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ORIGINAL RESEARCH

Evaluation of the Sealing Capability of the Internal Conical Connections of Implants with Titanium and Zirconia Abutments

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ABSTRACT

Aim: The purpose of this *in vitro* investigation was to evaluate the sealing capability of the conical implant–abutment interfaces under different abutment screw torque values using titanium and zirconia abutments with Morse taper designs.

Materials and methods: A total of 42 dental implants (n = 21 for titanium abutments and n = 21 for zirconia abutments) were inoculated internally with three bacteria. These assemblies were divided into four test groups (n = 10) based on screw fixation torques of 35 or 20 Ncm and placed in sterile broth; the remaining abutments were used as positive controls and torqued to 10 Ncm. Microleakage was quantified by enumerating the bacteria from the colony-forming units. An analysis of variance for the estimates of bacteria enumerated and microgaps was used with a *post hoc* analysis as indicated. A p-value of 0.05 was used as the level of significance.

Results: There was no statistically significant difference in microleakage among the four test groups; there were no significant effects of screw torque or abutment type on the bacteria enumerated. There was a significantly smaller mean microgap with the zirconia abutments.

Conclusion: The results of this study indicated no statistically significant difference in the sealing capabilities between titanium and zirconia abutments, having internal conical connections, after increasing the abutment screw torque.

Clinical significance: It is important for clinicians to follow the guidelines suggested by the implant companies to avoid biomechanical complications over time.

Keywords: Bacteria, Implant, Microgap, Sealing capability, Titanium, Zirconia.

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INTRODUCTION

Dental implants have become a viable and predictable treatment in restorative dentistry, and high success rates have been reported.[1] Long-term clinical success of implants has generated a widespread interest in implant placement and restoration throughout the dental community.[2]

Dental implants and abutments are usually made of commercially pure titanium or a titanium alloy, due to its biocompatibility and mechanical properties.[3] To mask the potential show through of metallic abutments, zirconia abutments are widely utilized in the anterior sites. Not only do zirconia abutments preserve natural tissue color but also according to Manicone et al,[4] tissues surrounding zirconia abutments demonstrate less inflammatory infiltrate, less microvessel density, and less vascular endothelial growth factor expression than tissues around titanium abutments. According to Watkin and Kerstein,[5] zirconia abutments demonstrate good biocompatibility, low corrosion potential, low thermal and electrical conductivity, and superior mechanical properties.

Abutments are seated on implants using either external or internal connections. These connections are designed to resist rotational and axial forces. The internal cone screw tapered connection was characterized by an 8° Morse taper in the mating angle between the implant and abutment, thus creating a mechanically sound and self-locking interface.[6] To maintain intimate implant–abutment contact under occlusal load, abutments are secured to implants through an abutment screw. The screw joint must have a preload necessary to maintain its integrity. The preload is the initial load in tension developed within the abutment screw when tightening torque is applied, creating a compressive clamping force between the abutment and the implant.[7] It should be noted that a true Morse taper exists at 2 and 4° and is self-locking without threads.[8,9] It relies on the frictional resistance of the dry, clean abutment post, and implant shaft for functional stability.[8] This metal-to-metal cold welding of the abutment against the implant wall has been shown to create an impenetrable seal against bacterial microleakage.[9]

Hecker and Eckert[10] demonstrated that, over time, an inaccurate fit of the implant–abutment connection might result in loosening or fracture of the abutment screw, leaving a microgap at the implant–abutment interface. Accumulation of bacteria at this interface[11,12] can be associated with peri-implant mucositis, which is defined clinically as erythema and edema of the soft tissue with bleeding on probing as an important feature.[13-16] Tabanella et al[16] found that the microflora most commonly associated with peri-implant bone loss included *Fusobacterium* species, *Tannerella forsythia*, *Campylobacter* species, and *Peptostreptococcus* micros. If there is a higher percentage of *Fusobacterium nucleatum* in diseased sites, Tabanella suggested that it is reasonable to assume that the bacterium may coaggregate *Porphyromonas gingivalis* and *P. intermedia*, as well as other periodontal pathogens, thereby participating in the formation of a pathogenic anaerobic polymicrobial community.[16]

