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Evaluating the microgap and sealing capability in four implant systems with different interlockings under different tightening torques: an *in-vitro* study

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PURPOSE. This study assessed the microgap width and adhesion of three bacterial species in four dental implants with different interlocks under four screwing torques. **MATERIALS AND METHODS.** Ten samples of four implant systems with various interlockings, including full-hexagonal (FHI), cylindrical-conical trilobe-index (TLI), Morse-taper with octagon terminal index (OI), and hexagonal interlock (slip-fit) (HI-SF), were used. The abutments were screwed to the fixtures under torques of 10, 20, 30, and 40 Ncm. The microgap between the abutment and the platform was assessed using a Scanning Electron Microscope (SEM). The leakage of 3 bacteria, including Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa, was evaluated under 30 Ncm torque. **RESULTS.** The TLI system showed the widest gap under all torques compared to others. There was no significance among all systems under different screwing torques. Regarding the leakage, there was no adherence to *E. coli* and *S. aureus* and 36.4% of *Ps. aeruginosa* to the HI-SF, followed by the OI system. The FHI and TLI systems showed the highest bacterial adherence. **CONCLUSION.** Even with low torque, the studied systems showed gap widths narrower than acceptable width. Implant systems with FHI and OI demonstrated misfits of less than 2 μm upon 10 Ncm and less than 1 μm when the torque increases, giving them priority to be used in areas with poor bone quality. The HI-SF demonstrated a high ability to resist the adherence to *E. coli* and *S. aureus*, followed by OI. However, *Ps. aeruginosa* demonstrated a high ability to adhere to all systems. [J Adv Prosthodont 2024;16:336-47]

KEYWORDS
Implant-abutment interface; Bacterial adhesion; *Escherichia coli*; *Staphylococcus aureus*; *Pseudomonas aeruginosa*

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INTRODUCTION

The causes of dental implant failures can be attributed to local and systemic factors. The local factors include infection, the loss of crestal bone level around the implant, the biological width, the gap at the implant-abutment interface (IAI), micromovement of the different prosthetic components, bacterial leakage, and stress factors. The systemic factors include uncontrolled diabetes mellitus, AIDS, osteoporosis, smoking, and medications such as corticosteroids and bisphosphonates.[1-7]

The stability of the IAI is an essential factor that influences load distribution to the marginal bone.[8] The microgap at the IAI could be a contributing factor in implant failure, as it can lead to other consequences, such as biological (such as peri-implant mucositis and peri-implantitis), mechanical (such as abutment screw loosening or fracture, abutment failure, and implant body fracture), and aesthetic consequences (such as veneering and framework fractures).[5,9] Several parameters, including the kind of IAI, the degree of adaptation between implant and abutment, the magnitude of applied torque, and masticatory forces, influence the stability of the implant-abutment connection (IAC).[6]

The manufacturers introduced different connection types, including external and internal connections, to provide a gapless IAI and eliminate the risk of the abovementioned complications. Anti-rotational geometries in the implant-abutment contact area, including hexagonal, octagonal, conical, butt-joint, tri-channel, spline, and Morse taper, were introduced to provide IAI with zero gaps.[10-12] Despite efforts to reduce the IAI gap to zero, inconsistencies and gaps are unavoidable, impacting the long-term stability of prosthetic components.[12] To date, no international agreement about an acceptable misfit in IAI can reduce the complications.[13,14] With no scientific evidence, a vertical misfit range from 10 to 150 μm was assumed.[15]

From a mechanical point of view, several studies have examined the microgap in implants with different materials, designs, types of connections, and magnitude of torques.[16-18] Morse taper has proven to be an appropriate connection for reducing the inner gap at the IAI, resulting in fewer mechanical and biological complications.[6,19,20] The polygonal design in internal hexagonal and octagonal abutments resulted in less micromotion than the internal conical abutment.[21] The transepithelial titanium-base abutments demonstrated lower microgap than the monolithic zirconia despite both microgaps being within the acceptable range.[22] Clinically, Morse taper was found to be less prone to prosthetic failure at an early and medium-term follow-up.[23] On the other hand, the implant system with internal connections (rather than implants with external connections) was found to be subjected to axial loads due to three causes, including machining errors, settling, and wedging effects. Besides, these systems did not show an anti-rotational concept, which determines the ideal location of abutments.[24,25] Indexes added to Morse taper abutments were found to decrease the frictional effect observed during preload.[26-28] However, indexed tapered abutments for single-crown restorations might represent greater biomechanical risk under function due to the decrease in the area of connections, which may result in gap formation.[29,30]

From a biological perspective, the microgap at the IAC can also increase bacterial adherence, leading to soft tissue inflammation, which may affect the peri-implant crestal bone level.[31] Premachined titanium abutments reduce the microgap and bacterial adherence compared to cast-on and castable abutments.[32] Implants with external hexagonal systems showed an inability to prevent bacterial microleakage in different loading conditions compared to internal hexagon implants.[9,33] Morse taper connections with conical shape exhibit more resistance to leakage than eight internal connections.[34,35] Similarly, "Tube-in-tube" interface implants were more resistant to bacterial colonization than "flat to flat" interface design.[17] Using internal index was found to have no influence on bacterial microleakage of Morse taper implants under static conditions.[34]

Depending on the literature, no IAI system was found to provide an ideal seal for bacterial adherence.[18,35] Although the conical interface designs showed microgap within the acceptable range, they did not demonstrate an absolute seal to bacterial adherence.[36-38] Varieties of bacterial species were exam-

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ined for the ability to adhere to IAC with external hexagonal designs. From 13 examined kinds of bacteria, the leakage was apparent for most bacteria.[39] This was confirmed by a recent study that showed that the bacteria [especially Pseudomonas aeruginosa (*Ps. Aeruginosa*)] could contaminate all types of connections, including hexagonal.[40]

Nowadays, there are a lot of brands that provide the dental market with internal IAI. These brands have started to use different interlockings to prevent rotation. The manufacturer's instructions focus on only one recommended tightening torque, ignoring the bone quality variation. Studies comparing interlocking in internal connection under different tightening torques regarding gap width and bacterial leakage, which may help prosthodontists choose effectively, are limited. The current study evaluated the microgap and bacterial sealing in four implant systems with different interlocking under four tightening torques. The null hypotheses were that all examined implant systems show no difference in the microgap under various tightening torques and no bacterial leakage at the IAI. The results of the study were compared to these null hypotheses.

