In modern prosthodontic dentistry, implant-supported restoration is considered to be a technique that is used to restore partially and fully edentulous cases. Development of the dental materials and the techniques used in implant-supported restorations has enhanced the outcome and success rate of these procedures. Peri-implantitis is considered to be a major fear of all practitioners, and it threatens the success of implant-supported restorations. Bacterial accumulation in the peri-implant tissue is considered to be the main etiology of peri-implant disease. Bacterial colonies in the peri-implant pocket and inside the implant system can result in an inflammatory response of the peri-implant tissue even in patients with an acceptable level of mouth care. Even if satisfactory osseointegration occurs between the implant and bone, the inflammatory response of the peri-implant tissue will lead to the early phase of implant failure.

There is a direct correlation between the presence of a gap in the implant system (implant-abutment and abutment-restoration interference) and intense inflammatory infiltration. The pumping effect of bacteria and endotoxins supports the recruitment of inflammatory cells to the peri-implant tissue. A deeper microgap is typically formed when a fixed implant-supported prosthesis is used in rehabilitation. Indeed, these include two gaps of interference. The first gap is the implant-abutment interference gap; the magnitude of this gap depends on the manufacturer, and it seems to be <50 µm for commonly used implant systems. The second gap, which is normally formed between the abutment and the restoration interference, is related to the restoration

Purpose: Despite the high success rate of implant-supported fixed restorations in dentistry, there is a lack of evidence on the marginal seal for dental cement. Thus, this study aimed to evaluate the marginal seal of implant-supported crowns and partial dentures cemented using four different dental cements. Materials and Methods: The study evaluated the marginal seal of implant-supported crowns and partial dentures cemented using zinc phosphate, resin-modified glass-ionomer, self-adhesive resin, and noneugenol, acrylic-urethane polymer-based temporary dental cements. After cementation and thermal cycling procedures, the samples were incubated in Escherichia coli suspension for 5 days at 37°C under an aerobic environment. After debonding the restorations under sterile conditions, sterile cotton swabs were used to obtain microbial samples from the inner surface of each restoration and abutment surface. To analyze the contamination, each sample was immersed in a brain-heart infusion culture medium and incubated at 37°C for 24 hours, and then, the colony-forming units were counted and recorded. Results: Regarding the number of colonies for Escherichia coli, the results revealed no substantial difference between the crowns and the fixed partial restorations (P = .25). However, the differences in the level of contamination between the cement groups were significant (P ≤ .001). The self-adhesive resin cement samples showed the lowest level of contamination, followed by the zinc phosphate and resin-modified glass-ionomer cements. The difference in the level of contamination between these groups was not significant. The temporary cement group exhibited significantly higher numbers of bacterial colonies in comparison to the other cement groups. Conclusion: Self-adhesive resin cement has better biologic properties for retaining implant-supported restorations than other types of dental cement. Int J Oral Maxillofac Implants 2021;36:910–916. doi: 10.11607/jomi.8824

Keywords: dental cement, dental implants, E coli, marginal leakage

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fabrication procedures and the retaining method of the prosthesis. Most dental practitioners prefer to use cement-retained abutments in fixed implant-supported restorations. There are many advantages to using a cemented abutment instead of a screw-retained abutment. A cement abutment enhances the passiveness of the restoration over the abutment, which is related to the ability of the dental cement to fill the microgaps along the margin between the dental restoration and the abutment. The mechanical strength and durability are greater for cement-retained restorations, which is related to the absence of a screw channel. Many types of dental cements are available that could be used to retain implant-supported restorations. The incidence of chewing load and temperature changes during normal function may lead to the washing out of dental cement in the margins of the restoration, assisting bacterial outflow. The contamination rate depends on the implant-abutment connections and the type of dental cement that is used to retain the restoration. The present study used Escherichia coli (E coli) for the microbiologic contamination, which is commonly used in in vitro studies for the purposes of contamination. These small Gram-negative anaerobic bacteria (diameter: 1.3 μm, length: 4 μm) are very suitable for evaluating gap-leakage, especially for implant-supported restorations. As rod-shaped bacteria, E coli are small enough to pass through the microgap, and the measurements are the same as periodontal pathogens. Thus, this present in vitro study aimed to evaluate the bacterial microleakage for implant-supported crowns and partial dentures cemented using four different types of dental cement, following thermal cycling.