Jaworski et al[6] concluded that the Morse taper connection provided a better sealing capability compared with external hex specimens in that less Morse taper specimens showed leakage at later points in time during their study. Other researchers have not been able to maintain a seal with this tapered geometry.[17,18] After evaluating the interfaces of an implant system in which the titanium and zirconia abutments created an external butt joint with the implant platforms, Smith and Turkyilmaz[19] found that the titanium abutments showed a smaller microgap (ranged from 2.0 to 6.6 µm) compared with the zirconia abutments (ranged from 7.4 to 26.7 µm). Furthermore, they found that increasing the abutment screw torque from 20 to 35 Ncm significantly decreased the microgap at the zirconia abutment–implant interface ($p = 0.017$).[19]

The purpose of this case–control *in vitro* investigation was to evaluate the sealing capability as well as the size of the microgap at implant–abutment interfaces, using two different abutment materials, consisting of internal conical connections under different abutment screw torque values. Three different types of bacteria, which contribute to periodontal disease-associated marginal bone loss and peri-implantitis, were used in this investigation. The null hypothesis was that there would be no significant difference in the sealing capability or microgap when abutment screw torque values were lower than that recommended by the manufacturer. By measuring the microgaps between the implants and abutments, the authors determined the effect of the abutment type and the effect of a clamping force generated by different torque values on the microgaps.

MATERIALS AND METHODS

A total of 50 implants (4.3 × 13 mm), regular platform, nobel active, Nobel Biocare USA, LLC, Yorba Linda, California, USA) with a 12° Morse taper within an internal hexagonal abutment connection, 25 zirconia (Zr) abutments (Procera Esthetic, Nobel Biocare USA, LLC, Yorba Linda, California, USA) and 25 titanium (Ti) abutments (Esthetic, Nobel Biocare USA, LLC, Yorba Linda, California, USA) were used. Three bacterial species were used to assess the microbial sealing effects: *P. gingivalis* ATCC strain 33277, *Prevotella intermedia* ATCC strain 25611, and *F. nucleatum* ATCC strain 10953. These microorganisms were chosen because they have been implicated as etiologic agents of peri-implantitis and periodontitis, and they are from different phyla, thus simulating the polymicrobial intraoral environment.[20,21] A mixture containing three bacteria in Todd–Hewitt broth supplemented with hemin and menadione (THB-HM) was prepared in the exponential, or log, phase of growth. The THB-HM media, such as brain heart infusion broth, supports the growth of these bacteria.[22,23]

One microliter of bacterial mix was placed, through an electronically controlled automated micropipette, at the apical end of 42 of the implant wells immediately after the implants were removed from their sterile packs. Twenty-one sterile Zr and 21 sterile Ti abutments were connected to the implants. Abutments were divided into test groups (n = 10) based on type and screw torque (Fig. 1). Group I consisted of Ti abutments tightened to 35 Ncm (ITi-35), which is the screw torque recommended by the manufacturer; group II consisted of Ti abutments tightened to 20 Ncm (IITi-20); group III consisted of Zr abutments tightened to 35 Ncm (IIIZr-35); and group IV consisted of Zr abutments tightened to 20 Ncm (IVZr-20). As positive controls, the two remaining inoculated implants were