MATERIALS AND METHODS

This study was ethically approved by the Local Committee of Bioethics, Jouf University, under reference number "4-10-44". The samples were separately calculated using the F-test [Repeated 1-way ANOVA (within-between interaction)], G*power software (3.1.9.4, University of Düsseldorf, Düsseldorf, Germany) for the microgap and bacterial seal. Introducing 5% for α-errors, 85% for β-error probability, 4 for the number of the groups, 4 for the number of measurements, and .25 for the effect size,[8] a total of 40 samples that were distributed equally into 4 groups were chosen with the actual power of .87. For bacterial leakage, 44 implants were divided into 4 groups to assess the leakage of 3 different kinds of microbiota using the same software and the same effect size,[32] using the Kruskal Wallis test for the statistical analysis, with actual power .85. The implant systems used in this study, including the connection and the recommended torque, according to the manufacturer's instructions, are shown in Table 1. The studied implants showed similar criteria, including straight abutments, conical connections, and a platform-switching concept. The main differences were in the interlocking features, including internal conical connections with full hexagonal interlock (FHI), trilobe-index (TLI), octagon terminal-index interlock (OI), and hexagonal interlock (slip-fit) (HI-SF) (Fig. 1).

The data collection was started by microgap assessment. The fixtures were removed from their plastic packaging and fixed to a sterile stainless-steel clamp. The abutments were then connected to the fixtures with a 10 Ncm torque value using a universal torque wrench (V139; Julldent, Mumbai, India). The implants were coated with a layer of gold using a Rotary Pumped Coater (Q150R S Plus; Quorum, East Sussex, UK), and the microgap was assessed using an SEM with a magnification of × 10,000 (Quattro; Thermo Fisher, Erlangen, Germany). The vertical distance of IAI was evaluated by calculating the vertical distance between two points, one on either side of the gap, using the Image J software (1.8.0; National Institute of

Table 1. Interlocking systems used in the study

Type of interlocking	Company	Recommended manufacturer torque (Ncm)
Internal conical connection with full hexagonal interlocking (FHI)	BioHorizons	10 - 30 The torque wrench snaps at the optimum torque
Cylindrical, conical internalconnection with intuitive trilobe-index (TLI)	Anthogyr Axiom	25
Morse taper conical connection with octagon terminal-index interlocking (OI)	Straumann	35
Internal conical with hexagonal interlock (slip fit) (HI-SF)	Implant Direct	30

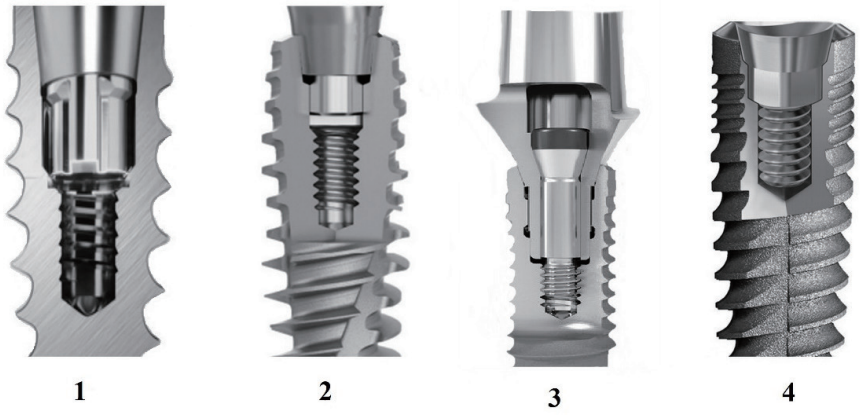

Fig. 1. The implant system used in the study, including 1: Internal conical connection with full hexagonal interlocking (FHI), 2: Cylindrical, conical internal connection with trilobe index (TLI), 3: Morse taper conical connection with octagon terminal-indexed interlocking (OI), and 4: Internal conical with hexagonal interlock (slip fit) (HI-SF).

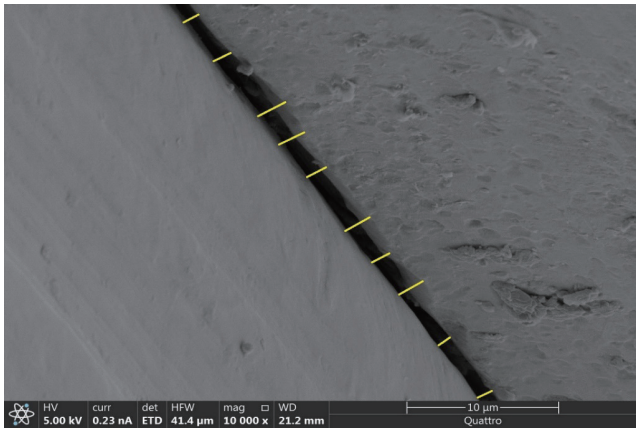

Fig. 2. Identification of the vertical gap using Image J software.

Health; Fig. 2). The same investigator conducted the measurements to reduce operator bias. Multiple measurements were done to the same implant system at different times to apply Cohen Kappa statistics for intra-rater reliability. Ten measurements were taken on the side facing the SEM, and the average of these measurements was used for each implant. After assessing the microgap in 10 Ncm, the implants were replaced in the clamp, and the torque was increased to 20, 30, and 40 Ncm, and the vertical gap was measured for each torque.

