Detection of Bacterial Colonization around Cobalt Chromium versus Zirconium Copings on Natural Teeth Supporting Overdenture. Two different in vitro studies

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Abstract: Purpose: The purpose of this study was to compare between two copings materials covering natural teeth abutments supporting overdentures including Cobalt-Chromium and zirconia as regard to bacterial adherence and biofilm mass formation. **Material & methods:** Sixteen completely edentulous patients with remaining lower canines of age ranged (50-65years) were selected according to bacterial sample inclusion criteria. The patients were divided into two equal groups(n=8):***First group*** had received complete maxillary dentures and tooth supported mandibular overdentures constructed with primary and secondary metal copings (Cobalt Chromium) .***Second group*** had received complete maxillary dentures and tooth supported mandibular overdentures constructed with primary and secondary zirconia copings. ***First in vitro*** **study (Quantitative assessment study)**: Microbiological swabs were collected from buccal, lingual, mesial and distal surfaces of the canines by using sterile endodontic paper points, Then the paper points were put immediately in vials containing sterile nutrient Broth Typicase Soy Broth (TSB). After incubation, bacterial colonies specially (Streptococcus sanguinus) counted in Colonial Forming Units(CFU/Ml)**. Second in vitro study (Bacterial adhesion assay)**: After incubation of bacterial colonies, an inoculums were then transferred to another fresh TSB broth, then bacteria were allowed to adhere to the prepared discs (12x12 x2mm^3) of Cobalt Chromium and Zirconium Oxide which finished and gradually polished like mirror surfaces, the tested biofilm mass adherence between the two materials was analyzed using microplate reader (Bio-Rad Laboratories, CA, USA). The data were collected and statistically analyzed.**Results**: The quantitative bacterial culture from each group of patients (n=8) had revealed higher percentage of bacterial count in (group1) of patients that were wearing overdentures with metal copings compared to the other group of patients (group 2) of zirconium copings with statistical significant difference(p<.0.001), The second *in vitro* study of both materials (Cobalt Chromium and Zirconium) according to the absorbent value that were investigated as regard to bacterial biofilm adherence revealed that, there was biofilm adherence for both materials, but that of Cobalt Chromium 0.400±0.08 was higher than of Zirconium material 0.100±0.03 with statistical significant difference (p<.0.001). **Conclusion**: Zirconium copings as regard to biological and bacterial adherence is much better for oral hygiene maintenance than metal Cobalt Chromium copings. Further studies are recommended by other experimental means like Electron Microscopy, other bacterial species to support this research.
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Key words: Overdenture; Coping materials; Bacterial biofilm.

1. Introduction

Bacterial colonization starts with the adhesion of early colonizers, called pioneer bacteria, to the salivary pellicle on teeth as well as on dental materials within minutes after tooth cleaning. The early colonizers, mostly streptococcoci, contribute to plaque development and ultimately to oral diseases. Investigations of dental plaque, including bacterial adhesion, employ various *in vivo* and *in vitro* models and use microscopic methods to assess surface phenomena.[1]

Oral streptococci have been known to bind to proteins such as alpha-amylase, proline-rich proteins and glycoproteins, and are recognized as early colonizers.[2] *S. sanguis* is thought to be one of the first bacterial species selectively adhere to teeth and colonize on saliva-coated teeth. This species appears in the human oral cavity after tooth eruption, and it becomes a normal inhabitant of the human mouth.[2,3]

Oral biofilm is the diverse microbial community found on the tooth surface, embedded in a matrix of polymers of bacterial and salivary origin. Oral biofilm has been known to be closely related with the occurrence of oral disease.[4]The formation of oral biofilm may lead to development of dental material surface biodegradation, secondary caries and periodontal inflammation, which considered the main reasons for the restoration replacement.[5,6]

Numerous factors have been identified to influence oral biofilm formation such as surface roughness and surface free energy. [7,8]