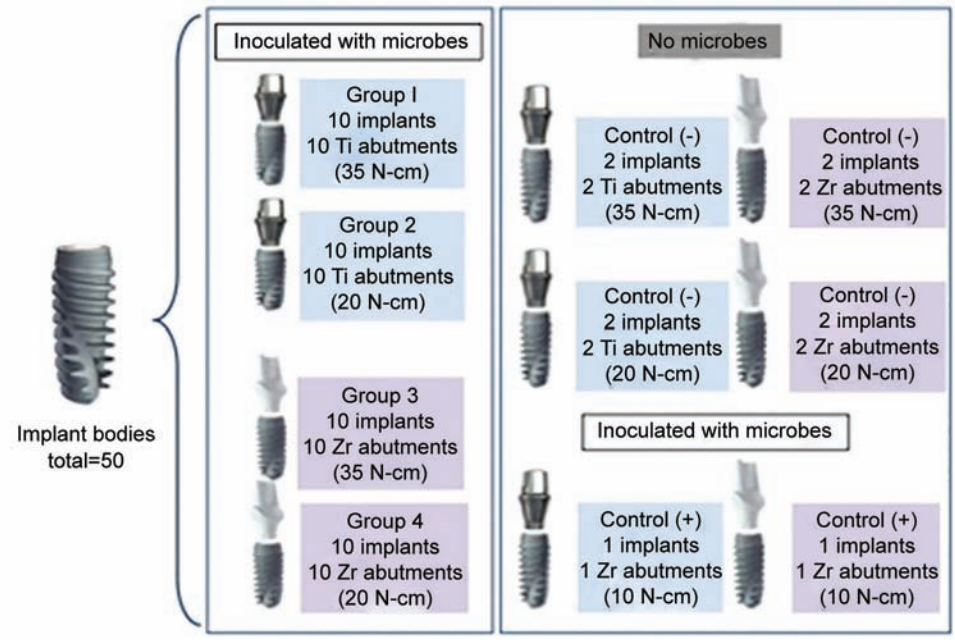


Fig. 1: Experimental and control groups

connected to a Zr and a Ti abutment and torqued to only 10 Ncm (n = 2) (Fig. 1). All abutment screw torquing was accomplished using a manual torque wrench, and counter torque was applied by holding the implant within its metal packaging. This was all accomplished under a cell culture hood to ensure a sterile environment.

After connecting the abutments, each assembly was submerged individually in 2 mL of THB-HM broth in 15-mL sterile plastic test tubes. To avoid potential microbial leakage through the occlusal access hole of the abutments, the broth was carefully measured to reach just past the implant–abutment interface (Fig. 2). The tubes were incubated in an anaerobic chamber (80% N_2, 10% H_2, and 10% CO_2) at 37°C. The positive controls were to demonstrate that the three bacteria were viable after placement into the implant wells and to show that they were, in fact, able to leak through an inadequately secured interface. As negative controls, two Ti and two Zr abutments, under each torque value tested, were connected to implants without bacterial inoculation (n = 8) and placed in tubes with sterile broth (Fig. 1). The THB-HM broth samples for the negative controls were cultured on *Brucella* H and K plates after 72 hours of incubation to exclude possible contamination.

Specimens were assessed each day following inoculation. Leakage was substantiated by turbidity of the normally clear THB-HM broth around the implant–abutment assembly (Figs 3 and 4). For each specimen that demonstrated leakage, a portion of the solution was diluted to a factor of 1/10,000; 100 µL of the diluted solution was spread onto Brucella H and K plates and incubated anaerobically at 37°C for 72 hours. Colony-forming

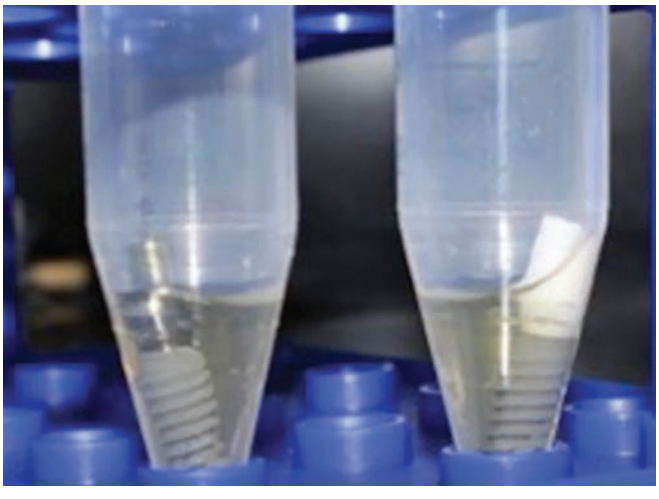


Fig. 2: Specimens assembled on day 0 of incubation

Fig. 3: Ti abutment specimens after incubation: Negative control (left) specimen showing leakage (right)

