Research Article

Bacterial accumulation of different telescopic crowns: (PEEK) versus Cobalt Chromium telescopes in implant supported mandibular overdenture.

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Abstract
Purpose: The propose of this study is to compare bacterial accumulation between telescopic overdentures fabricated by two different materials: PEEK (Polyetheretherketone) and Cobalt Chromium copings. Material &Methods: Ten completely edentulous patients were selected from the Outpatient Clinic of the Prosthodontics Department; Faculty of Dentistry, Minia University. Strict inclusion/exclusion criteria were applied. Also all required investigations were done for all patients. Each patient received two implants in the canine regions bilaterally. Implants in all participating patients were from the same manufacture having the same design and overdenture superstructure. The only difference was in telescopic coping material. The patients were randomly divided according to type of telescopic crowns into: Group (1): Patients received overdentures with cobalt chromium telescopic copings (CAD/CAM wax copings were constructed then these copings were transformed to Co/Cr by usual casting procedures). After six months all cobalt chromium copings were replaced by PEEK copings. Group (2): Patients received overdentures with PEEK (polyetheretherketone) telescopic copings fabricated by CAD/CAM. After six months all PEEK copings were replaced by cobalt chromium copings. The patients were recalled after 6 months and 1 year during which, the bacterial samples were isolated and the corresponding microbial alteration in the restored material were evaluated and data recorded and expressed as CFU/ml. Results: PEEK telescopic copings had greater affinity for bacterial accumulation more than metallic copings, and the differences were statistically significant. Keywords: implant supported overdenture, telescopes, PEEK, bacterial accumulation, CAD/CAM crowns.

Introduction
Since the introduction of modern dental implant; many problems were solved in case of complete edentulism. The problem of support and retention of complete denture can now be solved with dental implants.

Implant-supported overdenture treatment is very useful for patients undergoing full mouth rehabilitation. Implant supported overdenture prostheses can be divided into bar overdentures and single attachment overdentures. Single attachment elements for overdentures include single retentive anchors, single magnet anchors, and individually cast telescopic copings.

Among these treatment options; telescopic copings have the benefit of implant splinting found in bar overdentures and the retrievability of single attachment overdentures (Zarb et al., 2004)(3).

Chrome cobalt alloy can be used as a traditional treatment option, overcoming high economy of gold alloy, the most difficult yet important aspect is to produce well adapted surfaces and to maintain appropriate retentive forces over time (Preiskel, 1985)(2).

An alternative restorative material (poly-ether-ether-ketone) PEEK has been successfully used over the last years in the medical and dental fields.

This material presents high biocompatibility, good mechanical and physical properties, high temperature resistance, high polishing, low plaque affinity, good wear resistance, higher level of functional integration and provide a cheaper alternative to precious metal and other materials. Another important property is its resiliency (Kurtz, 2011)(3) (Passia et al., 2016)(4) (Costa-Palau, 2014)(5).
It is well known that, PEEK frameworks can be constructed by CAD/CAM manufacturing. The fabrication of computer aided dental prostheses has become common practice in dentistry and considered fundamentally important for patients seeking more rapid, accurate, and functionally efficient prosthetic rehabilitation (Najeeb et al., 2016)\(^6\) (Johansson et al., 2014)\(^7\) Here arises the question, Can PEEK telescopic crowns provide a good alternative to metallic telescopic crowns in implant supported mandibular overdenture?

**Subjects and Method**

Ten completely edentulous patients were selected from the Outpatient Clinic of the Prosthodontics Department; Faculty of Dentistry, Minia University.

The inclusion criteria were: cooperative patients, not having systemic diseases or medications that may adversely influence osseointegration, moderately developed residual alveolar ridge with sufficient width and height allowing for implant insertion with 3.6mm width and 14mm length, Class I Angle's classification with enough inter-arch space, absence of temporomandibular joint disorders. Patients having bad and abnormal habits were excluded from the study.

All patients involved in the study were told about the reality of the study, its objective, agreed to participate in it and submitted an informed consent reviewed by the faculty's research ethics committee.

For all patients, the following investigations were done: coagulation profile, Glycosylated hemoglobin (HgA1c), blood pressure, cone beam computerized tomography (CBCT).

**Patients grouping:**

Using computer generated list via JDistlib\(^8\) (Java Statistical Distribution Library), version 0.3.5 was used, to assign each participant number to either study groups, patients were randomly divided according to type of telescopic crowns into:

**Group (1):** Patients received overdentures with cobalt chromium telescopic copings (CAD/CAM wax copings were constructed then these copings were transformed to Co/Cr by usual casting procedures). After six months all cobalt chromium copings were replaced by PEEK copings.

**Group (2):** Patients received overdentures with PEEK (polyetheretherketone ) telescopic copings fabricated by CAD/CAM. After six months all PEEK copings were replaced by cobalt chromium copings.

**Pre-surgical procedures:**

**A) Construction of conventional complete dentures:**

Each patient was carefully examined, and a complete denture was fabricated. All steps for traditional complete denture fabrication were accomplished using the standard denture construction techniques.

