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Bacterialadhesion onCeramo-Metallic Versus Zirconia Crowns (Randomized Clinical Study).

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Abstract— Aim: The present study was done to evaluate and compare streptococcus mutansand sanguinis bacterial counton ceramo-metallic crowns and monolithic zirconia ceramic crowns at zero and 90 days follow up period. **Materials and methods:** Twenty healthy patients were chosen according to inclusion and exclusion criteria's and randomly distributed into two groups (n=10) according to material of the crown. A streptococcus mutans and sanguinis bacterial count(CFU/ml) were collected at zero- and 90-daysinterval from plaque, salivary swab and salivary collection samples. **Results:** No significant difference between the two groups except S. sanginus was significantly higher in plaque sample of zirconia crowns after 90 days follow up. **Conclusions:** Physical nature of bacteria may affect their adherence as Streptococcus sanguinis showed higher count on zirconia crowns than ceramo-metallic crowns after 90 days of follow up.

Keywords: plaque, saliva, mutans-sanginus media, caries,hydrophobic.

Introduction

Introducing of dental restorative materials in the oral cavity having different chemical and physical properties may change the nature of microorganisms' adhesion. Monolithic ceramic has been used recently to the overcome disadvantages of esthetic appearance of metallo-ceramic restorations which has proven to have superior performance from decades.[1-3]

Dental plaque is known to have a microbial population with high complexity, and key micro-organisms are generally recognized as closely associated with the development of human dental caries. *Streptococcus mutans*was reportedby**Coykendall**[4] as a group of seven species which are phenotypically similar causing dental caries and endocarditis. While*Streptococcus sanguinis,* one of the predominant species of the indigenous oral biota colonizing saliva and dental plaque, is usually associated with tooth surfaces free of caries.it is considered responsible for the initial formation of biofilm and one of the causative agents of infective endocarditis.[5,6]In the 1980s, Loesche[7-9]and others proposed that the *S. mutans* to *S. sanguinis* ratio might be indicative of caries outcome or risk for caries**.** In fact, several investigators using conventional culture methods have deducted from their studies that *S. sanguinis* may play an antagonistic role against *S. mutans* colonization[7,10,11]

Research question:

Will zirconia crown have better effect on lowering bacterial count of streptococcus mutans species more than metallo-ceramic crowns?

Statement of the problem:

limited quantitative and qualitative data available about adhesion of streptococcus mutans and sanginus around crown margin and in saliva which may predict caries risk and prosthesis survival.

Materials and methods:

Study design:
This was a prospective two arm parallel group, double blinded, randomized and clinical trial.

Inclusion and exclusion criteria:
Twenty patients having posterior teeth indicated for full coverage restorations were chosen for this study after clinical and radiographic examination. Having age ranges from 20-50 years old medical free, non-smokers, with the physical and psychological ability to tolerate conventional restorations and willing to return for follow-up examinations. Patients who were suffering from parafunctional habits and had a history of taking anti-inflammatories or antibiotics in the past three months were excluded from the study. The participants assigned informed consents.

Prothetic procedures:
Preparation:
Each patient was assigned randomly to a group where the patient was blinded about the crown's material he would receive. The crown preparations were done by a single operator to standardize the preparation who was not blinded about thecrown material. Secondary impressions were taken with additional silicone rubber base for try in and temporization.

Try in was done either by metal coping or PMMA (poly methyl methacrylate) according to the final crown material to check occlusal relation, marginal fit and proximal relation. Final restorations were fabricated and cemented using dual cure self-etch resin cement as in figure (1). The patients were instructed not to brush or use mouthwash for 24 hours and were recalled in the early morning of the next day for samples collection.

After samples collection the patients were instructed to brush and had oral hygiene measures regularly till the next follow up visit after 90 days.

Sample collection, culture and identification:
Supra-gingival plaque, salivary swab and unstimulated saliva were collected in reduced transfer fluid described **in table (1)**for transportation to the microbiology lab as soon as possible for streptococcus mutans and sanguinis culture. Thestatisticians and microbiologists did not know the crown's material nor the patients' names.

Mutans-sangius mediaas in table (2) was used as a selective media to differentiate between streptococcus mutans and sanguinis count. Media was prepared and poured into Petri dishes for sample cultures and bacterial count. The Petri dishes were incubated in an anaerobic incubator for 24 hours. Bacterial colonies were identified, differentiated and counted. Serial dilution of different samples was done according to bergey's methods[12] to be cultured.