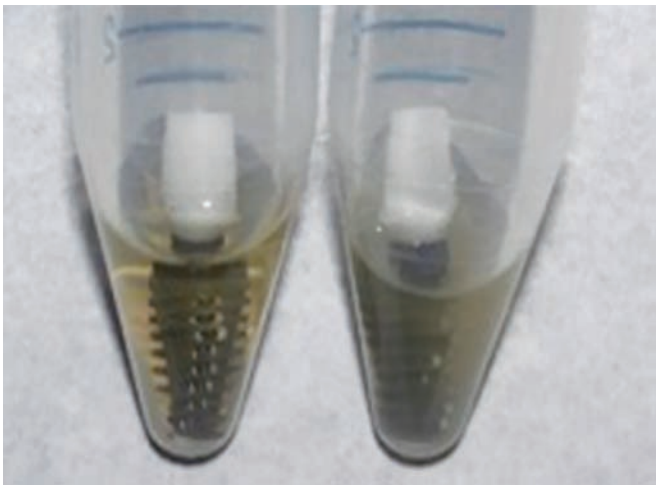


Fig. 4: Zr abutment specimens after incubation: negative control (left) specimen showing leakage (right)

Fig. 5: Implant–Ti abutment positioning for microscopic evaluation of interface, stereomicroscopic imaging (10×)

Fig. 6: Implant–Zr abutment positioning for microscopic evaluation of interface, stereomicroscopic imaging (10×)

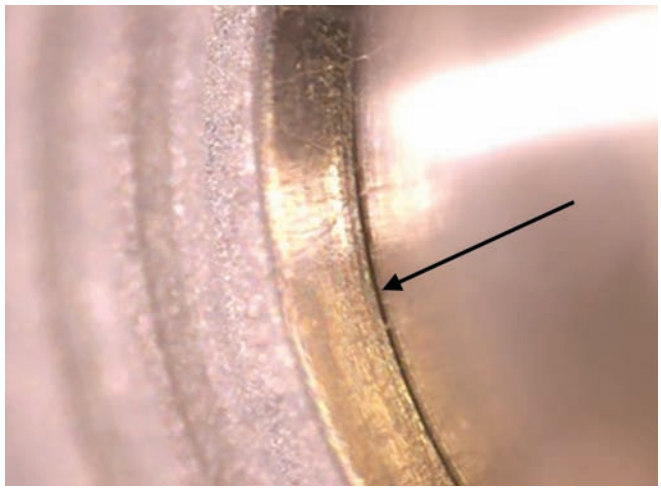


Fig. 7: Implant–Ti abutment interface (arrow), stereomicroscopic imaging (50×)

units (CFU) on each plate were then counted through an electronic colony counter (ProtoCol, Farmingdale, New York, USA). Then, based on the dilution factor, the bacteria were enumerated for each plate counted. Once a particular specimen showed leakage, there was no subsequent plating since no further information could be garnered.

After the incubation period of 14 days, all implant–abutment assemblies were rinsed extensively with sterile phosphate-buffered saline and evaluated with light microscopy at ×20 magnification (Nikon 7VL Digimicro MU-501c, Fryer Company, Huntley, Illinois, USA). Due to the emergence profile and internal connection of the abutments, each implant–abutment assembly was placed on the stage of the microscope and positioned at an angle to gain direct visualization of the implant–abutment interfaces. While looking through the microscope, the gap sizes between the implant platforms and abutments at the visible portions of the connections were measured with a digital readout system (Quadra-Chek 200, Heidenhain, Traunreut, Germany). Figures 5 to 8 show photographic examples of the visual assessment of implant–abutment interfaces obtained by a stereomicroscope (Axio Zoom. V16, Carl Zeiss MicroImaging GmbH, Jena, Germany).

