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In-vitro evaluation of the sealing capability of a novel cone-morse connection under dynamic loading

Abstract

The micro-gap between the implant and the abutment could allow pathogenic bacteria, toxins, acids, and enzymes to pass through it. The accumulation of toxic products at the implant-abutment interface could produce to peri-implant tissue inflammation. This process could lead, with time, to marginal bone loss and delayed implant failures. The literature has shown that the sealing ability of cone morse implants is correlated with the angle and length of the connection, which influence their stability during function. The purpose of this study was to evaluate the sealing capability and leakage. A total of thirty implants respectively characterized by a novel cone morse connection (CM) compared with external (EH), and internal hexagon (IH) and, under dynamic loading. 30 implants, 10 of each group, (AoN Implants, Grisignano di Zocco, Vicenza, Italy) with dimensions 3 have been investigated and immersed in a customized stub equipped with an internal liquid storage device containing toluidine blue. Following that, a Lloyd 30K universal testing device loaded each specimen in a cyclic loading mode. All the implants were screwed and tightened as suggested by Manufacturer. The connected im-plant/abutments were positioned inside the vials and filled with toluidine blue and distal water solution. Each group underwent 1 × 106 cycles loading in a Hsine shape at 4 Hz and a 30° angle. The sealing capability and leakage were quantified dichotomously, measuring the presence or absence of toluidine blue, inside the implant connection. The implants' internal chambers remained intact, proving there was no wear and tear in the connection areas. The Mann-Whitney test used for the statistical analysis showed no differences between CM and IH; on the contrary, EH showed a significantly higher infiltration (30% of the samples). Within the findings of the present in vitro investigation, the cone morse joint design seems to ensure an efficient connection joint under a simulated conditions with no evidence of components wearing and microleakage infiltration. Under a translational point of view, these advantages could support a more efficient mechanical and biological performances for a clinical application of CM implants.

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INTRODUCTION

The replacing missing or decayed teeth using dental implant restorations is a reliable treatment method that can be used for a single tooth or an entire arch (1,2). Successful oral rehabilitation using implants requires good bone integration, harmonization, and maintenance of peri-implant bone tissues (3–5).

Implantology has evolved into a predictable therapy with a high chance of success of 90% thanks to ongoing developments in implant design, operating protocols, and the creation of biologically orientated materials (6).

The fundamental drawback of two-piece medical implants is that despite the prosthetic abutment being secured to the implant with an abutment screw, a micro-gap still exists along the implant-abutment connection (7). Given the typical bacterial diameters of 0.2 to 1.5 µm in width and 1 to 10 µm in length, as well as the abovementioned micro-gap estimates, the area between the prosthodontic abutment and the dental implant serves as microbial storage (6,8).

To prevent bacterial colonization at the implant-abutment interface, the strengths have been focused on the production of antibacterial surfaces and the optimization of the sealing at the interface (9–14). The bacterial invasion that results in inflammatory cell response and pathological alterations to the tissues happens already less than an hour after surgery. Therefore, it may be beneficial to avoid the spread of bacteria during implant surgery, for instance, by using antimicrobial gels inside the implant platform or antibacterial-coated cover screws (10). Although they have already been designed, coatings for implants and abutments are still not clinically commonplace. Various surface treatments can be used to generate these surface alterations, which enable reproducible management of the necessary surface qualities on almost every area of an implant (15,16). A few examples of surface modifications to decrease bacterial colonization include titanium nitride, zirconium nitride, microwave assistant nano silver coating, doxycycline coating by cathodic polarization, silver coating by DC plasma sputter, and electrochemical surface modification of titanium by the anodic spark deposition technique (11,13,14,17,18). In particular, a nano-texture on the implant surface has been associated with lower bacterial colonization (14).

Over the years, the geometry and structure of implants have evolved, including mechanical, structural, and chemical changes, to increase the implants' stability, resistance, and longevity (19). One of the key elements controlling variations in peri-implant bone level has been identified as the implant-abutment connection (3). As a result, numerous platform designs are accessible in the market, including internal and external connections as well as cone morse (7,20).

Currently, there are two main types of implant-abutment connections: internal and external hexagon. Based on the geometric conformation of the inner part of the fixture platform, internal hexagon can be further classified in clearance-fit, conical, and combined connections (21).

