Original Article

Determinations of a Proper Covering Material for Fixture-Abutment Microleakage Evaluation Using Radiotracers and Gamma-Counter; A Pilot Study

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Abstract

Background and Aim: Radioisotopes adhere to any surface in which they come in contact. Covering the sample surface with a suitable material prior to submersion and isolation of the material before counting make it possible to evaluate the penetrating radioisotopes within the interfacial area. The aim of this study was to determine a suitable material to cover implant and abutment in evaluation of microleakage in implant-abutment interface using radiotracers and gamma counter.

Materials and Methods: In this in vitro study, 46 samples were selected and divided into two groups. The first group consisted of implant samples covered with putty, nail polish and putty-super glue with 1mm distance from the interface. The second group included non-implant samples covered with putty, autopolymerizing acrylic resin, and nail polish. This group was used for evaluation of adherence levels of the radioisotopes. Microleakage test was performed with thallium-201 and gamma counting in three phases: 1) after removing samples from thallium solution, 2) after washout of samples, and 3) after removing covering materials. In order to compare penetration of radioisotopes within the samples analysis of co-variance was carried out.

Results: There were statistically significant differences between three phases of gamma counting and between samples in different implant groups. Microleakage of implant-putty-glue was significantly less than that of implant-putty (217343.40±86007.926). Similarly, implant-putty showed a significantly less microleakage than implant-nail polish. (313247.20±67933.031).

Conclusion: The best material among the ones considered in this study turned to be putty sealed by super glue. Contrarily, nail polish was not considered suitable due to increased microleakage.

Key Words: Thallium-201, Radioisotope, Microleakage, Implant, Fixture-abutment interface

Introduction

Microleakage at the implant-abutment interface is a main cause in creating peri-implantitis inflammatory reactions. Prevention of microbial penetration in implant-abutment interface to limit these inflammatory reactions and maximizing bone stability in the implant's neck area is a major challenge.

To overcome this challenge a large number of stu-
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... have been performed to measure the amount of microleakage and to devise methods to reduce this phenomenon [1-3]. Broglini et al., in 2006, studied the distribution and density of inflammatory cells around the implants in cases where the implant-abutment interface were at the supracrestal, subcrestal, or crestal levels. In this study, all samples showed a similar inflammatory pattern around the implant with more density of neutrophils in subcrestal compared to supracrestal cases. Therefore, they concluded that inflammatory cells would congregate at the implant-abutment interface that would eventually result in alveolar bone resorption [4]. Furthermore, clinical studies have shown existence of live bacteria on the internal surfaces of the implant components [5].

Jansen et al., in 1997, showed that there is no way to prevent bacterial penetration and colonization even in implant systems with high compatibility between their components [6].

Bacteria need an appropriate environment for their growth and survival. Physical properties of the abutment-implant interface environment lend itself to such appropriate environments. When an abutment positions on an implant, around the threads, at the end of the screw and the bottom of screw chamber an appropriate environment is formed for growth and survival of bacteria. Through the interface gap liquids, macromolecules in saliva and bacterial toxin penetrate into these environments and provide bacteria with required materials for growth and survival [7].

Several methods have been used for assessment of microleakage including use of bacteria, compressed air, chemical tracers, electrochemical changes, autoradiographic studies, electron microscopy, and dyes penetration. Another method is use of radiotracers with several advantages that include features such as being noninvasive, quantitative, reproducible, and its precision and high penetration level due to small radioisotopes' sizes [8].

Given the permeability of radioisotope or radiotracer material one can evaluate the interfacial gap. Photon counting, performed by gamma-counter device, shows the microleakage characteristics of radioisotopes passing through the interface gap and entering the internal space of the implant [9]. Radioisotopes adhere to any surface that they meet. [10-16] Therefore, one needs to cover all surfaces except for the implant-abutment junction when the samples are immersed in radioisotope solution. This guarantees that the radiotracer does not directly contact any surface of the sample except for the implant-abutment interface. Therefore, after removing the cover, the detected gamma radiation would account only for the radioisotopes that penetrate through the interface. This provides a precise and reliable method of microisotope assessment.