The workflow of bacterial leakage assessment is demonstrated in Fig. 3. The experiment started by culturing the assigned microbiotas, including *E. coli* (ATCC 10536; Microbiologics, Saint Cloud, MN, USA), *S. aureus* (ATCC 25923; Microbiologics, Saint Cloud, MN, USA), and *Ps. Aeruginosa* (ATCC 10145; Microbiologics, Saint Cloud, MN, USA). Using filter papers on a laboratory balance scale (TP-214; Denver instrument, Shanghai, China), 14 gm of nutrient agar was added to flasks with 500 mL of distilled water. The flasks were shaken, covered with sterile cotton, sterilized at 121°C for 15 minutes [using a vertical sterilizer (VA-341; Gemmy Industrial Corp., Taipei, Taiwan)], and then poured into Petri dishes. The examined microbiotas were plated on nutrient agar and then incubated at 37°C in a laboratory incubator (MCO19AIC (UV); SANYO, Hampton, VA, USA) for 24 hours. Following incubation, each microbiota was determined microscopically using the Gram staining method. Because *E. coli* and *Ps. Aeruginosa* are Gram-negative, they are pink, while *S. aureus* is purple because it is Gram-positive. For automated verification of the bacteria, the VITEK 2 compact (SKU: 27630; bioMerieux Inc., Cambridge, MA, USA) set was used, following the steps of previous studies.[18,41,42] Briefly, a few swaps were carefully taken from the colonies on the agar plates and placed in a polystyrene tube. The tubes were then sealed, labeled, and mixed to produce a homogeneous solution using a Vortex-Mixer (Vortex

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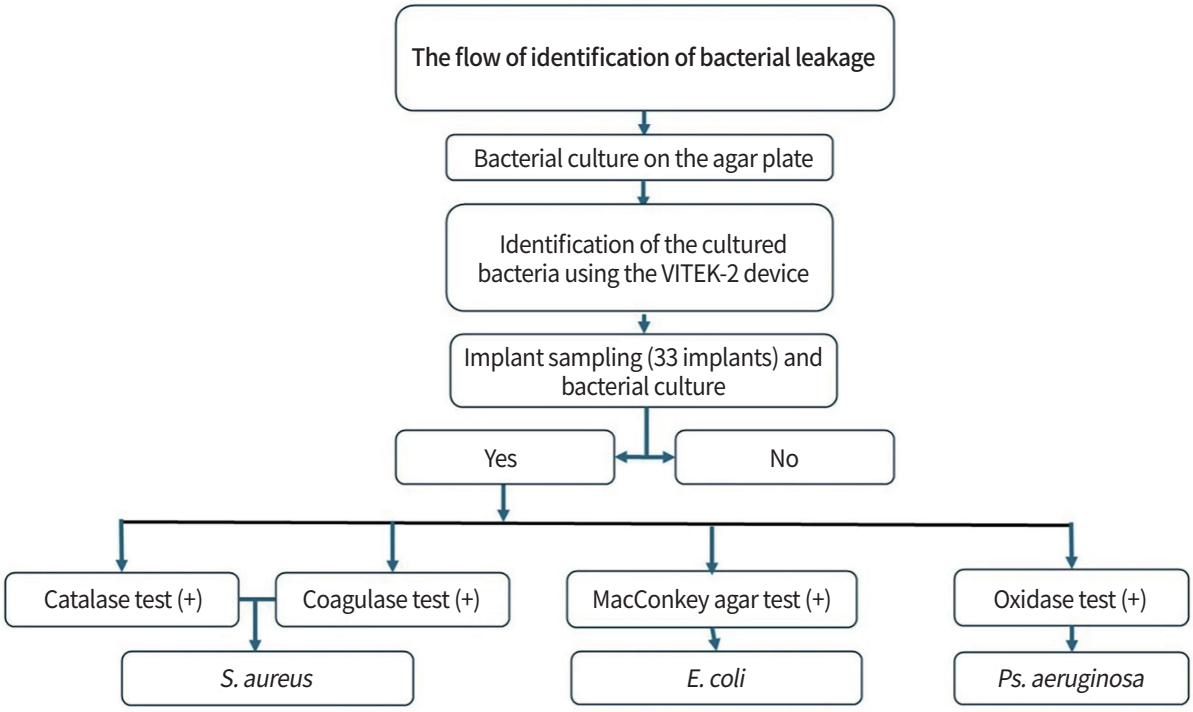

Fig. 3. Flow chart of the bacterial identification process in the study.

Genie Pulse, Bohemia, NY, USA). The tubes were then placed into a turbidimeter (DensiCHEK Plus, SKU 21255, bioMerieux, Cambridge, MA, USA) to check the turbidity and modify it by increasing or decreasing the concentration until it reached the desired McFarland Units. The desired turbidity values for *E. coli*, *S. aureus*, and *Ps. Aeruginosa* were 0.52, 0.49, and 0.56 McF, respectively (Fig. 4). The tubes were placed into the cassette case, and the VITEK-ID cards were warmed to room temperature and then placed in the cassette slot. After the preparation of the VITEK 2 device, the cassette with the cards and tubes was placed in the device and incubated at 35°C for nine hours, which resulted in the identification of the distinct microorganisms.

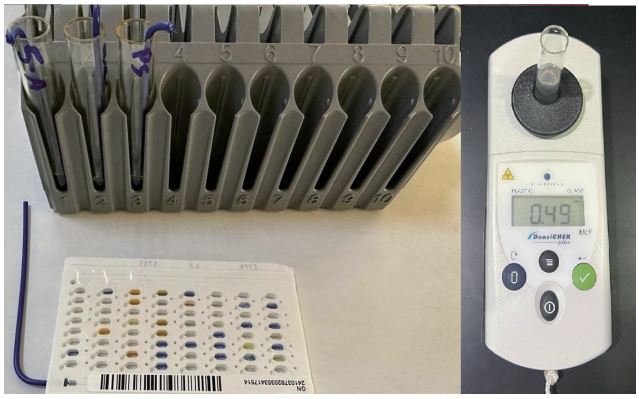

Fig. 4. Bacterial identification using the VITEK 2 Compact system and adjusting the turbidity of the solution of *S. aureus* using a turbidimeter.