The external hexagon connection was the first modern implantology system and the most common connection system for abutment and implant since the beginning of implantology (22,23). Although external hexagon implants are standard, this connection is unstable and has micromovement under horizontal load (24,25).

Internal hexagon is an upgraded and redesigned model of external hexagonal with more advantages than external hexagonal. Some of the internal hexagon design advantages are higher stability and resistance under lateral load, fewer micro-movements due to stress, and easier clinical management (23,26).

In clearance-fit connections, the internal aspect of the fixture and the abutment have parallel walls with a small gap to avoid friction between the components. To prevent the rotation of the prosthetic components, geometric features called “index” of different shapes can be added to these connections, with an anti-rotational function (21).

Morse taper or cone Morse connection is also a developed type of internal connection with higher stability, better sealing, and a better contact surface of abutment and implant (19,27). In cone morse connection, the inner part of the implant’s platform has a conical shape characterized by < 5° that create a tapered interface with the corresponding portion of the abutment, without the presence of an inner space. Also, in these connections, anti-rotational geometric features can be incorporated at the apical portion of the abutments (21).

Cone morse connection exceptional resistance to masticatory stresses due to the lack of the abutment screw and ensures the presence of an internal hexagon. This connection mechanism makes it easier to prepare abutments because they do not have a screw access hole, dramatically reducing prosthetic issues. An accurate transfer of implant position between the dental clinic and laboratory is also something that cone morse connection supports. As a result of the screw's absence, cone morse connection ensures that there are no micro-gaps, which results in a perfect microbiological seal, and no micro movements, which results in absolute stability, giving the option to place the implants subcrustal. Additionally, the presence of an internal hexagon enables quick and simple insertion of straight and angled abutments in any clinical setting, as well as simple and accurate implant placement with fewer components (28).

According to Ellakany et al. (29), depending on the type of connection used, the size of the micro-gap at the implant-abutment interface widens during pressure. Compared to static conditions, this phenomenon—referred to as the pumping effect—leaks more microorganisms. Various implant-abutment designs have been created to reduce microleakage through the micro-gap and improve the stability of prosthodontic abutments (30).

Abutment stability on the implant connections is widely influenced by mastication forces, which also can raise their connection's friction and anti-rotational stability (31). In addition, wear and degradation of implant materials can increase the magnitude of the implant-abutment connection, acting as bacterial reservoirs and affecting the implant treatment's long-term biologic effectiveness (32,33).

The microleakage between the implant and abutment allows pathogenic bacteria (predominantly anaerobic or microaerophilic species), acids, and enzymes to pass through it. This leads bacteria and toxic bacterial products to accumulate between the implant and abutment (23,34,35). The oral bacteria accumulation around the implant can cause peri-implant tissue inflammation. Therefore, adopting connections characterized by the decrease in the absence of this gap could be fundamental for avoiding bone loss at the implant/abutment interface (36,37).

A prolonged masticatory load could affect the connection, exacerbating structural deformity, possible infections in the peri-implant area, and producing malodor (33,38–42).

The shape, accuracy of the connections, and torque employed to secure the components can all affect the extent of the contamination (43–45); also, the compressive stress, tapering degree, and rigidity of connecting parts can affect how well a locking works (46). Recent years have seen a surge in interest in internal connection implants, resulting in their adoption of various implant systems and growth in the market.

The two primary internal connections are the conical and the internal hex ones, even though nearly every producer created and altered the connection style to distinguish their goods from those of rivals. However, so far, no carefully planned and executed clinical experiment has conclusively demonstrated the therapeutic superiority of any of these connections (47). It should be noted that Cone Morse connections are proven to have less abutment micromovement than external/internal hexagon systems, especially under axial and lateral loads, which is crucial for preserving the bone crest and minimizing bacterial leakage (48,49). However, bacterial permeability at the implant-abutment interface has been reported in investigations using various tapering techniques, both with (50–53) and without loading (20,43,54,55).

During the function, the implant-abutment connection is undergoing various adjustments that may result in a worsening of the sealing ability, that in cone morse connections is highly correlated with the angle and the lengths taper section of the abutment. Consequently, the evaluation of this parameter under the dynamic load could permit to achieve information more similar to the clinical behavior of the connection (56).