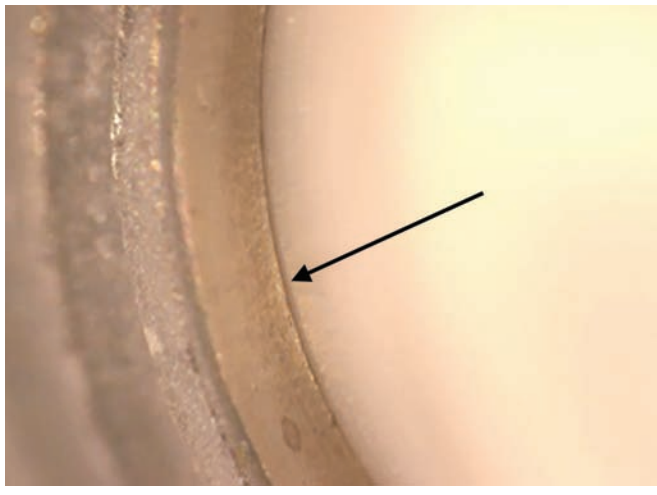


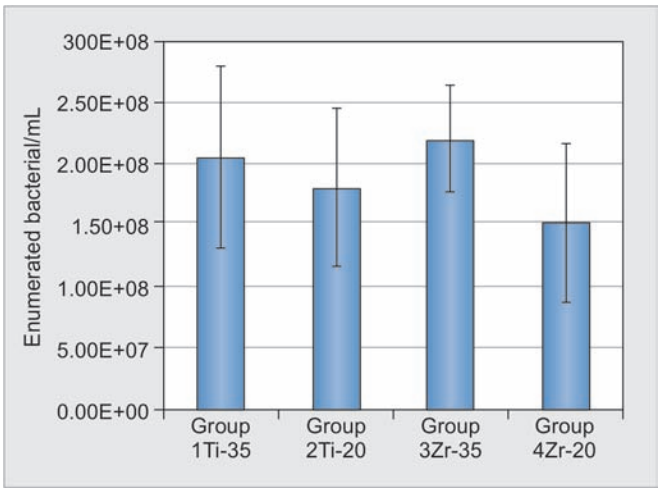
Fig. 8: Implant–Zr abutment interface (arrow), stereomicroscopic imaging (×50)

Table 1: Implant–abutment interface leakage

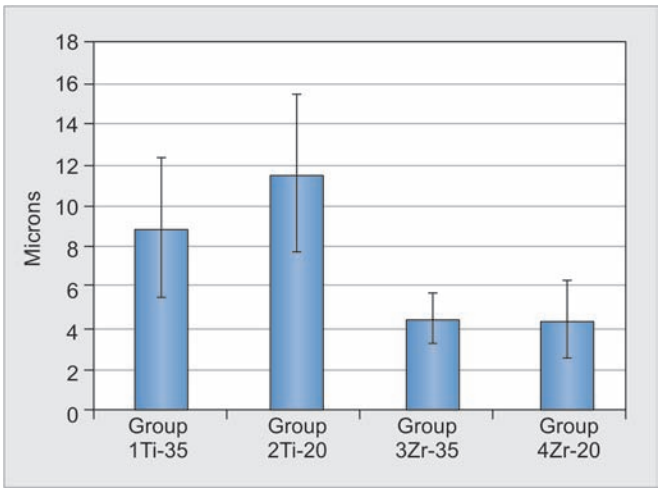
Test groups	*Implants showing leakage*	*Mean bacteria/mL*	*Range (bacteria/mL)*
Group ITi-35	9	2.05×10^8	1.09×10^8–3.27×10^8
Group IITi-20	4	1.81×10^8	1.13×10^8–2.52×10^8
Group IIIZr-35	8	2.20×10^8	1.33×10^8–2.57×10^8
Group IVZr-20	6	1.52×10^8	3.29×10^7–2.08×10^8

Table 2: Microgap size evaluation

Test groups	*Interfaces measured*	*Mean microgap (µm)*	*Microgap range (µm)*
Group ITi-35	10	8.98	3.73–13.70
Group IITi-20	10	11.59	5.20–16.40
Group IIIZr-35	10	4.43	2.53–6.40
Group IVZr-20	9	4.41	2.67–8.80



Graph 1: Microleakage assessments



Graph 2: Microgap measurements

Statistical Management of Data

The leakage evaluation consisted of comparisons of leakage amounts among the four test groups. The microgap measurements among the test groups were also compared. The amount of leakage was quantified by enumerating the bacteria following the counting of the CFU. The outcome variables of leakage and microgap were expected to be continuous and normally distributed. Therefore, these data were analyzed using analysis of variance (ANOVA), with a *post hoc* analysis as indicated.