**MATERIALS AND METHODS**

A total of 84 direct abutments (T2 Analog, NucleOSS) were placed vertically in an auto-polymerizing resin block (HinriPress, Ernst Hinrichs Dental) using a dental surveyor for proper placement. The specimens were divided into two main groups (n = 28), relying on the design of the metal restoration: a single-unit molar (coded as C) and a three-unit posterior denture (second premolar as a pontic and first premolar and first molar as abutments, coded as B). The samples were further divided into eight subgroups (n = 7) based on four different types of cement used to retain the crowns and the partial dentures (Fig 1). The manufacturer information for each type of dental cement is provided in Table 1.

**Table 1 Description of Dental Cements Used in This Study**

<table>
<thead>
<tr>
<th>Commercial name</th>
<th>Cement type</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harvard</td>
<td>Zinc phosphate cement</td>
<td>Harvard Dental International, Germany</td>
</tr>
<tr>
<td>RelyX U200 Automix</td>
<td>Self-adhesive resin cement</td>
<td>3M ESPE dental products, USA</td>
</tr>
<tr>
<td>RelyX Luting Plus</td>
<td>Resin-modified glass-ionomer cement</td>
<td>3M ESPE dental products, USA</td>
</tr>
<tr>
<td>Dentotemp</td>
<td>Noneugenol, acrylic-urethane polymer-based temporary cement</td>
<td>Itena Clinical, France</td>
</tr>
</tbody>
</table>

**Fig 1** Flowchart for study group blocks.
Metal restoration wax was made using computer-aided design/computer-aided manufacturing (CAD/CAM) technology. First, all the specimens were scanned using an extraoral lab scanner (inEos X5, Dentsply Sirona); then, a full anatomical restoration was digitally designed (inLab CAD software, Dentsply Sirona). The film thickness of dental cement was set at 50 µm in the occlusal and axial walls except for the margins, which were planned to be precisely adapted to the abutment. Then, the wax models were milled (inLab MC X5, Dentsply Sirona) using wax disks (98.5-mm diameter and 14-mm thickness; on dent, Wax-on, WGS-9814). A wax loop was joined occlusally to the single-crown samples, and two loops were connected to the occlusal surface of the molars and premolars in the partial denture samples. The design of the study samples and the abbreviation system used to code the study samples are shown in Fig 2. The loops were used to attach the samples to the superior arm of the universal machine to debond the restorations from the abutments before the biologic tests were conducted. The established wax patterns were invested (Sherafina-Rapid, Shera Werkstoff-Technologie), then cast in a base metal alloy (Eisenbacher, Dentalwaren). After divesting and cleaning, the established metal cast was sterilized by the autoclave. Four available luting agents were evaluated in this study: zinc phosphate cement (Harvard); noneugenol, acrylic-urethane polymer-based temporary cement (Dentotemp); resin-modified glass-ionomer cements (RelayLuting Plus); and dual-curing, self-adhesive resin cement (RelayX U200 Automix). Zinc phosphate cement is a well-known standard with a long history of use; thus, it was used to provide reference points. In this study, each type of cement was used as reported by the manufacturers' recommendation and used to retain the restorations.