This work aimed to evaluate the sealing capacity of a novel cone-morse connection characterized by a 3° total taper, a positioning hexagon, a stabilizing through the screw, and 20 mm2 of the contact surface. This experimentation has been performed under dynamic load to mimic masticatory forces and turbulences that characterize the oral cavity during the function. Results have been compared with two implant connections of the same Manufacturer, an internal and an external hexagon.

MATERIALS AND METHODS

The sample size calculation has been performed, and the calculated specimens for statistical significance were 30 implants [effect size: 0.6; α err: 0.05; 1-β: 0.80; n groups: 3]. The sample size analysis has been conducted using a flexible statistical power analysis approach by the analytical package G*Power 3.1 (Heinrich-Heine-Universität Düsseldorf, Germany). A total of 30 specimens (AoN Implants, Grisignano di Zocco, Vicenza, Italy), 3.30 × 11.5 mm implants were used in this *in-vitro* study:

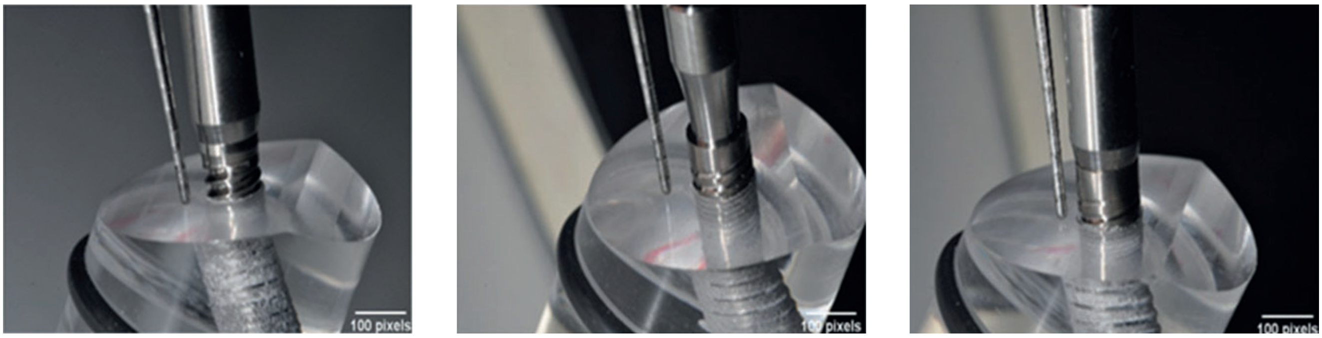


Fig. 1 Stub equipped for implant fixation and the distance of 3 mm from the platform to the implant's exposed location to simulate a critical case of bone resorption.

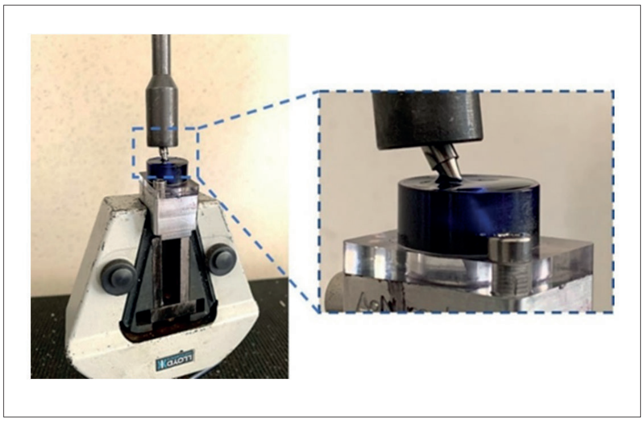


Fig. 2 Loading of samples by an electronically controlled cyclic loading machine Lloyd 30K universal testing machine.

- 10 with a screw-retained External Hexagon connection (Group EH)
- 10 with a screw-retained Internal Hexagon connection (Group IH)
- 10 with a screw-retained Conical Cone Morse REVCON (Group CM).