Streptococcus mutans and streptococcus sanguinis were identified according to the shape and size of the colonies as in figure (2). They were grey, white or colourless,1-3 mm in diameter. S mutans formed rough, heaped, irregular colonies resembling frosted glass. Mostly crumbly, although whole colonies were picked off the agar, which was white, grey or yellow and 0.5-2 mm in diameter S.sanguis formed smooth or rough, hard and rubbery colonies, which adhere strongly to agar, making them difficult to remove with a loop.

Results:
Data were collected, tabulated and analyzed. Statistical analysis was performed with SPSS 20®, Graph Pad Prism® and Microsoft Excel 2016. Comparison between 2 different groups was performed by using the Mann-Whitney test. Comparison between more than 2 dependent groups (follow up) was performed by using. Freidman's test.

Exploration of the given data was performed using Shapiro-Wilk test and Kolmogorov-Smirnov test for normality. As Listed in **Table (3)** it was revealed that the significant level (P-value) was shown to be insignificant at P-value <0.05, which indicated that data originated from non-parametric data.
Regarding ceramo-metallic crowns, different bacterial counts on salivary swab, salivary collection and plaque are presented as mean and standard deviation in **Table (4)**. Comparison between 2 species was performed at different follow up intervals and revealed insignificant difference at $P > 0.05$ in different samples (salivary swab, salivary collection, and plaque).While, comparison between different follow up intervals revealed insignificant difference in both species regarding salivary swab, salivary collection, and plaque at $P > 0.05$.

While for zirconia ceramic crowns comparison between 2 species was performed at all intervals and revealed insignificant difference at $P > 0.05$ in different samples (salivary swab, salivary collection, and plaque). Also, comparison between different intervals revealed insignificant difference in both species regarding salivary swab, salivary collection, and plaque at $P > 0.05$ except in case of mutans in salivary swab there was a significant difference at $P < 0.05$ (after 3 months was significantly higher than baseline) as shown in **table (5).**

Comparison between group I & II is presented as mean and standard deviation in **Table (6)**. At baseline, there was insignificant difference between them in all bacterial counts in different samples (salivary swab, salivarycollection, and plaque) cultured on selective media at $P > 0.05$.

Comparison between group I & II is presented as mean and standard deviation in **Table (7)**. After 3 months, there was insignificant difference between them in all bacterial counts in different samples (salivary swab, salivarycollection, and plaque) cultured on selective media at $P > 0.05$, except in case of Sanguis in plaque was significantly higher in group II (zirconia ceramic crowns) than group I (ceramo-metallic crowns) at $P < 0.05$.

Discussion:
With the continuous evolution of new materials for computer-aided design/computer -aided manufacturing (CAD/CAM) systems giving alternatives for the clinicians in patients rehabilitations. As the CAD/CAM technology gave the chance to reduce patient visits and fabrication time[13]. **I Susic et al., (2017).**

Although metallo-ceramic restorations were considered the gold standard, attempts to use monolithic materials to overcome problems such as metal shadow, veneering chipping and delamination resulting in failure of restoration[14]**Rekow et al., (2011)**

The patients included in the present study were medically free, non-smokers and didn't take any antibiotics before and during the treatment and follow up. **Grine et al., (2019)**[15]found that smoking has a mechanism that alters microbial ecology, including increasing acidity of the saliva, depleting oxygen and impairing hot immunity. While antibiotic may destroy the balance between specific bacterial populations throughout the ecosystem as mentioned by **Cheng et al.,(2022)**[16]**.**

Different samples (salivary swab, salivary collection and plaque) for bacterial growth been assessed in the current study as **Kann et al., (2022)**[17]stated that different sample had different composition recommending collection of same samples for proper comparison.

Selective media (Mutans-sangius agar) was used in the current study for culture of streptococcus mutans and sanguinis as both have a significant function in the oral cavity. **Redrigo et al. (2015)**[18]reported that Sanguinis competes with S.mutans ,which may lessen or prevent dental caries or can be a pioneering colonizer facilitating the attachment of succeeding pathogens.

Regarding of higher count of streptococcus mutans in salivary swab in relation to zirconia crowns may be due to its high prevalence in saliva and may indicate for caries risk assessment follow up as mentioned by **Parampreet et al (2013)**[19]

Concerning the results of streptococcus sanguinis count in the plaque of zirconia ceramic group was found to be significantly increased than that of ceramo-metallic crowns after three months follow up interval. These findings are concise with **Waassamann et al., (2017)**[20]**,** who found Sanguinis is hydrophobic bacteria and stated that hydrophobic bacteria adhere to hydrophobic material and zirconia ceramic found to be hydrophobic.