A small spatula was used to apply the cement on the axial wall of the restorations to reduce the hydrostatic pressure during the placement of the restoration. Hand pressure was held over the restoration for 10 seconds followed by immediate placement of 6-kg static load pressure with the long axis of the restorations, which was maintained for 10 minutes. The specimens were kept in 100% humidity at 37°C for 24 hours. Then, the samples underwent 10,000 thermal cycles (between 6.5°C and 60°C, dwell time 45 seconds). Following thermal cycling, one sample from each group was incubated for 5 days in distilled water to confirm the colonization and disclosing techniques. The swab was picked up from the internal wall of the restorations after debonding of the samples under sterile conditions. These samples were used as negative controls. The remaining six samples from each group were put into an E.coli suspension (ATCC 25922) at a McFarland standard 5 (1.5 x 10^8 CFU/mL) and incubated for 5 days at 37°C under aerobic conditions. The culture medium was changed every 48 hours. After debonding the restorations under a sterile environment, sterile cotton swabs were used to obtain the microbial samples from the internal surface of the metal restorations. To analyze the degree of contamination, each sample was immersed in a brain-heart infusion culture medium and incubated at 37°C for 24 hours. After that, the colony-forming units (CFUs) were counted and reported.

Statistical assessment was done using a readily available statistical program (IBM SPSS Statistics 21, SPSS, an IBM Co). Levene's test was applied to analyze the continuous variable data (P < .05). To measure the variables with more than two dimensions, two-way analysis of variance (ANOVA) was applied to identify the differences between the groups.

RESULTS

CFUs for E.coli were not detected in any of the samples in the negative group. Concerning the number of CFUs for E.coli, the results revealed no substantial difference between the crowns and the fixed partial restorations (P = .25). The differences in the CFUs between the cement groups were significant (P ≤ .001). The resin cement samples had the lowest level of contamination (Fig 3, Table 2). All the crown samples that were cemented with resin cement were free of any contamination. Two of the six self-adhesive resin cement partial denture samples had no bacterial contamination. The samples that were cemented using zinc phosphate cement and resin-modified glass-ionomer cement had the same amount of bacterial contamination (4,000 CFU/ML), but the difference between these groups was not significant. The temporary cement samples were found to have significantly higher CFUs for E.coli in comparison to the other cement groups. The crown samples that were cemented using temporary cement had a higher number of colonies for E.coli (55,000 CFU/ML) than the
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Partial denture samples that were cemented using the same dental cement (5,500 CFU/ML). The CFUs for the E coli were lower for the zinc phosphate cement samples than the temporary cement and resin-modified glass-ionomer cement samples.

**Discussion**

Cement-retained implant-supported restorations have a higher success rate than screw-retained implant-supported restorations; however, according to the literature,10,11 implant-supported restorations showed 9.8% decementation under normal function in 3 years of usage. The multifactorial medium of the mouth is the reason for the retention failure. The restoration is exposed to a broad spectrum of germs, thermal changes, and leverage forces in a high-humidity environment. These factors decrease the bond strength of the cement and raise the frequency of bacterial leakage.10 The bond strength and marginal leakage of dental cement are strongly associated with many factors. The cement type, cement film thickness, morphology of the abutment, the surface treatments of the abutment and restoration, temperature changes, and the load cycle of the restoration during function are known to have an impact on the bond strength of dental cement.12 Previous studies have conducted mechanical and biologic evaluations of implant-supported restorations using different dental cements, implant systems, and adhesion protocols7,13–15; however, these in vitro studies only examined single designed implant-supported crowns.

The present study was designed to evaluate the microbiologic contamination of different restoration designs (single crown and fixed partial dentures) that were retained with four different types of cement. CAD/CAM technology was used to mill the wax for casting the metal restoration to standardize all the other parameters that might affect bacterial leakage, such as the cement film thickness, abutment, and surface treatment applied to the restoration. For this purpose, the inlay wax disks were milled with attention to the cement film (50 μm) thickness and marginal adaptation. The conventional method of metal casting, which depends on the die spacer used to arrange the space for the dental cement and the manual wax buildup, is a multistage process in which more errors can occur.16 Consequently, the conventional method of wax buildup.