The manufacturer pre-assembled the implant’s prosthetic components according with the protocol. Each group underwent 1 × 10^6 loading cycles. A hemispheric loading cell was used to transmit the loading pressure to the abutment. The implant was held in a specially designed stub with an inbuilt liquid storage device. To represent a critical case of bone resorption, there was a 3 mm gap between the implant platform and the exposed position of the implant (Figure 1). The load was supplied to the abutment at a 30° angle, and the implant's exposed position was 11 mm from the hemisphere's center. Then, using the Lloyd 30K Universal Testing Equipment (Lloyd Instruments Ltd, Segensworth, UK), which controls the 20 to 100 N/cm cycle loading in a Hsine shape at 4 Hz, each sample was placed in a cyclic loading phase (Figure 2). The deepest region of the interior compartment of the three different implant sys-tems was then filled with 0.7 µl of toluidine blue using an electrically controlled auto-mated pipette. The small pipette tip allowed for simple insertion through the entire depth of all implant systems' internal threads, making this process more accessible. The abutments were attached to the implants following the manufacturer's instructions after placing the color marker within the implant connection. In 15 ml vials that had previously held 3 ml of distilled water, the attached implant abutments were put inside for 5 minutes. At the end of the experimental tests, the specimens were unloaded and the abut-ment was removed. The toluidine marker infiltration has been detected through a pa-per point inserted into the internal chamber of the implant fixture. A total of one paper point for each implant has been used for the present study. The paper point has been positioned in the internal chamber of the implant for 1 minute due to its capillarity property.

Statistical analysis

The sealing ability and leakage were quantified dichotomously, as the presence or absence of

Fig. 3 Toluidine marker infiltration of the implant connection, detected through a cone paper from the EH group.

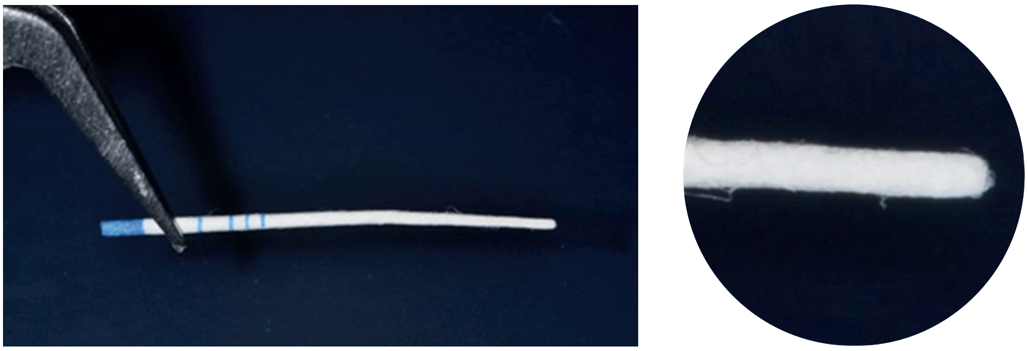
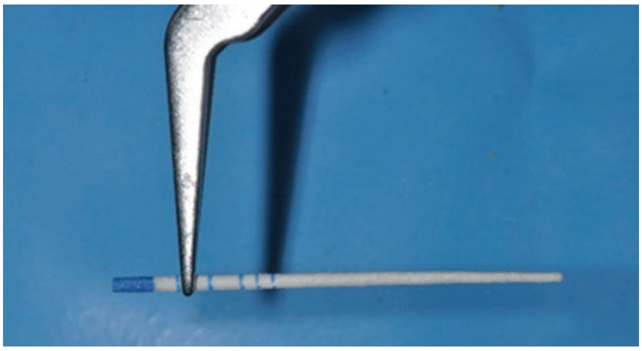


Fig. 4 Toluidine marker infiltration through a cone paper from the IH group.



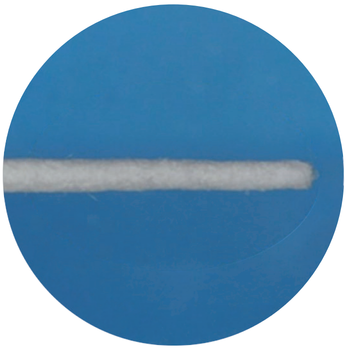


Fig. 5 Toluidine marker infiltration through a cone paper from the CM group.

toluidine blue, inside the implant connection. The study data has been col-lected in a specially designed form by Excel software package (Microsoft, Redmond, WA USA) and statistically analyzed by Graphpad 9.0 (Prism, San Diego CA USA).