**B) Surgical guide fabrication:**

1. The lower denture was duplicated and the duplicate was used as a radiographic stent after 10 gutta percha markers were obtained on the tissue surface Fig. (1).

2. CBCT machine (Scanora 3d, sorex. Nalikelantie 160, P.O.Box 148, FI-04301 Tuusula, Finland) and 3D printer( 3d printer Envisiontec micro DGP,USA) were used for surgical guide construction. Fig. (2).

![Fig. (1) Radiographic stent with gutta percha markers.](image1)

![Fig.(2): The fixed surgical guide in patient](image2)
Implants insertion:
In each patient two root type implants (Dentium, 10F, Dongsung Building, 21, Tehran-ro87-gil, Gangnam-gu, Seoul, Korea) with a length of 14 mm and 3.6 diameter were inserted in the mandibular canine regions using CAD/CAM surgical guide.

Overdenture Construction:

A) Construction of metallic framework substructure: Two months after implant insertion, two straight dual abutments (Dentium, 10F, Dongsung Building, 21, Tehran-ro87-gil, Gangnam-gu, Seoul, Korea) Fig. (3) were attached to the implants. Open meshwork design as a substructure for over denture was waxed up then cast with base metal alloy according to manufacturer's instructions Fig. (4).

B) Construction CAD/CAM telescopic crowns: PEEK, wax patterns for metal copings were constructed using CAD/CAM (15DWX-51D, Roland, Shizaoka, Japan) Fig 5,6. Then, wax patterns were transformed to metallic copings using usual casting procedures. Then usual steps for overdenture construction were completed. Finally, pick up was done using chair side intraoral pick up material (Reparon, GC, Tokyo, Japan material) material.

Follow-Up and Evaluation:
Microbial samples from internal margin of the copings were isolated using sterile paper points. The paper points were placed in sterile saline and transferred in icepack containers and processed in the laboratory immediately. The interval between collection and processing of specimen was less than an hour.

The samples were serially diluted (10 fold dilution) in sterile tryptic soy broth (TSB) (and 10µl from 10-2, 10-3, 10-4 and 10-5 were
spread on to the surface of Muller-Hinton and blood agar plates and de Man, Rogosa and Sharpe (MRS) agar for the isolation of Lactobacillus Spp.

Undiluted samples were inoculated on to Muller-Hinton, blood agar and chocolate agar plates. All these plates were incubated at 37°C. After 24–48 h incubation the total numbers of colonies were counted and the viable count determined. The isolated colonies were further processed and identified microscopically (Light microscope, Optika, Italy) with their biochemical characteristics Fig. (7).

The patients were recalled after 6 months and 1 year during which, the bacterial samples were isolated and the corresponding microbial alteration in the telescopic coping material were evaluated. Then data was recorded and expressed as CFU/ml (Leung et al., 1998 and Leung et al., 2003)\(^{(8),(9)}\).

**Bacterial adherence on the surfaces of both agents:**

Four pieces of each of the tested material were incubated in bacterial suspensions that contained 1X10\(^6\) cfu/ml of bacteria (Streptococcus mutans ATCC 35668 or Lactobacillus acidophilus ATCC 4356) in 5 ml of Trypticase soy broth (TSB, BBL, USA) to allow bacterial adherence and biofilm formation. After incubation at 37°C for 24 h, materials were removed and rinsed three times with phosphate buffer saline (PBS). Then, placed in 10 ml fresh sterile saline and sonicated for 30 seconds to dislodge the sessile adherent cells. Serial dilutions of the sonicated saline were cultured. The number of sessile bacteria that indicates degree of adherence was determined by the viable count technique (Reid et al., 1994)\(^{(10)}\)

**Scanning electron microscopy (SEM)**

Streptococcus mutans ATCC 35668 and Lactobacillus acidophilus ATCC 4356 cells were suspended in saline solution containing 0.2% Tween-80 and incubated with the tested material at 37°C. After 24 h, the tested material was then washed and fixed in tris-acetate buffer containing 1.5% glutaraldehyde, and then freeze-dried. Each bacterial culture was observed by SEM (JSM-840 SEM (JEOL Ltd, Tokyo, Japan) at different magnifications Fig.(8) (Soboh et al., 1995)\(^{(11)}\)

**Fig. (7):** Bacterial colonies under light microscope.

**Fig. (8):** SEM images of PEEK copings (A) and cobalt/chromium copings (B).
Results
In vivo results of bacterial counting showed that PEEK telescopic copings had greater affinity for bacterial accumulation more than metallic copings, and the differences were statistically significant. (table 1 and figure 9)

Table 1: Comparison of in vivo bacterial culture count between Metal and Peek.

<table>
<thead>
<tr>
<th></th>
<th>Metal (n=20)</th>
<th>Peek (n=20)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial count</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>1.2x10⁴ (1x10³-1.3x10⁵)</td>
<td>5.4x10⁵ (2.9x10⁴-1.9x10⁷)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

* significant level at p value <0.05
Data presented as median (Range), analyzed by Mann-Whitney U test

Fig. (9): Comparison of in vivo bacterial culture count between Metal and Peek.