RESULTS

Initial analysis consisted of compiling descriptive, univariate statistics on all the data to determine normality and ranges of data. Results can be found in Tables 1 and 2. Graphs 1 and 2 depict graphical methods to display the data. The data appeared to be normally distributed; therefore, a two-factor ANOVA was performed on both the bacteria count data and the microgap measurements data. There was no need for *post hoc* analysis because there were only two levels for each factor. A $p = 0.05$ was used as the level of significance for rejecting the null hypothesis.

The seal between implants with internal conical connections to prefabricated Ti and Zr abutments was evaluated in the first portion of the investigation. One implant package was empty; thus, one specimen was excluded from group IV Zr-20. Table 1 shows the mean bacteria/mL enumerated for each test group as well as the number of specimens that showed leakage. Although groups I Ti-35 and III Zr-35 showed the highest mean bacteria/mL, there was no statistically significant difference between the four groups ($p > 0.05$). Table 2 shows the mean microgaps for each test group. Groups III Zr-35 and IV Zr-20 showed significantly smaller mean microgaps ($p < 0.05$) compared with the Ti groups.

Results from the ANOVA can be found in Tables 3 and 4. There was no significant difference in mean bacteria/mL enumerated, regardless of the effect of abutment type alone ($p > 0.05$), the effect of screw torque alone ($p > 0.05$), or the interaction of the two factors ($p > 0.05$) (Table 3). The ANOVA also showed that screw torque alone had no significant effect on the mean microgap

Table 3: Effects of test factors on bacterial leakage

Source of variation	*p-value*
Abutment type	>0.05
Torque	>0.05
Interaction (torque × abutment type)	>0.05

Table 4: Effects of test factors on microgap size

Source of variation	*p-value*
Abutment type	<0.05
Torque	>0.05
Interaction (torque × abutment type)	>0.05

($p > 0.05$), as well as no significant interaction between screw torque and abutment type ($p > 0.05$) (Table 4). The effect of abutment type alone, however, was a statistically significant factor in mean microgap size ($p < 0.05$), with the zirconia abutments having smaller microgaps.

DISCUSSION

In this investigation, increasing the abutment screw torque to the manufacturer recommended 35 Ncm did not significantly influence the sealing capability or the microgaps with either Ti or Zr abutments. Therefore, the authors failed to reject the null hypothesis. One of the treatment considerations involved with placing implant-retained restorations is minimizing the bacteria that colonize the transmucosal portion of the restoration. The implant–abutment interface is of particular importance, as Broggini et al[24] reported that the peri-implant inflammatory cells associated with the implant–abutment interface resulted in a significant crestal bone loss. In a histomorphometric analysis of two-piece submerged and nonsubmerged and one-piece submerged implant systems in the mandibles of Foxhound dogs, Broggini et al[25] found significantly less inflammatory cell infiltration around the one-piece implants. Other studies showed crestal bone loss resulting from the creation of a microgap even at 1 mm coronal to the alveolar crest.[26] Hermann et al[27] reported that crestal bone loss around two-piece nonsubmerged implants was significantly greater compared with one-piece implants even when the microgap was <10 µm.

The present investigation involved implant–abutment interfaces consisting of internal conical connections. It tested the effects of abutment type and abutment screw torque on the permeability of the interface to bacteria and the effects that abutment type and screw torque had on the size of the microgap at the interface. The results from the first portion of this investigation indicated that neither the abutment type nor the abutment screw torque was significant in creating a hermetic seal at the implant–abutment interface. Visual assessment of turbidity of the media surrounding each implant–abutment assembly indicated bacterial growth resulting from microleakage through the interface from within the implants, indicating a lack of microbial seal in most of the implant–abutment connections.