The Chi-Square test was used to compare the differences in toluidine blue pres-ence or absence. Statistically significant differences were set at $p<0.05$.

RESULTS

Group EH (External Hexagon connection)

In the samples of group EH, toluidine blue was found in 3 of 10 specimens (Figure 3). The other specimens did not report any evident toluidine blue infiltrations. All ex-ternal hexagon implants showed no macroscopic signs of deformations and damage after the cyclic loading.

Group IH (Internal Hexagon connection)

The samples of group IH reported no infiltrations of toluidine blue marker (10/10 samples) (Figure 4). All internal hexagon implants showed no macroscopic signs of de-formations nor damages to the internal chambers after the cyclic loading.

Group CM (Cone Morse connection)

The specimens of group CM reported no evident infiltration of toluidine blue marker (10/10 samples) (Figure 5). All Cone Morse implants showed no macroscopic signs of deformations nor damages to the internal chambers and implant/abutment joint under the cyclic loading.

Table Analyzed	
	Chi-square
Chi-square, df	6.667, 2
p value	0.0357
p-value summary	*
One- or two-sided	NA
Statistically significant (p< 0.05)?	Yes

Tab. 1 Summary of the statistical analysis test output. [Chi-Square test; *$p<0.05$].

No differences that could be statistically significant between the IH and CM groups were found after 1×10^6 loading cycles. The group EH reported a significantly higher positivity for the toluidine leakage compared to the group CM ($p=0.0357$) and group IH ($p< p=0.0357$) (Tab.1).

DISCUSSION

The micro-gap between the implant and abutment can cause mechanical and biological problems. Micromovement is a mechanical defect that causes the loosening or fracturing of screw-retained abutments in implants (57). In addition, biological problems like the accumulation of bacteria and their products can lead to peri-implant tissue inflammation (36,52). In various studies, these issues have been investigated. Also, the micro-gap and leakage of the external hexagon, internal hexagon, and Morse taper connection have been compared in the studies.

The implant-abutment micro-gap has mainly been investigated in the literature, and many studies support the conclusions that cone morse implants are characterized by no detectable separation at the implant-abutment interface, that is characterized by an absolute congruity without any micro-gaps between the parts (58,59). Nevertheless, the investigations asserted that both IH and CM systems showed no bacterial infiltration under dynamic loading and 30-degree forces. It is good to mention that many studies have compared implant systems with different methodologies and achieved diverse outcomes. These contrasting results are due to the different features that characterized each implant system. Original cone morse connections were characterized by a taper angle 2°50 with the mathematical relation that tang 2°50=5%, but the different morse tapers on the market are characterized by different angles and contact lengths (60). Consequently, there is great variability in the performance of the different implant systems on the market.

Sahin et al. (61) used a modified fluid filtration method to compare the leakage at CM and IH implant-abutment interface. Similarly, there was no significant difference in the microleakage of both systems, for what concerning titanium implants. However, the zirconia IH systems revealed 5 times greater microleakage and torque loss than titanium ones [62]. More specifically, Naser Mostofy et al. (62) evaluated the effect of zirconia and titanium CM on the implant-abutment interfaces microleakage under oblique cyclic loading. Their samples were subjected to 75 N oblique cyclic loading at an angle of 30±2° to the longitudinal axis (500,000 cycles, 1 Hz). Like previous results, they observed more microleakage at the interfaces of zirconia abutments (62).

So, these findings proved the importance of choosing appropriate dental materials for specific applications, especially implants exposed to masticatory loads over time. Moreover, considering the outcomes in this research, the material used in the implant plays a more significant role in microleakage than their connection type.