For bacterial leakage assessment, the implants and abutments were tightened and then autoclaved to ensure the absence of bacterial adherence. The nutrient broth was made by adding 6.5 gm of broth powder to 500 mL of distilled water, sterilizing it at 121°C for 15 minutes, and distributing it into Eppendorf tubes (which were labeled into a test, positive control, and negative control groups). The implants were then placed in the tubes except for the positive ones, and then 200 mL of nutrient broth was added to be at a level just above the IAC. 4 mL of *E. coli* suspension was added to the tested tubes except for the negative control, and the tubes were then incubated for 48 hours at 37°C. After incubation, the fixtures were

detached from the abutments. The IAC was then rinsed with 8 μL of normal saline, and the rinse was then cultured in a nutrient agar plate and incubated at 37°C for 24 hours. The plates were then examined for the presence of bacterial growth.

Additional microbiological tests were employed, including MacConkey agar to differentiate *E. coli* bacteria from the other coliform bacteria,[43] the oxidase test to differentiate *Ps. Aeruginosa* from the other bacilliform bacteria,[43,44] and catalase, and coagulase tests to distinguish *S. aureus* from other staphylococcus bacteria.[45] The microorganisms were treated and disposed of using the Medical College, Jouf University lab protocol.

The statistical calculations were done using Statistical Package for Social Sciences (SPSS 20.0; IBM Corp, Armonk, NY, USA). The normality test was made using the Shapiro-Wilk test, a comparison between different groups was done using 1-way ANOVA, and post-hoc Tukey tests were done to identify the significant differences in the gap widths among and within the different groups. Regarding the bacterial leakage, a comparison between different groups was done using the Crosstab Chi-Square Test with statistical significance set at $P < .05$.

RESULTS

Regarding the size of the gap at IAC among the different implants, Table 2 compares the gaps between and within the assigned implant systems under different torques. Significant differences were noted among the studied implant systems for torque magnitudes. The widest gap was reported in TLI in all types of torques ($P \leq .001$). For 10 and 20 Ncm torque, the widest gap in the IAI was recorded in the TLI, followed insignificantly by the HI-SF (3.53 ± 1.01 and 16 ± 1.62 under 10 Ncm, and 2.78 ± 1.04 and 1.18 ± 0.88 μm for TLI and HI-SF respectively; Fig. 5). On the other hand, there were no significant differences among HI-SF, OI, and FHI systems ($P \geq .21$). As for 30 Ncm, the widest gap was demonstrated by TLI (2.32 ± 0.66 μm; $P \leq .001$). On the other hand, there were no significant differences among the other three implant systems ($P \geq .92$). Similar to 30 Ncm, although the OI system demonstrated the narrowest (.17 ± .31) and the TLI showed the widest gap (1.16 ± .43; $P \leq .001$), there were no significant differences between OI and the two interlocking systems ($P \geq .35$; Fig. 6).

Regarding measuring the gap width within the same systems under different tightening torques, a decrease in the gap width of all systems as the torque increased was apparent, except for the FHI system, which showed no significance in the gap under different torques ($P = .09$; Table 2). Regarding the TLI system, the gap width decreased insignificantly as the torque increased from 10 Ncm to 20 Ncm ($P = .19$). Although at 30 Ncm, the gap showed an insignificant

Table 2. The width of the gap in assigned interlocking systems under different torques

Magnitude of torque (Ncm)	Type of the implant				*P*-value$
	FHI Mean ± SD	TLI Mean ± SD	OI Mean ± SD	HI-SF Mean ± SD	
10	1.44 ± 1.63[b][w]	3.53 ± 1.01[a][w]	1.13 ± .76[b][w]	2.16 ± 1.62[ab][w]	.001$
20	.89 ± .76[b][w]	2.78 ± 1.04[a][wx]	.53 ± .47[b][wx]	1.18 ± .88[ab][wx]	.001$
30	.62 ± .29[b][w]	2.32 ± .67[a][x]	.50 ± .38[b][x]	.60 ± .25[b][x]	.001$
40	.42 ± .23[b][w]	1.16 ± .43[a][y]	.17 ± .31[b][x]	.49 ± .61[b][x]	.001$
P-value*	.09	.001*	.002*	.002*	

FHI, full hexagonal interlock; TLI, trilobe-index; OI, octagonal interlock; HI-SF hexagonal interlock with slip-fit system; SD, standard deviation
[a,b,c] Represent 1-way ANOVA, Post-hoc Tukey test among the different groups in the same torque while [a] is the highest and [c] is the lowest ($P < .05$). The variable with the same letter shows non-significant differences ($P \geq .05$).
[w,x,y] Represent 1-way ANOVA, Post-hoc Tukey test among the different torques in the same group while [w] is the highest and [y] is the lowest ($P < .05$). The variable with the same letter shows non-significant differences ($P \geq .05$).
* Sig. difference between the different magnitudes of the torque for each type of implant.
$ Sig. difference between the different types of implants for each magnitude of torque.

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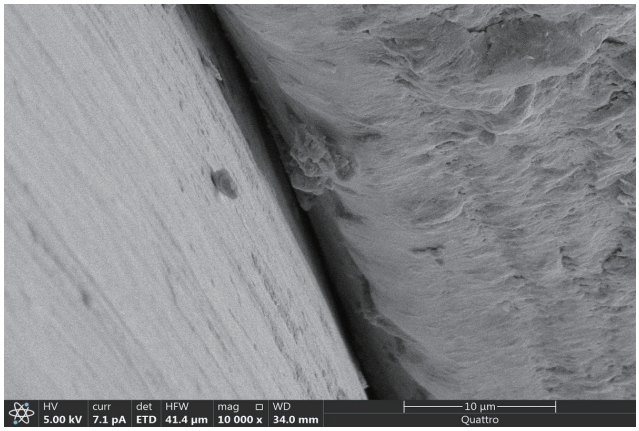

Fig. 5. Gap width in Anthogyr system for torque value of 10 Ncm.