Microscopic examination of early plaque formation on teeth showed the adhesion of the initial colonizing bacteria along cracks and pits in the enamel,

suggesting the influence of surface structure on bacterial adhesion.[6]

Various affinities of oral bacteria adhesion have been reported for different materials including Titanium, Cobalt chromium, resins and ceramics.[8-12] Bacterial adhere more readily to Cobalt Chromium than to alumina ceramic.[12]

Dental ceramic materials also applied to broad range of clinical practice, specially, zirconia has been introduced to improve esthetics for natural teeth and implant prostheses because of its biocompatibility, high resistance to wear and fracture by fatigue loading.[10-14]

The use of high strength ceramics, both as a permucosal abutments on implants and as a copings for ceramic crowns, is increasing. Zirconia (ZrO2) is especially promising because of its high fracture toughness and favorable light dynamics. To date, there is only limited information available with respect to the clinical and biological performance of ZrO2-based restorations[15-17]

The purpose of this study is to compare between copings different material including cobalt-Cromium and zirconia as regard to bacterial adherence and biofilm mass formation.

2. Material and Methods:

Selection of Patients:

Sixteen male patients their ages ranged between 50-65 years. They were completely edentulous patients with remaining lower canines that used as abutment for the tooth supported mandibular overdenture and opposing conventional complete upper dentures were constructed.

They were selected from out -patient clinic of the Prosthodontics department. Faculty of Dentistry , Suez Canal University.

The patients were divided into two equal groups(n=8):

First group: had received complete maxillary dentures and tooth supported mandibular overdentures constructed with primary and secondary metal copings (Cobalt Chromium) .

Second group received complete maxillary dentures and tooth supported mandibular overdentures constructed with primary and secondary zirconia copings

-The following criteria were taken into consideration before taking microbiological specimens: -

-All patients were free from any systemic diseases, as diabetes, renal or liver disease.

-Patients with severe clenching, bruxism, , alcoholic or drug abuse were excluded.

-The edentulous ridges were covered with healthy, firm mucosa, free from any severe bony undercut and with adequate inter arch distance.

-The canines were free from caries or periodontal diseases or any periapical lesions examined through clinical and radiographic assessment.

-cases with poor oral hygiene were excluded; no dental plaque was visible

-No history of antibiotic within two months before taking microbiological specimens.

- No history of using bacterial disinfection before taking microbiological specimens.

Microbilogical specimen collection

-Supragingival plaque if detected was removed and dried with sterile cotton pellets

-Microbiological swabs were collected from buccal, lingual, mesial and distal canine surfaces by using sterile endodontic paper points(Densply Dental,Tianjin) which were gently inserted into the canine sulcular depth until feeling of resistance, they kept in place for few seconds (Fig .1)

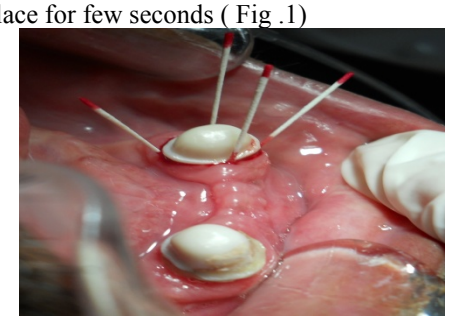

Fig .(1) :Microbiological swap

-The paper points were put immediately in vials containing sterile nutrient Broth Typicase Soy Broth (TSB) supplemented with1% yeast extract (BD diagnostics)

***In vitro* bacterial culture:**

-The vials with paper points were centrifuged at 3500 g for 5 minutes to help in bacterial concentration

-Ten microns of centrifugated solution was taken by using calibrated loop and plated on MacConkey and Dextrose agar plates (Fig.2),then incubated at 37c°, for 48 hours

-Streak plate method was used for isolation and semi-quantification of bacterial colonies.