The goal of this study was to determine an appropriate material to cover implant and abutment when evaluating microleakage characteristics of implant-abutment interface using a radiotracer and gamma-counter device.

**Materials and Methods**

A number of candidate materials were considered for this study:

1) Putty (Speedex – Putty, Silicone Impression Material, Coltene/Whaledent, Germany),
2) Autopolymerizing acrylic resin (Luxatemp; DGM, Hamburg, Germany),
3) Nail polish (Bourjois, Paris),
4) Cyanoacrylate glue.

The main criteria in selecting these materials were the following: ease of use, lack of technical insensitivity, resistance against penetration of the radiotracer, ease of detachment from the sample after being immersed in radioisotope solution, and lack of any adverse effects on the samples.

The method was designed to achieve two goals to find the best covering material among the ones considered:

1) Evaluating the penetration level of radiotracer into the covering material,
2) Evaluating the adherence level of the radiotracer to the covering material and penetration depth.

There were a total of 46 samples grouped into two categories (Fig 1). The samples in the first category were used to evaluate penetration level of the
radionuclide when covering implants. These samples, called Implant Samples (IS), were implant analogues attached to impression coping (Fig 2).

The IS samples include the following: Five fixture analogues, Replace implant replica RP (Noble Biocare, Goteberg, Sweden) and Replace impression coping closed tray 4.3 mm (Noble Biocare, Goteberg, Sweden) with putty coverage. Five fixture analogues and impressions with putty cover super-glued at its borders’ gap due to putty shrinkage. Five fixture analogues and impressions with nailbrush cover. It should be noted that all covers were placed 1 mm away from the analog-impression interface so that they did not block the gap or alter penetration of radioisotopes through the interface. One fixture analogue and impression was used as control to evaluate the level of radioisotope adherence without any cover. It is noteworthy that there was no need for negative control.

The samples in the second category, called Cylindrical Samples (CS), were used to evaluate adherence level and penetration depth of the raddiatracers (Fig 3). There was no need to use expensive implant samples in this category. The CS samples included the following: Ten samples of putty material. Ten samples of putty material covered by a nail polish layer. Ten samples of autopolymerizing acrylic resin.

A stainless steel cylinder, 5 mm in diameter was used as a cast to build the non-implant samples. This guaranteed all CS samples were the same size and surface area.

Microleakage test in IS samples and radioisotope adherence in CS samples have been performed in three phases. In the first phase, the samples were immerged in thallium chloride-201 radionuclide solution of 2 mCi (milli Curie) in 500 cc water for 24 hours. Then the samples were removed and dried all in the same position. The samples were placed in specially designed test tube for gamma photon counting. A gamma counter (Kontron, Gammatronic, Switzerland) with Photo pick adjustment for Thallium-201 (77 kev) and an energy window of 15% was used in an interval of one minute to count the photons simultaneously [17].

In the second phase, samples (CS, IS) were washed by detergent solution for one minute and then distilled water using a microbrush. Caution was taken...
to make sure the analogue impression junctions were not washed in IS samples. Then, the samples (CS, IS) were placed in the test tube designed for gamma counting in the same position and the countings were performed for one minute simultaneously. All results were documented in gamma counts per minute (cpm).

In the third phase CS samples were evaluated to determine the depth of radionuclides penetration. Cylindrical putty samples and putty with nail polish were cut all around by scalpel at a 1-mm width. Then 1 mm of acrylic resin was removed from acrylic samples by acrylic bur. In IS implant samples with putty or putty and glue the entire putty was detached. Nail polish was removed from implant samples with nail polish. Then gamma counting was performed again for samples in both CS and IS groups under the same condition as in the previous phases. At the end, samples (CS, IS) were quarantined in a lead cap for 12 days to protect the environment from radioactive contamination. It should be noted that half-life of Thallium is 72 hours.

In this study penetration levels of radioisotopes in samples were evaluated using covariance analysis test considering the initial penetration level as the covariance.

Results
All statistical analyses were performed using SPSS software, version 11.5 considering statistical error of first type of 0.05 (p ≤ 0.05).