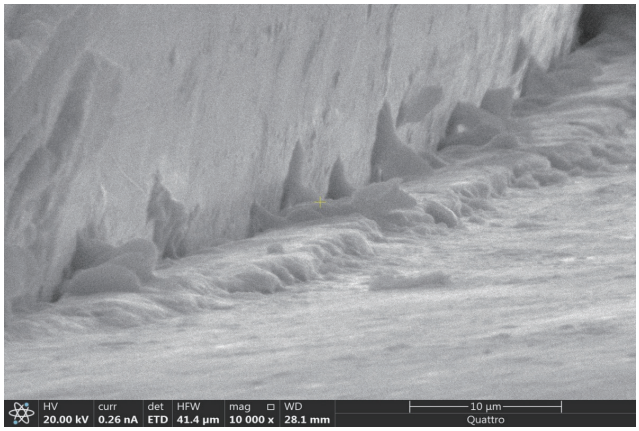

Fig. 6. Gap width in Straumann implant system for torque value of 40 Ncm.

decrease in the width (P = .61), it is significantly less than those under 10 Ncm (P = .01). Upon reaching 40 Ncm, the gap demonstrated the least width compared to the gap width under lesser torques (P = .001). As for the OI systems, the widest gap width was shown by torques of 10 Ncm (P = .002), followed insignificantly by 20 Ncm (P = .06). On the other hand, after increasing the torque more than 20 to 40 Ncm there were no significant differences in the gap widths (P = .42). The same as for OI system, the gap width in HI-SF system was the widest at 10 Ncm (P = .002), while no significant differences among other torque were apparent ($P \geq .13$).

Table 3 compares the bacteria adherence among and within the assigned implant systems. The HI-SF system showed no leakage to *E. coli* and *S. aureus* compared to the other systems. In contrast, the OI system showed the least leakage to *Ps. Aeruginosa* (P = .02), which was significant. Among the studied implant systems, the FHI and TLI systems showed the highest leakage to *Ps. Aeruginosa* and *E. coli*, and the TLI system demonstrated the highest leakage to *S. aureus*.

The FHI system showed no significance in the adherence to the tested bacteria, although it showed more adherence to *S. aureus* and *Ps. Aeruginosa*

Table 3. Percentage of bacterial adherence to the assigned interlocking systems at a torque of 30 Ncm

The bacteria		Type of the implant				*P*-value[$]
		FHI N (%)	TLI N (%)	OI N (%)	HI-SF N (%)	
E. coli	Yes	4 (36.40)	4 (36.40)	1 (9.10)	0 (0.00)	.06
	No	7 (63.60)	7 (63.60)	10 (90.90)	11 (100.00)	
S. aureus	Yes	7 (63.60)	11 (100.00)	7 (63.60)	0 (0.00)	.001[$]
	No	4 (36.40)	0 (0.00)	4 (36.40)	11 (100.00)	
Ps. aeruginosa	Yes	7 (63.60)	7 (63.60)	1 (9.10)	4 (36.40)	.02[$]
	No	4 (36.40)	4 (36.40)	10 (90.90)	7 (63.60)	
P-value*		.33	.006*	.004*	.01*	

FHI, full hexagonal interlock; TLI, trilobe-index; OI, octagonal interlock; HI-SF hexagonal interlock with slip-fit system; SD, standard deviation
Qualitative data were statistically represented in terms of numbers and percentage using the Crosstab Chi-Square Test, with significance when $P < .05$.
* Sig. difference between the different magnitudes of the torque for each type of implant.
[$] Sig. difference between the different types of implants for each magnitude of torque.

than *E. coli* (P = .33). As for the TLI system, *S. aureus* demonstrated absolute ability to adhere to the implant (100%), followed by *Ps. Aeruginosa* (63.3%), while *E. coli* showed the least adherence (P = .006). For the OI system, *S. aureus* was the highest adherent bacteria (63.6%) compared to *Ps. Aeruginosa* and *E. coli*, which showed a lack of ability to adhere to this system (P = .004). *Ps. Aeruginosa* showed 36.40% adherence to the HI-SF system but not for the other two bacteria (P = .01).

DISCUSSION

It was agreed that the variations in the implant abutment connections influence gap width and bacterial adherence.[6,17] However, to the authors' knowledge, the studies that have been delivered to compare the influences of terminal interlocking on the gap width and bacterial leakage were limited. Bone density varies in humans, depending on gender, age, health, physical activities, smoking, alcohol, and the various locations of the jaw in the same person.[46] An initial insertion torque of 30 Ncm or implant stability quotient (ISQ) value (≥ 60) was recommended as a minimal value for successful loading protocols.[47-49] However, in some clinical scenarios where the implant is inserted in low bone quality (such as posterior maxilla), the osseointegration may not fully mature, and the required torque or quotient values may not be fully achieved, the prosthodontist may feel cautious when applying the manufacturers' recommendation regarding the abutment screw tightening torques.[50] Increasing the abutment screw tightening torque beyond the bone density may result in increasing the stress on the implant surrounding bone. The stress concentrated around the IAC area can lead to marginal bone loss.[23] Lower abutment tightening torque (than loading torque) may be helpful for subjects with low bone density. Because of that, the authors used four tightening torques to check their influence on the gap width, starting from the lowest torque (10 Ncm) to the highest (40 Ncm).

Substantial variations were observed when comparing the width of the microgap among the assigned systems under the same torques and between the same implants under different torques, so the first null hypothesis was rejected. TLI system showed the widest vertical gap at torque magnitudes of all tightening torques, compared to the other interlocking system, which showed no significance at the same torque values. Although the TLI system revealed the widest gap compared to the other system, it is still within the permissible misfit (< 10 μm).[13-15] The important finding in this investigation is that the gap width in all concepts was within the limits of the misfit, even at 10 Ncm. That may release the worry of the prosthodontist regarding following the manufacturers' recommendation during abutment tightening, especially in areas with poor bone density, where the implant loaded with < 30 Ncm or ISQ < 60.[50]

When tightening of 10 and 20 Ncm was used, both FHI and OI demonstrated a gap width of less than 2 μm and 1 μm for 10 and 20 Ncm, respectively, giving them advantages over the other types of interlockings when 10 Ncm was considered. By increasing the tightening torque to 30 and 40 Ncm, FHI, OI, and HI-SF achieved a misfit of less than 1 μm. This result puts these systems first when considering a tightening torque of 30 Ncm.