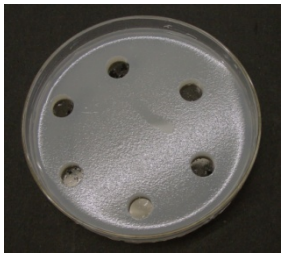

Fig. (2): MacConkey and Dextrose agar plates

Quantitative assessment:

After incubation, bacterial colonies, specially (Streptococcus sanguinus) they were counted in Colonial Forming Units(CFU/Ml) and compared for each group with identification of other aerobic bacterial growth by using the following methods[18], (Colonial Morphology Gram Staining Catalase test, Oxidase test, Bichemical reaction & API20EStreptococcipanel (Biomerieux,France).

***In vitro* bacterial adhesion assay:**

After incubation of bacterial colonies, an inoculums was then transferred to another fresh TSB broth with dilution 1:50 and grown at 37c°, for 16 hours ,this culture was sonicated for1 minute(30W, Sonifier Ultrasholl,\desintegrator, Branson Sonic power Co, Berlin Germany) and washed with physiologic Saline then harvested by centrifugation at 8000 g for five minutes and resuspended in human saliva with specific density 10^8-10^9 cells/ml

Whole saliva was pooled from two healthy volunteers who did not show any caries or periodontal diseases and sterilized by Millipore filter (Millipore,MA,USA)

Density of bacteria per ml in bacteria- saliva mixtures was determined by using Spectrophotometer and readymade turbidity standard (Siemens.Microscan Supply,Germany)

Prior to seeding into study material, sterilized specimens were placed into a 24-well culture plate and were incubated with saliva for 2 hours at 37℃

The material under study including Cobalt Chromium and Zirconium Oxide prepared as (12x12 x2mm^3) discs as obtained from the manufacturer (VitaZahnfabrik, Bad Säckingen, and Bego Co Germany) which finished and gradually polished like mirror surface.

The surface roughness (Ra) and topography were m easured by the confocal laser microscope (Zeiss, Germany). The static water contact angle of each surface of specimen was measured with a Phoenix 300 contact-angle meter (Surface ElectroOptics, Korea) at room temperature

Before the adhesion experiments the material slides were decontaminated with ethanol and exposed to the sterile human saliva at room temperature for 15 min.The bacteria were allowed to adhere to the surfaces during one hour at room temperature. The test specimens were removed, washed with Phosphate Buffer saline(PBS),then air- dried and stained by applying 1% Crystal Violet solution(CV) (Sigma-Aldrich MD,USA) followed by10 minutes incubation time, Then, the excess of unbound dye was removed by washing the plates with deionized water. The bound CV was extracted with destaining solution (80% ethanol, 20% acetone).

The tested biofilm mass adherence between the two materials was analyzed using microplate reader (Bio-Rad Laboratories, CA, USA). The background staining was corrected by subtracting the mean value for CV bound to negative controls.

The data were collected and statistically analyzed using SPSS software version16 (one way Anova and t-student test, the calculated data were expressed as a mean ± standard deviation (SD). a *P*-value of<.05 was considered statistically significant.

3. Results:

The surface of Cobalt Chromium was rougher than Zirconium they were 133.91 ± 40.92 nm for Cobalt Chromium and 0.064 ± 0.020 nm for Zirconium. Bacterial culture from both groups as shown in table 1 had revealed presence of microorganisms as follows: in group1 with metal copings eight bacterial species were identified as Streptococcus sanguinus , six species were identified as other varidans Streptococci that include both Streptococcus mutans and Streptococcus oralis, three species of Staphilococcus epidermis, three species were identified as Enterococcus fecalis,three species as *Candida albicans*, two species as Staphilo coccus aureus ,two species as Enterobactria cocci and one case was identified as Pseudomonus aeroginosa. On the other hand in the second group with Zirconium copings it was found only five species of *Streptococcus sanguinus*, five species were identified as other varidans Streptococci , also there were lower numbers of bacterial species include two species of *Candida albicans*, two species Staphilococcus epidermis, and one Enterococcus fecalis.