![Image](221x541 to 535x727)

**Table 2** Numerical Value of CFUs for E coli

<table>
<thead>
<tr>
<th>Dental cement</th>
<th>Partial denture</th>
<th>Crown</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resin-modified glass-ionomer cement</td>
<td>4,000 CFU/ML</td>
<td>4,000 CFU/ML</td>
<td>.25 *</td>
</tr>
<tr>
<td></td>
<td>SD = 4,647.580</td>
<td>SD = 4,647.580</td>
<td></td>
</tr>
<tr>
<td>Self-adhesive resin cement</td>
<td>00 CFU/ML</td>
<td>6.67 CFU/ML</td>
<td>.002 ***</td>
</tr>
<tr>
<td></td>
<td>SD = 0.000</td>
<td>SD = 5,164</td>
<td></td>
</tr>
<tr>
<td>Temporary cement</td>
<td>5,500 CFU/ML</td>
<td>55,000 CFU/ML</td>
<td>.001**</td>
</tr>
<tr>
<td></td>
<td>SD = 4,929.030</td>
<td>SD = 49,295.030</td>
<td></td>
</tr>
<tr>
<td>Zinc phosphate cement</td>
<td>2,020 CFU/ML</td>
<td>55 CFU/ML</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD = 3,937.710</td>
<td>SD = 49,295</td>
<td></td>
</tr>
</tbody>
</table>

*The statistical difference value between crown and partial denture samples. **The statistical difference value between the four cement groups. ***The statistical difference value between all the subgroups of the study (8 groups).
has been replaced by CAD/CAM to ensure that all the samples are an exact copy of each other. According to other in vitro studies, the film thickness of dental cement should be wide enough to provide a good fit for the restoration, but not too thick to cause marginal leakage. The acceptable marginal gap amount for fixed partial dentures is 50 μm. The required thickness for the dental cement (50 μm) has been determined to be 30 μm for cement film and 20 μm for shrinkage of the inlay wax.

In a study that was conducted by the author of the present study, the same sample design was used. The present study also used the same methodology as that study, except for the medium of incubation, which was the basic fuchsine dye, so the highest marginal leakage value was seen in the resin-modified glass-ionomer and the temporary cement groups. However, no significant difference was observed between the marginal leakages in these two types of cement. The self-adhesive resin cement samples were found to have the lowest level of leakage, followed by the zinc phosphate cement. These results are similar to the results of the present study. In the previous study, the direct method was used to make the measurements; that method is most often used because it allows for repeatable and accurate measurements. The stereomicroscope is the tool most often used to evaluate direct method measurements. The high image magnification feature of the stereomicroscope enables precise measurements.

There are different opinions about which method is best for evaluating marginal leakage. In in vitro studies, the marginal leakage of the samples was calculated by measuring the distances between the margins of the restoration and the deepest point for dye penetration using the direct stereomicroscope method. In the present study, the evaluation method depends on detecting the number of bacterial colonies under the cemented restoration and to record the number of bacteria under sterile conditions. The pathologic manifestation resulting from bacterial accumulation in the implant sites is explained as peri-implant mucositis and peri-implantitis. Peri-implant mucositis is defined as peri-implant soft tissue inflammation, whereas bone resorption is also recorded in peri-implantitis. They are primarily caused by bacterial accumulation in the peri-implant area, and they may even result in implant loss.

In implant-supported restorations, the marginal leakage of the restoration is not the only bacterial passage. Implant-abutment interference is also considered to be a gap for bacterial transition. This leakage has been discussed in various in vitro studies. Many types of implant-abutment interfaces were tested with combinations of conventional materials did not hinder bacterial leakage. The conical abutment interface has advantages over other systems (internal- and external-hexagon implant-abutment interface). The design of the connection between the implant and abutment decreases the width of the microscopic gap, leading to a minimizing in bacterial movement through the interface. Nevertheless, even conical abutment interfaces tested with combinations of conventional materials did not achieve absolute bacterial tightness. In contrast, absolute bacterial tightness was achieved with conical PEEK abutment on conventional titanium implants. This is most likely due to the high elasticity of the PEEK materials. In the present study, to prevent contamination from the bacterial transition through the implant-abutment interference and to evaluate the level of contamination of the margins of restoration, a direct abutment was used. Moreover, the direct abutment was fabricated as a one-piece abutment (abutment-analog), eliminating any gap resulting from the implant-abutment connection.