Comparing the microgap in the same implants under different torque, it was apparent that the microgap decreased as the torque increased. These results were comparable to the outcomes of other studies that showed that high torque is usually associated with higher fit and intimate contact in the IAI.[15,18] Regarding implant systems with FHI and OI, the gap width demonstrated less than 1 μm at 20 Ncm or more. Making other factors constant, these results recommend using these interlocking systems with low bone quality (where the insertion torque was less than 30 Ncm or when the ISQ < 60). Similarly, the implants with HI-SF systems showed a gap width of less than 1 μm at 30 Ncm. That makes these implants adequate for use when 30 Ncm was used for implant insertion. Respecting the TLI system, the gap width was near 1 μm when the torque was highest (40 Ncm). It is recommended that this system be used with adequate bone quality. Despite varying torques, all interlocking concepts maintained a microgap of less than 10 μm, providing mechanical and biological benefits as previously reported.[13-15]

Perio-pathogenic bacteria typically measure 0.2 to

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2 μm in width and 1 to 10 μm in length.[51-53] However, bacteria and calculus have been found to fill the 1 - 5 μm space between the implant and the healing abutment in retrieved human implants.[54] The misfit in the IAI does not imply a misfit in the interior surface of the implant-abutment connection. Significant differences were also found when comparing bacterial leakage among studied systems. Thus, the second null hypothesis was also rejected. The adhesion of perio-pathogenic bacteria, including *S. aureus*, *E. coli*, and *Ps. Aeruginosa* was evaluated in the assigned systems at the tightening torques identified by the manufacturers. These bacteria were chosen mainly due to their reduced size, frequent isolation in periimplantitis, and easy preparation.[9]

The first bacterium employed was *Ps. Aeruginosa*, an opportunistic human pathogen frequently linked to implant failure. It is commonly detected at implant sites and in periodontal diseases and can build biofilms that cause peri-implantitis. This pathogen demonstrated the ability to adhere to all tested interlockings. This result was consistent with the findings that isolated *Ps. Aeruginosa* from all internal conical screwed and cemented connections,[55] and those who examined its adherence to external and internal connections.[53] That can be explained by the fact that *Ps. Aeruginosa* is characterized by a smaller size (less than 1 μm), which allows this bacteria to adhere to minor gaps at the implant-abutment connection.[40]

The second bacterium employed was *E. coli*, an opportunistic human pathogen infrequently linked to implant failure.[56] Although the abutments were linked to the implants following the manufacturer's instructions, *E. coli* leakage was observed in 36.40% of the internal connection of FHI, TLI, and, to a lesser degree, OI (9.10%). Previously, the same bacterium was found to adhere to the internal conical implants after 24 hours.[57] Conversely, no leakage was reported with the HI-SF system.

S. aureus are present within the oral cavity, and their isolation from peri-implant infection as they are frequently responsible for diseases associated with metallic biomaterials. They demonstrated an ability to adhere and colonize on the surfaces of dental implants with subsequent infections.[58] *S. aureus* adherence was observed in the FHI, TLI, and OI with varying degrees, unlike the HI-SF, which showed no adherence. These findings are consistent with those that showed bacterial leakage in most of the IAI, irrespective of the type of connections.[9] However, the slip fit of the HI-SF may provide an adequate seal to the bacterial leakage. That was consistent with the finding that the conical connections and internal hexagonal interlocking provide an adequate seal against *S. aureus*.[59,60]

The current investigation found that although the implant systems were designed with a high-precision fit, they could not entirely avoid bacterial leakage. However, the slip-fit of the HI-SF design provided an effective seal against certain germs, including *S. aureus* and *E. coli*. As a result, it has been proposed as a typical interface that can seal the IAI properly.

One of the limitations of this study is the use of SEM in assessing the gap in the implant-abutment connection from the exterior surface, although the microgap externally does not imply that the same gap occurs on the inner surface. This study includes only 3 types of bacteria, which shows another drawback. Additional time-extended studies can be performed to better understand the stability of the examined implants under masticatory efficiency and different bacteria that may impact the performance of these systems.

CONCLUSION

Depending on the findings of the current study, all the studied interlocking connections showed an acceptable misfit in the IAI (< 5 μm). However, both FHI and OI demonstrated gap widths of less than 1 μm upon 20 Ncm, prioritizing them for use in surgical areas with low bone quality. The TLI shows low misfit (around 1 μm) upon 40 Ncm, making them adequate only for use in areas with high bone quality. From a bacterial adherence point of view, the HI-SF provided a higher seal against bacterial leakage than other assigned systems. However, no interlocking system could completely seal the adherence to *Ps. aeruginosa*.