Table1: Shows presence of microorganisms after paper points culture from both groups (Quantitative assessment study)

Microorganism	Group1(n=8) Metal copings	Group2(n=8) Zirconiacopings	Total
Streptococcus sanguinus	8	5	13
Varidans Streptococci	6	5	11
Staphilococcus epidermis	3	2	5
Enterococcus fecalis	3	1	4
Enterobacteria cocci	2	-	2
Candida albicans	3	2	5
Staphilococcus aureus	2	-	2
Pseudomonus aeroginosa	1	-	1

The quantitative bacterial culture from each group of patients (n=8) as shown in table 2 had revealed higher percentage of bacterial count in group 1 of patients that wear overdenture with metal copings

compared to that of group 2 of zirconium copings with statistical significant difference $p<.0.001$ regarding bacterial colonization specially Streptococcus sangius strains with means ($9x10^5$±2.95CFU/ml) for metal copings and ($4x10^4$±1.86 CFU/ml) for zirconium copings

Table.2: Means and SDs of quantitative bacterial culture CFU/ml between groups

Groups (n=8)	Means of quantitative culture CFU/ml	SD	*P* value
Group1(metal copings)	$9x10^5$ CFU/ml	2.95	$p<.0.001$
Group2(Zirconium copings)	$4x10^4$ CFU/ml	1.86	

CFU=Colony Forming UNits

The second *in vitro* study of both groups according to the absorbent value that were investigated as regard to bacterial biofilm adherence the results as shown in table 3 revealed that, there was biofilm adherence for both coping materials, but that of Cobalt Chromium 0.400±0.08; was higher than Zirconium material 0.100±0.03 with statistical significant difference at p value($p<.0.001$).

Table.3: Means and SDs of Absorbent value to reflect biofilm mass of Cobalt Chromium and Zirconium coping material

Material	Absorbant value to reflect biofilm mass (Mean±SD)	*P* value
Cobalt Chromium	0.400±0.08	
Zirconium	0.100±0.03	$p<.0.001$

4. Discussion:

Zirconia has been used to manufacture primary and secondary copings due to its good mechanical and biocompatible properties including esthetics and low both thermal and electrical conductivity as compared to Cobalt Chromium(CoCr) or Gold copings and the observation in this present study can explain that zirconia has low bacterial adherence affinity as compared to (CoCr).Streptococcus sanguinus bacteria was chosen for this study as it the most earlier bacterial colonizer on both tooth surface and restorative materials ,also it can be easily isolated and identified by simple and low cost experimental tools.

Bacterial adhere more readily to CoCr than to Zirconium. An explanation for that COCr have a greater surface roughness and hydrophorbicity compared to Zirconium as *S. sanguis* is highly hydrophobic microorganism, both, hydrophobic sites of the bacterial cells and sites complementary to saliva pellicle seemed contributing to bacterial adherence to the surfaces. Also CoCr have greater electric conductivity and galvanic action than Zirconium which can considered as insulator so, the surface charge on CoCr can attract the microorganisms to be adhered to the charged surface .Plaque accumulation on tooth or coping surfaces induces an inflammatory reaction in the gingival and alveolar mucosa around teeth leading to periodontitis and subsequent both alveolar bone resorption and periodontal pocket formation .Pockets are the main reservoir for bacterial colonization specially in partially edentulous patients which can be a source of spreading inflammation.[10] This observation agreed with the few studies that have examined removable dentures retained on teeth or implants using zirconia for the fabrication of copings.[9-14]

It has been further asserted that zirconia copings can help stabilize soft tissue against inflammation and contribute to the stability of the crestal bone level around the natural teeth under overdenture

Conclusion:

Zirconium copings as regard to its biological and bacterial adherence is much better for oral hygiene maintenance than metal Cobalt Chromium copings.
Further studies are recommended by other experimental means like Electron Microscopy, other bacterial species to support this research.

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