Results of electron microscope scanning (SEM):
An in vitro study confirmed also as in vivo study that PEEK telescopic copings had greater affinity for bacterial accumulation more than metallic copings by using viable cell counting.

Under electron microscope the surface of the polymeric plastic PEEK material was more rough than metallic ones.

Also scans of electron microscope proved that PEEK material had more affinity for bacilli species more than cocci, unlike metallic material which had more affinity for cocci more than bacilli. (table 2, figure 10).

Table 2: Comparison of in vitro bacterial culture count between Metal and Peek.

<table>
<thead>
<tr>
<th></th>
<th>Viable cell counts (Mean cfu X 10⁵ ± S.E.M)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PEEK material</td>
<td>Metal material</td>
</tr>
<tr>
<td><em>Streptococcus mutans</em> ATCC 35668</td>
<td>19 ± 0.32</td>
<td>7 ± 0.11</td>
</tr>
<tr>
<td><em>Lactobacillus acidophilus</em> ATCC 4356</td>
<td>20 ± 0.41</td>
<td>4 ± 0.24</td>
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Discussion

By randomizing patients to a sequence of treatments, crossover studies offer many potential advantages over parallel designs, including the ability to ascertain clinical significance and elicit patient preferences, intra-individual comparisons, increased statistical power, faster completion, lower cost, and equality for trial participants (Hui et al., 2015). Washout periods were not applied in our study, because periods of washout would adversely affect bone indirectly through decreased dietary intake by patient.

Also, in this study, the metallic meshwork was open not closed design to allow for replacement of telescopic crowns after six months during crossover. In our study, we used CAD/CAM technology in the fabrication of telescopic crowns. CAD/CAM technology offered many of benefits such as; reduced time, reduced work and quality assurance (Patil M et al., 2018).

The samples were serially diluted (10 fold dilution) and 10µl from 10-2, 10-3, 10-4 and 10-5 were spread on to the surface of the culture media. These dilutions were done to obtain the most comfortable and accurate dilution for bacterial counting.

Tryptic soy broth (TSB, BBL, USA) was added to bacterial suspensions in the in vitro study for incubating pieces of the tested material. Tryptic Soy Broth (TSB) is a nutritious medium that will support the growth of a wide variety of microorganisms. Saliva pellicle was not used because on the one hand, the saliva facilitates bacterial adhesion, but on the other hand, it also contains antibacterial proteins that inhibit bacterial growth and adhesion (Hannig, Hannig., 2009).

During in vitro study of bacterial accumulation, pieces of the tested material were placed in 10 ml fresh sterile saline and sonicated for 30 seconds to dislodge the sessile adherent cells and enable viable cell counting under light microscope.

In vivo study of bacterial accumulation may be subjected to multiple variables that may jeopardize standardization of the research such as: immunity of each patient participating in the research, some patients may undergo some medication that may affect bacterial growth, also type of food and variability in accurate application of oral hygiene instructions by some patients. For all of the previous, we preferred to make in vitro culturing of bacterial species resulted from in vivo study on the surface of the different tested telescopic coping materials.

These in vitro cultures were counted also under light microscope using viable cell count technique, and also scanned under electron microscope. Streptococcus and lactobacilli species were predominant during in vivo study, for this we confirmed these results by culturing these species in vitro on the surfaces of the different tested materials.
Also, scans of electron microscope proved that PEEK material had more affinity for bacilli species more than cocci on the other hand metallic material had more affinity for cocci more than bacilli. The mechanism and the reason for this variation need more investigations in relation to surface physical characteristics of each material.

Increase in surface roughness and surface free energy facilitates biofilm formation on surfaces. Surface chemistry and the design features of the dental restorative material configuration also play a significant role in biofilm formation. (Subramani et al., 2009)(15).

According to Teughels et al., (2006)(16), increased surface roughness increases the amount of bacteria in the biofilm compared with a smoother surface.

Although material composition may play a role, the larger surface area created by the porous surface is likely to be the more influential parameter because it affords a greater surface area and bacteria become entangled and trapped in the surface irregularities (Barkarmo et al., 2019)(17).

According to Hahnel et al., (2015)(18); there was almost no available literature on the adhesion and proliferation of clinically relevant oral bacteria on PEEK.

Also, till now other than this study, there were no direct studies in the literature comparing directly between bacterial accumulation and surface characteristics of metallic cobalt chromium versus PEEK. But there were studies related to each material alone or comparing each material with other restorative materials.

Conclusion
Under the limitations of the current study we can conclude that:

- PEEK material has more affinity for bacterial accumulation more than cobalt chromium metallic material and this mainly may be attributed to its plastic rough surface. The in vivo and in vitro differences in bacterial accumulation values are statistically significant between PEEK and cobalt chromium groups.

Recommendations
- The outcomes of this study should be confirmed and generalized by further studies with greater sample size and conducted in different areas of the mouth. Also, more follow up period should be applied

References:
10. Reid G, Sharma S, Advikolanu K, Tieszer C, Martin RA, Bruce AW.: Effects of


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