Conclusions:

- Bacteria may have physical and chemical properties that affect their behavior to different prosthetic materials.
- Streptococcus sangius adhered higher to zirconia crown more than ceramo-metallic crown after 90 days.

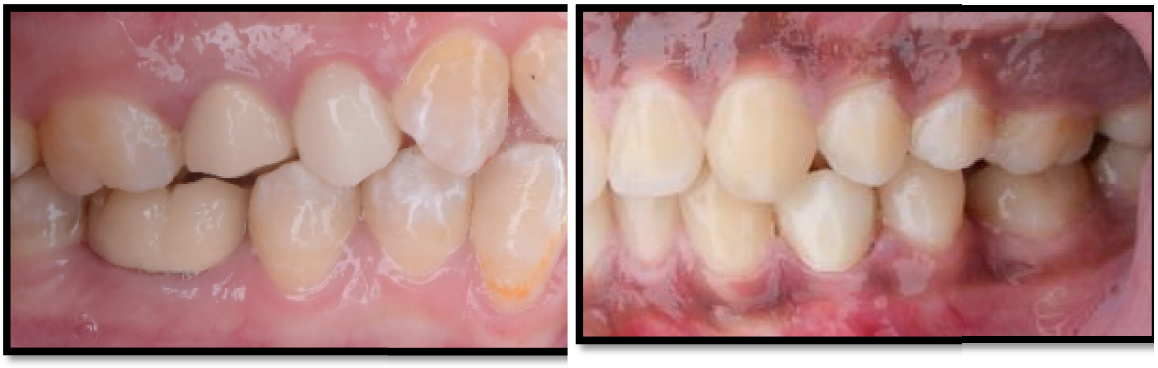

Figure (1): Postoperative lateral view of ceramo-metallic crown of lower 1st molar and zirconia crown of lower 1st molar.

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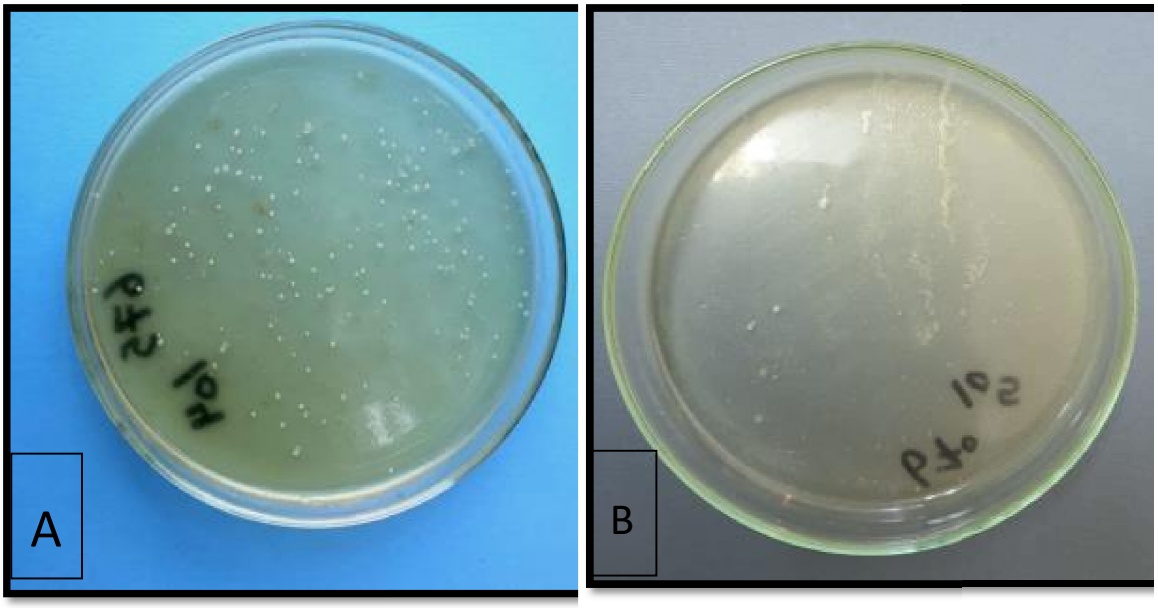

Figure (2) A: Small colonies of streptococcus sanguinis. B: Ground glass appearance of streptococcus mutans