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REFERENCES

1. Kochar SP, Reche A, Paul P. The etiology and management of dental implant failure: a review. Cureus 2022;14:e30455.
2. Romanos GE, Delgado-Ruiz R, Sculean A. Concepts for prevention of complications in implant therapy. Periodontol 2000 2019;81:7-17.
3. Samara W, Moztarzadeh O, Hauer L, Babuska V. Dental implant placement in medically compromised patients: a literature review. Cureus 2024;16:e54199.
4. Monje A, Kan JY, Borgnakke W. Impact of local predisposing/precipitating factors and systemic drivers on peri-implant diseases. Clin Implant Dent Relat Res 2023;25:640-60.
5. Zheng Z, Ao X, Xie P, Jiang F, Chen W. The biological width around implant. J Prosthodont Res 2021;65:11-8.
6. Bishti S, Strub JR, Att W. Effect of the implant-abutment interface on peri-implant tissues: a systematic review. Acta Odontol Scand 2014;72:13-25.
7. Sghaireen MG, Srivastava KC, Shrivastava D, Ganji KK, Patil SR, Abuonq A, Mousa MA, Dar-Odeh N, Sghaireen GM, Kamal MA, Alam MK. A CBCT based three-dimensional assessment of mandibular posterior region for evaluating the possibility of bypassing the inferior alveolar nerve while placing dental implants. Diagnostics (Basel) 2020;10:406.
8. Gehrke SA, Shibli JA, Aramburú Junior JS, de Val JE, Calvo-Girardo JL, Dedavid BA. Effects of different torque levels on the implant-abutment interface in a conical internal connection. Braz Oral Res 2016;30:S1806-83242016000100233.
9. Mishra SK, Chowdhary R, Kumari S. Microleakage at the different implant abutment interface: a systematic review. J Clin Diagn Res 2017;11:ZE10-5.
10. Lauritano D, Moreo G, Lucchese A, Viganoni C, Limongelli L, Carinci F. The impact of implant-abutment connection on clinical outcomes and microbial colonization: a narrative review. Materials (Basel) 2020;13:1131.
11. Ceruso FM, Barnaba P, Mazzoleni S, Ottria L, Gargari M, Zuccon A, Bruno G, DI Fiore A. Implant-abutment connections on single crowns: a systematic review. Oral Implantol (Rome) 2017;10:349-53.
12. Tsuge T, Hagiwara Y, Matsumura H. Marginal fit and microgaps of implant-abutment interface with internal anti-rotation configuration. Dent Mater J 2008;27:29-34.
13. Katsoulis J, Müller P, Mericske-Stern R, Blatz MB. CAD/CAM fabrication accuracy of long- vs. short-span implant-supported FDPs. Clin Oral Implants Res 2015;26:245-9.
14. Jokstad A, Shokati B. New 3D technologies applied to assess the long-term clinical effects of misfit of the full jaw fixed prosthesis on dental implants. Clin Oral Implants Res 2015;26:1129-34.
15. Pan Y, Tsoi JKH, Lam WYH, Pow EHN. Implant framework misfit: a systematic review on assessment methods and clinical complications. Clin Implant Dent Relat Res 2021;23:244-58.
16. Kano SC, Binon PP, Curtis DA. A classification system to measure the implant-abutment microgap. Int J Oral Maxillofac Implants 2007;22:879-85.
17. Baggi L, Di Girolamo M, Mirisola C, Calcaterra R. Microbiological evaluation of bacterial and mycotic seal in implant systems with different implant-abutment interfaces and closing torque values. Implant Dent 2013;22:344-50.
18. Alshubrmi H, Mousa MA, Taher IT, Sghaireen MG, Ganji KK, Issrani R, Alzarea BK. Influences of different torques on the size of the microgap and bacterial leakage in different implant systems: an in-vitro study. Int J Prosthodont 2024;0:1-25.
19. Goiato MC, Pellizzer EP, da Silva EV, Bonatto Lda R, dos Santos DM. Is the internal connection more efficient than external connection in mechanical, biological, and esthetical point of views? a systematic review. Oral Maxillofac Surg 2015;19:229-42.
20. Vinhas AS, Aroso C, Salazar F, López-Jarana P, Ríos-Santos JV, Herrero-Climent M. Review of the mechanical behavior of different implant-abutment con-

nections. Int J Environ Res Public Health 2020;17:8685.
21. Saidin S, Abdul Kadir MR, Sulaiman E, Abu Kasim NH. Effects of different implant-abutment connections on micromotion and stress distribution: prediction of microgap formation. J Dent 2012;40:467-74.
22. Cascos R, Celemín-Viñuela A, Mory-Rubiños N, Gómez-Polo C, Ortega R, Agustín-Panadero R, Gómez-Polo M. Influence of the use of transepithelial abutments vs. titanium base abutments on microgap formation at the dental implant–abutment interface: an in vitro study. Materials 2023;16:6532.
23. Lorusso F, Alla I, Gehrke SA, Carmine MD, Tari SR, Scarano A. Effect of different dental implant prosthetic joints on marginal bone loss: emerging findings from a bayesian network meta-analysis (NMA) and systematic review. Prosthesis 2024;6:186-205.
24. Back S, Kim S, Park J, Pyo SW, Chang JS. Comparative study of axial displacement in digital and conventional implant prosthetic components. Sci Rep 2024;14:19185.
25. Kim KS, Lim YJ. Axial displacements and removal torque changes of five different implant-abutment connections under static vertical loading. Materials 2020;13:699.
26. Goyeneche VP, Cortellari GC, Rodriguez F, De Aza PN, Maria da Costa E, Scarano A, Júnior NB, Gehrke SA. Does the index in Morse taper connection affect the abutment stability? An in vitro experimental study. PLoS One 2024;19:e0298462.
27. Zhang WT, Cheng KJ, Liu YF, Wang R, Chen YF, Ding YD, Yang F, Wang LH. Effect of the prosthetic index on stress distribution in Morse taper connection implant system and peri-implant bone: a 3D finite element analysis. BMC Oral Health 2022;22:431.
28. Villarinho EA, Cervieri A, Shinkai RS, Grossi ML, Teixeira ER. The effect of a positioning index on the biomechanical stability of tapered implant-abutment connections. J Oral Implantol 2015;41:139-43.
29. de Oliveira Silva TS, Mendes Alencar SM, da Silva Valente V, de Moura CDVS. Effect of internal hexagonal index on removal torque and tensile removal force of different Morse taper connection abutments. J Prosthet Dent 2017;117:621-7.
30. Shen L, Dong C, Chen J, Bai X, Yang F, Wang L. The mechanical and clinical influences of prosthetic index structure in Morse taper implant-abutment connection: a scoping review. BMC Oral Health 2023;23:775.
31. Romanos GE, Biltucci MT, Kokaras A, Paster BJ. Bacterial composition at the implant-abutment connection under loading in vivo. Clin Implant Dent Relat Res 2016;18:138-45.
32. Rismanchian M, Hatami M, Badrian H, Khalighinejad N, Goroohi H. Evaluation of microgap size and microbial leakage in the connection area of 4 abutments with Straumann (ITI) implant. J Oral Implantol 2012;38:677-85.
33. Bittencourt ABBC, Neto CLMM, Penitente PA, Pellizzer EP, Dos Santos DM, Goiato MC. Comparison of the morse cone connection with the internal hexagon and external hexagon connections based on microleakage - review. Prague Med Rep 2021;122:181-90.
34. Resende CC, Castro CG, Pereira LM, Prudente MS, Zancopé K, Davi LR, Penatti MP, das Neves FD. Influence of the prosthetic index into morse taper implants on bacterial microleakage. Implant Dent 2015;24:547-51.
35. Canullo L, Penarrocha-Oltra D, Soldini C, Mazzocco F, Penarrocha M, Covani U. Microbiological assessment of the implant-abutment interface in different connections: cross-sectional study after 5 years of functional loading. Clin Oral Implants Res 2015;26:426-34.
36. Cavusoglu Y, Akça K, Gürbüz R, Cehreli MC. A pilot study of joint stability at the zirconium or titanium abutment/titanium implant interface. Int J Oral Maxillofac Implants 2014;29:338-43.
37. Sahin C, Ayyildiz S. Correlation between microleakage and screw loosening at implant-abutment connection. J Adv Prosthodont 2014;6:35-8.
38. Smith NA, Turkyilmaz I. Evaluation of the sealing capability of implants to titanium and zirconia abutments against Porphyromonas gingivalis, Prevotella intermedia, and Fusobacterium nucleatum under different screw torque values. J Prosthet Dent 2014;112:561-7.
39. do Nascimento C, Miani PK, Pedrazzi V, Muller K, de Albuquerque RF Jr. Bacterial leakage along the implant-abutment interface: culture and DNA Checkerboard hybridization analyses. Clin Oral Implants Res 2012;23:1168-72.
40. D'Ercole S, Dotta TC, Farani MR, Etemadi N, Iezzi G, Comuzzi L, Piattelli A, Petrini M. Bacterial microleakage at the implant-abutment interface: an in vitro