In another research, D'Ercole et al. (54) tracked bacterial leakage of CM and IH implants for 28 days, in static conditions. They did not apply any load on the implant systems and just placed them into the bacterial suspension of *Pseudomonas aeruginosa* (PS) and *Aggregatibacter actinomycetemcomitans* (AA) species. They found that IH systems' bacterial contamination was nearly double that of CMs (54). These results have also been confirmed in another study by D'Ercole et al. (20), in which *Streptococcus Oralis* and *Pseudomonas Aeruginosa* has been inoculated in different implant platforms in static conditions. Cone morse connections showed better performances, and respect EH and IH systems. *Pseudomonas Aeruginosa* showed a higher ability to infiltrate all implants connection, with respect *Streptococcus Oralis*. It is good to mention that the experimental condition in the study was approximately like D'Ercole's research, but we exerted a dynamic cyclic loading on implant systems. The oblique force was used in the current investigation as cyclic loading to replicate the clinical circumstances of occlusal force transmission in the oral environment on the abutments. A 30° angle of force was used to imitate the occlusal loading applied to the maxillary roots and mandibular incisors (63). As a comparison, since no bacterial infiltration was found in the findings of CM nor IH, it could be concluded that cyclic load (like in vivo masticatory load) would positively affect implant-abutment sealing in IH types. The different results between static bacterial leakage and dynamic sealing evaluation are due to the other behavior of the connections during function. It has been shown that the stability of conical connection during function is related to the angle of the taper and the length of contact between the implant platform and the abutment (60).

Vertugo et al. (23) studied microleakage of abutment/implant interface compared External hexagon connection and Morse taper. They performed a tightening process and then put the samples under load cycling and thermocycling. Mechanical load by 2000 cycles of 10 k for each 0.5 s and thermal cycle for two times was performed. Also, they put the samples in water at 5° C for 5 s and 50° C for 5 s in 300 cycles. After the mechanical and thermal process, they put the samples in methylene blue at a concentration of 0.2% at 37°C for 24 h with no mechanical load. They observed that the internal connection of the Morse taper implant has a lower level of microleakage compared to the external hexagon connection. This study asserted that a bigger micro-gap leads to a higher microleakage. Also, it can be concluded that Morse tapers have better and more sealing against oral microbes with a lower level of microleakage compared to External Hexagon connection. They reported that Morse taper would be less likely to inflammation the periodontal tissues, and, due to better sealing Morse taper implants have more prevention of bacteria accumulation and present a higher rate of success in the treatments (23).

Scarano et al. (52) used toluidine blue to detect the presence of leakage between implant-abutment connections. They performed different mechanical cycling loads of 10^6, 3×10^6, and 6×10^6 on Morse taper and extra hexagonal and investigated the level of leakage. They observed that the level of leakage was directly related to the performed mechanical load. The samples loaded with 3×10^6 and 6×10^6 cycles showed more difference in leakage but the samples in 10^6 load showed no significant difference. They asserted that the Morse taper group had lower toluidine blue leakage than the external hexagon group. As a comparison, since the amount of leakage increased with the increase of cycling load on Morse taper and external hexagon, it can be concluded that in external hexagon implants, the load on the implant has a negative effect on its sealing properties and can lead to an increase in the micro-gap and leakage level. In this study, we also observed the leakage in the external hexagon after performing a circular load, which can confirm this issue.

Pereira et al. (34) evaluated the removal torque and the biofilm penetration at the implant-abutment interface of the Morse taper and external hexagon after the fatigue test. They performed a normal force of 50 N at 1.2 Hz for 500,000 cycles in a growth medium containing human salvia for 72 h. before the fatigue test, they tightened the Morse taper abutment screws by a torque of 15 Ncm and the external hexagon abutments using a torque of 32 Ncm. The gap sizes before the fatigue test were 1.7 ± 0.4 µm for Morse taper implants and 1.5 ± 0.4 µm for external hexagon implants. After the fatigue test t. The gap sizes were 3.2 ± 0.8 µm for Morse taper implants and 8.1 ± 1.7 µm for external hexagon implants. Both cases had an increase in the size of the micro-gap, but the increase in the size of the micro-gap in external hexagon samples was clearly higher than in Morse taper samples. Therefore, it can be concluded that at high loads external hexagon has less resistance than Morse taper for the increase of the micro-gap size.

Interestingly, da Silva-Neto et al. (64) compared microleakage applying toluidine blue and cyclic loading (300.000 cycles, 50 N, 1.2 Hz), between specially designed CM, EH, and IH. They discovered that microleakage generally increased under nonaxial forces in the following order: CM < EH < IH. Contrarily, comparing to groups with conical connections, Ricomini Filho et al. (65) discovered that the EH systems had a better bacterial seal. These researchers realized that the EH connection may have been a physical barrier, preventing bacterial infiltration into the implant's interior (65). Therefore, the microleakage level depends on the technique utilized for evaluation. Even studies using similar methodologies showed high variability in microleakage among the different implant systems (66). It is important to highlight that a possible variable could be represented by the characteristics of toluidine blue used for the tests, like the water contact angle, the concentration, and the solubility. Since it is not easy to maintain environmental conditions for the survival of microorganisms and aseptic conditions during cyclic loading, few studies mimic loading circumstances using bacteria (67).