Statistical analysis showed that microleakage level in all three phases of counting between the groups were significantly different (diagram 1). This observation indicated that:
1. Washing the samples significantly reduced radiotracers in both groups (CS, IS).
2. In IS samples, all three methods reduced the number of radiotracers that reach the samples.
3. In CS samples, after removing 1 mm from the samples surfaces it was determined that penetration of radiotracers in this depth (1 mm) was significantly reduced.

Post hoc Tukey LSD test showed that, in all three groups with IS implant, microleakage level was significantly lower in glue compared to putty and it was significantly lower in putty compared to nail polish (p ≤ 0.05)

### Table 1. Mean and standard deviation of radioisotope microleakage in the third phase of gamma-counting in two sample groups (CS, IS)

<table>
<thead>
<tr>
<th>Coating Media</th>
<th>Std. Deviation (cpm)</th>
<th>Mean (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catagory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cylindrical Samples (CS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Putty</td>
<td>410/121</td>
<td>369/30</td>
</tr>
<tr>
<td>Putty with nail polish</td>
<td>2236/563</td>
<td>1558/70</td>
</tr>
<tr>
<td>Implant with putty</td>
<td>420/969</td>
<td>367/60</td>
</tr>
<tr>
<td>Implant with nail polish</td>
<td>86007/926</td>
<td>217343/40</td>
</tr>
<tr>
<td>Implant Samples (IS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Implant, putty and adhesive</td>
<td>67933/031</td>
<td>313247/20</td>
</tr>
<tr>
<td>Acrylic resin</td>
<td>23909/586</td>
<td>76379</td>
</tr>
</tbody>
</table>

Diagram 1. Microleakage comparison between covering materials in three phases of gamma-counting process (Phase 1: Initial counting; Phase 2: Counting after washing; Phase 3: Counting after cutting putty and removing acrylic resin and nail polish).
Discussion
Periimplantitis as an inflammatory reaction that occurs along with losing supporting bone around the implant and it is defined as a dysfunctional phenomenon [18]. Poor hygiene around implant directly relates to accumulation of bacterial plaque and peri-implant mucositis in humanbeings. This provides a suitable environment for the microbial flora, which are common periodontal pathogens.

According to Broggini, et al., in 2006, micro-gaps in bone-level implants along with stable bacteria and bacterial leakage cause concentration of inflammatory cells, initiation and growth of osteoclasts, and alveolar bone resorption [2].

Radioisotope provides a precise, relatively inexpensive, and totally reproducible method that lends itself to quantitative measurements of microleakage. The samples used in this method are completely reusable so they can be used in several other tests. However, to make the method more precise, all surfaces of the samples except for the interface should be covered [23]. The covering materials that can be used include putty, cyanoacrylate glue, acrylic resin, and nail polish. Based on the results of non-implant samples, adherence and penetration power of radiotracers were lower in nailbrush compared to putty and they were lower in putty compared to acrylic resin with no statistical significance. However, these differences in implant analogue (IS) group were significant. Considering the fact that when acrylic resin is used to cover the implant analogue surfaces, its handling and detachment from the samples are extremely difficult and therefore, it is not a viable option for implant samples. In IS samples with nail polish, distributing the nail polish evenly on the samples’ surfaces is challenging. Further, this requires some time to get nail polish completely dried. Also, removing the entire nail polish from the implant samples (IS) is extremely difficult. Also, there is a high possibility that the radiotracers contaminate other areas and cause error in the study results.

Using putty compared to nail polish and acrylic resin has is advantageous due to its ease of attachment from the samples. Also, handling putty is very simple. The only drawback is that the edges of putty do not stick to the samples. Due to shrinkage, there is a gap between putty and the sample, which causes leakage from this gap. This can be fixed by applying glue on the edges. However, one shall make sure that glue does not cover or penetrate into the analogue impression interface.

Conclusion
Within the limited nature of this study and based on the results, one can conclude that in microleakage evaluation best material for covering implant samples (IS) is putty with cyanoacrylate glue on the edge of putty.

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References