The contamination level is remarkably higher under screw-retained implant-supported restorations than cemented restorations. According to in vitro and in vivo studies, the cement serves as a barrier to prevent bacterial transition between the oral cavity and
the implant system. An in vitro study reported a high concentration of bacterial colonies inside the implant lumen and under the screw-retained restoration. In other studies, bacterial colonies were detected under the restoration and around the screw of the abutment in screw-retained implant-supported restorations. There is a relationship between the clinical complications of peri-implantitis and the presence of bacteria (Helicobacter pylori and Staphylococcus aureus) for screw-retained implant-supported restorations. The contamination of screw-retained implant-supported restorations may be related to leakage in the occlusal filler, which seals the restoration screw channel. The absence of the screw hole in cement-retained implant-supported restorations gives them a biologic advantage over screw-retained restorations.

The bacterial contamination level in cemented restorations is related to the type of dental cement. Phillips showed that zinc phosphate cement has no antibacterial effect. Schwartzman et al reported that the low pH level of zinc phosphate cement renders the atmosphere under the restoration unsuitable for bacterial colony formation, which is considered to be a mild antibacterial effect for this cement. In this study, the presence of bacterial colonies under the restoration cemented using zinc phosphate cement demonstrated that this cement has no antibacterial effect. The bonding ability between the zinc phosphate cement and the underlying titanium abutment and the durability of this cement explains why its marginal sealing is better than the resin-modified glass-ionomer and temporary cements in the present study.

According to an in vitro study, the anti-bacterial effect of resin-modified glass-ionomer cement is limited to 7 days after the cementation procedure. In the present study, the bacterial incubation followed the thermal cycling, which extends the incubation period for 13 days; this explains the high level of contamination seen in the resin-modified glass-ionomer cement in comparison to the self-adhesive resin cement and the zinc phosphate cement. Clinical symptoms of peri-implantitis are associated with bacterial colonies in the peri-implant pockets (Prevotella intermedia, Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis). Bacterial colonies have been shown to range between 4.6 × 10⁴ and 3.1 × 10⁶ CFU/mL in peri-implant pockets. In the present study, the number of bacterial colonies detected for the samples cemented with the self-adhesive resin, zinc phosphate, and resin-modified glass-ionomer cements were less than the number of colonies associated with the peri-implantitis clinical feature. The number of bacterial colonies in the temporary cement restorations was found to be close to the number of bacterial colonies in the peri-implant pockets, so the use of temporary cement is contraindicated for implant-supported restorations. Temporary dental cement is unable to provide a hermetic seal to the underlying abutment or withstand all the thermal, mechanical, and chemical factors, so it is expected that marginal leakage would be detected. The presence of marginal leakage leads to bacterial transition from the oral cavity to the area under the restoration. According to several in vitro studies and in vivo studies, the increase in the incidence of microleakage will increase the bacterial transition. Most of the bacteria in the oral cavity are 10 μm in size, so they can pass easily from the marginal gap of the restoration. This accumulation of bacteria is considered to be a pre-factor for mucositis and periodontitis.

CONCLUSIONS

This study aimed to provide a standard methodology for similar in vitro studies to enable dental practitioners to select the dental cement that provides the best mechanical and biologic properties to enhance the success of implantology treatment. Self-adhesive resin cement found to have biologic advantages over the other dental cements used in the study. Self-adhesive resin cement was found to provide the best marginal seal against bacterial transition, followed by zinc phosphate cement and then resin-modified glass-ionomer cement. Temporary cement had the highest incidence of bacterial leakage during bacterial incubation.

In light of the biologic evaluation conducted in this study and the mechanical evaluation conducted by the same author in a previous study, self-adhesive resin cement is the best choice for retaining a single crown and partial implant-supported dentures. In the future, more in vivo designed studies are recommended to evaluate the biologic and mechanical performance of different dental cements for retaining implant-supported restorations.

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