In this survey, however, the connections have been subjected to a cyclic dynamic load that dissipated on the fixture-abutment complex and could cause fractures (68). The friction between the implant and abutment interface would guarantee their tight junction. Increasing the loads on the abutment during the time would cause wear and erosion in the interface, which enlarges the micro-gap in that area (69,70). Following that, liquids in the oral cavity and microorganisms would leak into the micro-gap. This phenomenon has been reported in EH and IH implants considerably more than in CM based on various research (52,70). However, the previous finding stated that the amount of load does not directly influence bacterial penetration (52).

Regarding the results, all 3 implant types were exposed to the same load, and neither was a sign of deformation nor damage in the connections interface. Therefore, there could be no considerable correlation between the cyclic load, implant types, and implant-abutment connection. Besides, findings demonstrated that the inner chambers of implants have remained untouched, which proved no wear and erosion in the junction areas. As far as the deformation of implant-abutment connection and integrity can increase the risk of micro-gap formation in many studies, this result is significant (32,42,51). Moreover, regarding marginal bone loss, the presence of micro-gap is a crucial factor (71,72). Considering the experiment condition to stimulate a critical case of bone resorption, these achievements are notable. The main advantage of a continuous cyclic loading under submerged toluidine blue study model is determined by the pump effect produced by the prosthetic connection mismatch. In addition, the present study model presents decreased exposure to standardization biases and weak points correlated to the fluid leakage determination. Due to the study design, the universal testing machine is a fully automated instruments that provide a highly standardized cyclic loading protocol in a controlled environment and the calibration protocol is not applicable for this test.

One of the limitations of this study could be represented by the sensitivity of paper points to reveal the presence of toluidine blue inside the implant platform: potentially false negatives could be detected. However, this method has been largely described in the literature, and for this reason, the results are comparable with previous works (52).

One of the vital issues mentioned in previous studies is the presence of micro-gap in the screw-retained implants, which significantly affects microorganisms' infiltration and crestal bone loss (72,73). In a comparative study between the internal hexagon and cone morse implant-abutment connections, micro-gaps were evaluated with X-ray 3D microtomography (58). Results depicted frequent gaps in the implant-abutment junction of the IH, while no measurable separation for cone morse implants was seen [59]. Moreover, the earlier research proved that cyclic loads would cause micro-gaps in external hexagon implants sooner than other cone morse (40). So, a suitable condition for bacterial penetration and inflammation would provide. A recent publication confirmed that conical implant-abutment joint is correlated to an increase of the prosthetic stability in favor of a more consistent prevention of the bacterial microleakage under the function (74). In the present study, the investigation of toluidine blue infiltration has compared the groups. While in the other two groups, internal hexagon, and cone morse, no sign of dye infiltration has been seen.

CONCLUSIONS

According to the findings in this study, cone Morse and Internal Hexagon implants tested in this study showed an extreme sealing capability and no leakage because no signs of toluidine blue presence were found inside the implant platform, after performing 1×106 cycling loading at a 30° angle in toluidine blue solution. On the contrary, the external hexagon (EX) showed infiltration in 30% of the samples. These results suggest that internal hexagon (IH) and cone Morse (CM) could guarantee greater reliability even against bacterial infiltration compared with an external hexagon (EH).

Author Contributions

Conceptualization, AP, AS, FL, LC, and MP; methodology, FL, AS.; software, FL, AS.; validation, S.D; formal analysis, FL; investigation AS, FL, FT, STR; resources, AP; data curation, TCD, NE, MRF writing—original draft preparation, TCD, NE, MRF, MP.; writing—review and editing, AP, AS, SD, LC; visualization, AP, AS, LC; supervision AP, AS, SD, LC, MP; project administration, AP funding acquisition, AP, MP, AS, SD. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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