In an *in vitro* study, in which cast on, castable, solid, and synocta abutments were connected to Straumann implants and submerged in a bacterial solution, Rismanchian et al[28] found no significant difference in microleakage. Although Jaworski et al[6] reported that Morse taper implant systems provided a better seal compared with external hexagonal systems with respect to the amount and time of bacterial leakage, Duarte et al[29] found that bacteria penetrated the internal aspect of implants from the outside media regardless of the internal or external hexagonal connection. The results of the present investigation agree with those reported by Jansen et al,[30] in which the three conical abutment interfaces evaluated showed evidence of leakage. In a systematic review, Schmitt et al[31] reported that although no connection is 100% effective, conical connections seem to provide a superior bacterial seal. In contrast, however, Dibart et al[9] found no bacterial penetration of the interface from within the implant or from the surrounding bacterial solution, when evaluating the seal of the 1.5° locking taper design at the interface.

In the second portion of the present investigation, there was a significantly smaller mean microgap associated with the Zr abutment groups. These results are in contrast to those found by Baldassarri et al,[32] in which three different Zr abutment systems and a Ti abutment system were custom milled, and the Ti abutment group had significantly smaller microgaps than those of all of the Zr abutment groups. The present investigation also found no significant effect of the abutment screw torque on the mean microgap size associated with either the Zr or the Ti abutments. This was in contrast to the results of Smith and Turkyilmaz,[19] in which increasing the abutment screw torque from 20 to 35 Ncm had a significant effect on the microcap seen with Zr abutments.

One limitation to the present investigation includes possible false positives in leakage assessment in the abutment groups. Deposition of bacterial solution was a highly technical process in which improperly placed bacterial solution could be displaced coronally toward the implant platforms due to liquid adhesion. However, the primary investigator (DB) performed many trial solution depositions before the actual test implants were seeded, starting with groups I Ti-35 and III Zr-35. Still, it is also possible that as specimens in groups II Ti-20 and IV Zr-20 were assembled, the assembly and inoculation became more streamlined and more of the depositions of bacterial solution were placed at the apical aspects of the implant wells, as intended, well away from the platforms. Furthermore, contamination of the implant–abutment assemblies was minimized using sterile gloves, sterile instruments, and working under a sterile hood. Lack of bacterial contamination of negative controls suggests that inadvertent contamination was unlikely.

Another limitation to this study involves the assessment of the microgap. Due to the geometry of the connection and the emergence profile on the pre-fabricated abutments, visualizing and measuring the interfaces to assess microgaps were difficult. The assemblies had to be oriented at an angle to gain direct visualization of the interface from a coronal view. Microcomputed

tomography was not able to visualize the interfaces. Hence, the best method was the use of light microscopy to help measure the interfaces with direct vision. However, those measurements were only at the most coronal aspects of the implant platforms, and there was no way to visualize the more apical connections without sectioning the specimens. As mentioned by Rismanchian et al,[28] the microgap is a three-dimensional space and the measurements obtained at the outer portion of this space cannot be generalized to the remaining portions, which may be otherwise inaccessible. Even though the findings showed that bacterial microleakage was not affected by abutment screw torque, following the manufacturer's recommended screw torque may be more critical from a biomechanical standpoint.

CONCLUSION

Within the limitations of this investigation, the following conclusions were made:

- There was no significant effect on the sealing capability of Ti or Zr abutments with internal conical connections when the manufacturer's recommendation of abutment screw torque was followed.
- There was no significant decrease in microgap within either Ti or Zr abutment groups when the manufacturer's recommendation of abutment screw torque was followed.
- There was a significantly smaller microgap associated with Zr abutments with the internal conical connection compared with Ti abutments of the same geometry, regardless of abutment screw torque.

This investigation focused on the potential for bacterial leakage through the microgap formed at the implant–abutment interface and their colonization within the internal aspects of the implant. Bacterial leakage seems to occur even when the abutment screw is tightened to the manufacturer recommended torque when using an implant system with an internal conical connection.

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