study. Bioengineering (Basel) 2022;9:277.
41. Mousa MA, Lynch E, Kielbassa AM. Denture-related stomatitis in new complete denture wearers and its association with Candida species colonization: a prospective case-series. Quintessence Int 2020;51:554-65.
42. Mousa MA, Alam MK, Ganji KK, Khader Y, Lynch E, Kielbassa AM. Prospective case series on possible effects of local factors on the development of halitosis in new complete denture wearers. Quintessence Int 2022;53:218-25.
43. Jacob ME, Keelara S, Aidara-Kane A, Matheu Alvarez JR, Fedorka-Cray PJ. Optimizing a screening protocol for potential extended-spectrum β-lactamase Escherichia coli on MacConkey agar for use in a global surveillance program. J Clin Microbiol 2020;58:e01039-19.
44. Shields P, Cathcart L. Oxidase test protocol. American Society for Microbiology 2010:1-9.
45. Fernandes Queiroga Moraes G, Cordeiro LV, de Andrade Júnior FP. Main laboratory methods used for the isolation and identification of Staphylococcus Spp. Rev Colomb Cienc Quim Farm 2021;50:5-28.
46. Krall EA, Dawson-Hughes B. Heritable and life-style determinants of bone mineral density. J Bone Miner Res 1993;8:1-9.
47. Papaspyridakos P, Chen CJ, Chuang SK, Weber HP. Implant loading protocols for edentulous patients with fixed prostheses: a systematic review and meta-analysis. Int J Oral Maxillofac Implants 2014;29 Suppl:256-70.
48. Schimmel M, Srinivasan M, Herrmann FR, Müller F. Loading protocols for implant-supported overdentures in the edentulous jaw: a systematic review and meta-analysis. Int J Oral Maxillofac Implants 2014;29 Suppl:271-86.
49. Schrott A, Riggi-Heiniger M, Maruo K, Gallucci GO. Implant loading protocols for partially edentulous patients with extended edentulous sites-a systematic review and meta-analysis. Int J Oral Maxillofac Implants 2014;29 Suppl:239-55.
50. Herbst PE, de Carvalho EB, Salatti RC, Valgas L, Tiossi R. Influence of different screw torque levels on the biomechanical behavior of tapered prosthetic abutments. Int J Oral Maxillofac Implants 2018;33:536–40.
51. Enkling N, Marder M, Bayer S, Götz W, Stoilov M, Kraus D. Soft tissue response to different abutment materials: A controlled and randomized human study using an experimental model. Clin Oral Implants Res 2022;33:667-79.
52. Nascimento C, Pita MS, Santos E de S, Monesi N, Pedrazzi V, Albuquerque Jr RF, Ribeiro RF. Microbiome of titanium and zirconia dental implants abutments. Dent Mater 2016;32:93-101.
53. D'Ercole S, Tripodi D, Ravera L, Perrotti V, Piattelli A, Iezzi G. Bacterial leakage in Morse Cone internal connection implants using different torque values: an in vitro study. Implant Dent 2014;23:175-9.
54. Scarano A, Assenza B, Piattelli M, Iezzi G, Leghissa GC, Quaranta A, Tortora P, Piattelli A. A 16-year study of the microgap between 272 human titanium implants and their abutments. J Oral Implantol 2005;31:269-75.
55. Assenza B, Tripodi D, Scarano A, Perrotti V, Piattelli A, Iezzi G, D'Ercole S. Bacterial leakage in implants with different implant-abutment connections: an in vitro study. J Periodontol 2012;83:491-7.
56. Carvalho ÉBS, Romandini M, Sadilina S, Sant'Ana ACP, Sanz M. Microbiota associated with peri-implantitis-a systematic review with meta-analyses. Clin Oral Implants Res 2023;34:1176-87.
57. Koutouzis T, Mesia R, Calderon N, Wong F, Wallet S. The effect of dynamic loading on bacterial colonization of the dental implant fixture-abutment interface: an in vitro study. J Oral Implantol 2014;40:432-7.
58. Heitz-Mayfield LJ, Lang NP. Comparative biology of chronic and aggressive periodontitis vs. peri-implantitis. Periodontol 2000 2010;53:167-81.
59. Discepoli N, Ferrari Cagidiaco E, Landini G, Pallecchi L, Garcia-Godoy F, Ferrari M. Sealing effectiveness against Staphylococcus aureus of five different implant-abutment connections. Am J Dent 2018;31:141-3.
60. Nassar HI, Abdalla MF. Bacterial leakage of different internal implant/abutment connection. Future Dent J 2015;1:1-5.