1 (2.6)	38 (100)

All symbols and abbreviations are in Table 2.

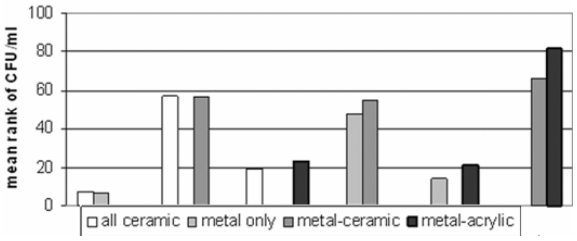

Figure1: Mean rank values of Lactobacillus species across 4 types of crown material combinations. According to Mann Whitney U test, significant differences lie only between metal ceramic crowns and metal acrylic crowns (P=.003).

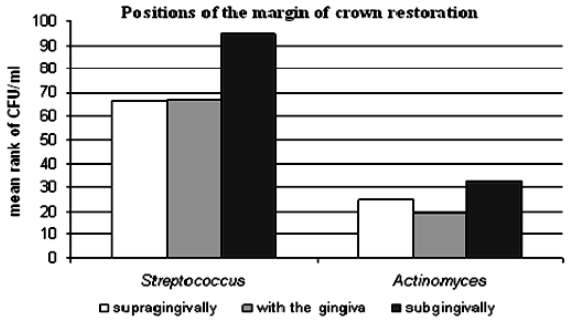

Figure 2 : Significant differences in bacterial counts (CFU/ml) of Streptococcus and Actinomyces species across 3 positions of crown restoration margin. Subgingival margin has highest counts of Streptococcus (P=.001) and Actinomyces species (P=.032) compared to supragingival margin and margin at gingival level.

4. Discussion

The present study aimed to evaluate the effect of crown restorations made from different combination of materials on the level of the most cariogenic bacteria compared to

the bacterial level on natural teeth sites. The number of CFU/ml of plaque was enumerated on each selective media for each patient and the proportions of each cariogenic bacteria were estimated. Low counts of Lactobacillus species in dental plaque were found in this study and this is expected since the Lactobacillus species are found in high numbers in samples taken from a caries lesion (Ahumada et al., 2003), which is not the case of the present study.

An interesting and significant result obtained is the difference in the lactobacillus counts in supragingival plaque on natural teeth compared to the crowns. There was no significant difference in the count of both Streptococcus and Actinomyces species between teeth sites. It is well known in most studies regarding the effect of dental restorations on oral bacteria that their surface roughness increases bacterial accumulation (Hannig, 1999; Weiman and Eames, 1975). Moreover, some of the elements that might be released from the restoration may have an influence in the bacterial adhesion and growth (Khalichi et al., 2004). In this study because there were various types of crown restorations involved, all the increased level of Lactobacillus could be explained to both the surface roughness and the physico-chemical properties of the restorations.

It was found also that the materials of crown restorations have a significant effect on the count of bacteria, where the differences lie between the metal-ceramic and metal-acrylic restorations (P=.003). The metal-ceramic had lower count than metal-acrylic. In spite of the fact that resin restoration is widely used in dental practice due to its low cost, it was reported in many studies that resin restoration promotes the accumulation of bacteria more than any other restoration, because their surface is highly rough (Weiman and Eames, 1975), and their released biodegradation by- products may stimulate bacterial growth. (Khalichi et al., 2004)

The current study showed that subgingival margin has the highest counts of Streptococcus and Actinomyces. The enamel close to margin restoration may be rapidly affected by secondary caries formation (Savarino, 2002; Mjor, 1985). The incomplete sealing between the enamel surface and the margin could lead to marginal leakage that allows the penetration and colonization of bacteria along the margin of the restoration and because the cleaning and removing of this accumulated bacteria become difficult when the restoration margin is below the gingival level.

Also this study revealed that the counts of Lactobacillus species from posterior teeth were significantly higher than from the anterior teeth. The accessibility of cleaning the anterior teeth may interfere with plaque accumulation and resulted in a reduction in bacterial counts compared to the posterior teeth. Differences in cariogenic bacterial counts between upper and lower teeth were not observed (P>.05).

The present study shows that persons with chronic periodontitis have the highest bacterial counts. The cariogenic bacteria have an indirect role in the periodontitis, by the interaction with bacterial species that cause periodontitis, Porphyromonas gingivalis (species that cause periodontitis) can co-aggregate with Streptococcus species (Cook et al., 1998). This study indicated that the age of the crown restoration had a significant effect on bacterial counts, the older the crown the higher the counts of cariogenic bacteria found. Previous studies showed that dental materials stimulate the accumulation of bacteria (Satou et al., 1988; Weiman and Eames, 1975; Khalichi et al., 2004), so it could be concluded that the older the restoration, the larger the accumulation of bacteria that could be found. This also could be due to the deterioration of the marginal seal between the restoration and the tooth margin, which subsequently enhance the conditions for the bacteria to accumulate and multiply.

Finally, it should be stated that in this study it was difficult to have a standard plaque sample size during the clinical sampling; therefore the quantitative comparison should be interpreted with caution. It's recommended for future research to standardize many parameters including; the plaque sample size, the condition of the periodontal tissue, and the level of oral hygiene of the patients.

5. Conclusions

Under the conditions of this study high counts of cariogenic bacteria was found to be associated with crown restoration vs. natural tooth, subgingival margin vs. supragingival and margin with the gingival level, metal acrylic crown vs. metal ceramic crown, as well as, the age of the crown and the periodontal inflammation.

Acknowledgment

This study was supported by the Deanship of graduate studies and research at Yarmouk University-Jordan, grant No. 2/2006.

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