Table (1): Reduced transfer fluid composition:

Reduced transfer fluid	Composition
	• 0.045% K_2HPO_4 • 0.045 % KH_2PO_4 • 0.09 % NaCl • 0.09% $(NH_4)_2SO_4$ • 0.018 % $MgSO_4$ • 0.038 % EDTA • 0.04 % NA_2CO_3 • 0.02% Dithiothreitol

Table (2): Mutans-sangius media composition:

	Composition
Mutans-sangius media	• Casein enzymic hydrolysate 15.000 • Yeast extract 5.000 • L-Cystine 0.200 • Sodium sulphite0.100 • Sodium chloride 1.000 • Disodium phosphate 0.800 • Sodium bicarbonate 2.000 • Sodium acetate 12.000 • Sucrose 50.000 • Agar 12.000 • Final pH (at 25°C) 7.3±0.2

Table (3): Results of the Normality exploration of the data from both tested groups:

	Group I	Indication	Group II	Indication
Mutans-sanginius	<0.05*	Non-parametric	<0.05*	Non-parametric

Table (4): Mean and standard deviation values of ceramo-metallic crowns on bacterial count in different samples at different follow up intervals:

Group I PMF		Mutanssanguinis				P value
		Mutans		Sanguinis		
		M	SD	M	SD	
Salivary swab	Baseline	0.00	0.00	0.00	0.00	------
	After3 months	2.34	3.02	2.34	3.02	1.00
	P value	0.066		0.059		
Salivary collection	Baseline	3.05	3.23	3.67	3.18	0.68
	After3 months	3.46	3.04	4.60	3.22	0.21
	P value	0.766		0.51		
Plaque	Baseline	1.52	2.49	3.55	2.68	0.12
	After3 months	3.05	2.73	1.73	2.30	0.28
	P value	0.237		0.173		

Table (5): Mean and standard deviation of zirconia ceramic crowns effect on bacterial count in different samples at different follow up intervals.

Group II Zirconia		Mutans-sanginus				P value
		Mutans		Sanginus		
		M	SD	M	SD	
Salivary swab	Baseline	0.00	0.00	0.00	0.00	----------
	After 3 months	4.65	2.56	1.98	3.20	0.21
	P value	0.012*		0.109		
Salivary collection	Baseline	1.48	2.38	2.92	3.15	0.23
	After 3 months	3.78	3.29	4.56	3.18	0.51

	P value	0.063		0.208		
Plaque	Baseline	1.96	2.63	2.40	3.15	0.71
	After 3 months	2.12	2.78	4.24	3.03	0.11
	P value	0.500		0.386		

M: mean SD: standard deviation
P: probability level which is significant at P ≤ 0.05

Table (6): mean and standard deviation of the comparison between the effect of group I and group II on bacterial count in different samples at baseline (mean and standard deviation):

Baseline		Mutanssanginus media			
		Mutans		**Sanginus**	
		M	SD	M	SD
Salivary swab	Group I	0.00	0.00	0.00	0.00
	Group II	0.00	0.00	0.00	0.00
	P value	**1.000**		**1.000**	
Salivary collection	Group I	3.05	3.23	3.67	3.18
	Group II	1.48	2.38	2.92	3.15
	P value	**0.14**		**0.51**	
Plaque	Group I	1.52	2.49	3.55	2.68
	Group II	1.96	2.63	2.40	3.15
	P value	**0.75**		**0.55**	

M: mean SD: standard deviation
P: probability level which is significant at P ≤ 0.05.

Table (7): Mean and standard deviation of the comparison between the effect of group I and group II on bacterial count in different samples at 3 months follow up interval:

After 3 months		**Mutans -sangius media**			
		Mutans		**Sanguis**	
		M	SD	M	SD
Salivary swab	**Group I**	**2.34**	**3.02**	**2.34**	**3.02**
	Group II	**4.65**	**2.56**	**1.98**	**3.20**
	P value	**0.23**		**0.91**	
Salivary collection	**Group I**	**3.46**	**3.04**	**4.60**	**3.22**
	Group II	**3.78**	**3.29**	**4.56**	**3.18**
	P value	**0.52**		**0.98**	

Plaque	**Group I**	**3.05**	**2.73**	**1.73**	**2.30**
	Group II	**2.12**	**2.78**	**4.24**	**3.03**
	P value	**0.56**		**0.03***	

M: mean SD: standard deviation

P: probability level which is significant at P ≤ 0.05

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