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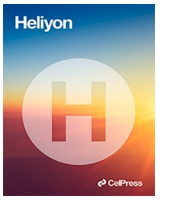

Research article

Biomechanical evaluation of abutment stability in morse taper implant connections in different times: A retrospective clinical study compared with an in vitro analysis

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ABSTRACT

Objectives: Micromotion between a dental implant and abutment can adversely affect clinical performance and compromise successful osseointegration by creating a bacterial harbor, enabling screw loosening, and imparting disruptive lateral forces on the cortical bone. Thus, the aim of the present study was to measure the abutment stability evolution using resonance frequency analysis (RFA) in vivo at four different times (baseline, 3, 4, and 12 months), and compare these data obtained with the RFA measured after mechanical cycling (in vitro) corresponding to the proposed times in numbers of cycles.

Methods: To evaluate the abutment stability, RFA was performed in 70 sets of implant/abutment (IA) with a total of 54 patients (31 women, 23 men). These IA sets were divided into three groups, according to the abutment angulation: straight abutment (Abt1 group), 17-degree angled abutment (Abt2 group), and 30-degree angled abutment (Abt3 group). Abutment stability was measured immediately at implant placement and the abutment installation (T1), 3 (T2), 4 (T3), and 12 months (T4) later. For the in vitro analysis, ten sets of each group were submitted to mechanical cycling: T1 = 0 cycles, T2 = 90,000 cycles, T3 = 120,000 cycles, and T4 = 360,000 cycles. All data collected were statistically evaluated using the GraphPad Prism 5.01 software, with the level of significance was $\alpha = 0.05$.

Results: In vivo, the overall data of implant stability quotient (ISQ) values obtained for all groups in each evaluation time were 61.5 ± 3.94 (95% CI: [60–63]) at T1, 62.8 ± 3.73 (95% CI, [61–64]) at T2, 63.4 ± 3.08 (95% CI: [61–64]) at T3, and 65.5 ± 4.33 (95% CI: [63–68]) at T4. Whereas in vitro, the ISQ were 61.5 ± 2.66 (95% CI: [59–63]) at T1, 63.2 ± 3.02 (95% CI, [61–65]) at T2, 63.9 ± 2.55 (95% CI: [62–66]) at T3, and 66.5 ± 2.97 (95% CI: [64–68]) at T4. In both evaluations (in vivo and in vitro), the data showed a significant difference (ANOVA test with $p < 0.0001$).

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Conclusions: The RFA to measure the abutment stability used in this study showed that there was a progressive increase in stability among the predetermined times for the measurements, in both analysis (in vivo and in vitro). Furthermore, the values at each time point were similar, with no statistical difference between them.

1. Introduction

The replacement of missing teeth through implant-supported rehabilitations has been a treatment option widely used today due to its high predictability and success rate. The implant osseointegration success are fundamentally depends on adequate primary [1,2]. If the implant's primary stability is not sufficient, micromovements may occur, preventing optimal healing conditions and leading to the formation of fibrous tissue and implant loss. Adequate primary stability reduces interfacial displacements of the implant and newly formed bone tissue, thus allowing bone apposition on the implant surface [2]. The instrumental methods to measure the dental implant primary stability are thought of resonance analysis frequency (RFA) method (eg., Periotest and Osstell devices), and insertion and removal torque (this in animal models).

This rehabilitation model (called a dental implant) is traditionally composed of an endosseous implant to which a transmucosal abutment is attached, forming a two-piece set, where the dental crown is fixed [3,4]. Different types of connections can be used, the most frequent being external and internal hexagon, and Morse taper. Morse taper connection systems vary in terms of the angulation of the internal cone. However, the Morse taper connection present greater contact between the internal walls of the implant and the abutment, thus presenting mayor stability of the sets when compared to hexagonal systems [5].

Regardless of the form of connection used between implant and abutment (IA), this fitting should favor load distribution and biological response, in addition to hindering the bacterial proliferation [6–8]. This IA interface has been referenced as a significant factor for the longevity of dental implant rehabilitation treatment [3,9]. Different factors related to the manufacture of implant components and their use and/or clinical and laboratory indication can contribute to a misfit of this interface [4,8,10]. This misfit (microgap), which are microscopic spaces between the implant and the prosthetic abutment, can generate micromovements that allow the passage of microorganisms and their produced fluids [3,6,9,11]. The presence of bacteria and their fluids in this area (IA interface) can generate inflammation of the peri-implant tissues and, consequently, bone loss around the implants, which may progress to *peri*-impantitis and/or even loss of the implant [4]. On the other hand, the presence of micromovements at the AI interface can, in addition to causing tissue changes, generate wear of the parts by friction, loosening of the fixation screw and fracture of the components [6,8,12].

Internal conical connections (Morse taper type) promote intimate contact between the surface of the implant and the abutment, promoting better mechanical stability of the abutment, and avoiding micromovements [13,14]. The fixation and stability of this system does not occur as a function of the screw, but by the frictional contact between the conical parts of abutment and implant, promoting greater flexural resistance of the IA interface [15]. In vitro studies analyzed the micromotion of the AI interface in different systems, showing a greater presence of micromotions and rotational motions in hexagonal abutments, compared to Morse taper connection abutments [11,16,17].

On the other hand, it is suggested that the adequate torque value may play an important role in the formation of microgaps and bacterial infiltration, being one of the main factors in determining the stability of the abutments [10]. Several in vitro studies have investigated the union of the AI interface through microtomography, scanning electron microscopy (SEM) or optical microscopy [11, 17,18]. In this sense, in a recent study published by our research group, it was demonstrated through radiographic images that Morse taper connection implants present better stability between abutment and implant when subjected to different values of loads [11]. In addition, several in vitro studies have shown that in most implant systems that feature a Morse taper connection, the microgap that initially exists after insertion and torque of the abutments to the implant is reduced with the application of loads [19,20].

To date, few studies have evaluated micromovements in AI sets in clinical situations. With the emergence of sensors (SmartPegs) for use on transmucosal pillars, the possibility was created to evaluate the behavior of these AI sets directly in patients, without causing any type of damage or discomfort. Thus, the objective of this study was to evaluate and measure the initial stability of abutments installed at the time of surgery and their behavior after different periods of time in function. However, it was necessary to carry out an in vitro test (mechanical cycling) to be able to analyze the approximate values of implant stability quotient (ISQ) evolution and compare with the in vivo obtained data. This comparison was performed to ensure that the ISQ values measured on the abutments corresponded to the abutment and not the implant.

2. Materials and methods

2.1. In vivo evaluation

This retrospective study was performed in the Bioface/PgO/UCAM clinic in Montevideo, Uruguay. As the institution is a post-graduate center, all patients who undergo any type of procedure sign a free and clarified term that all data collected during treatment can be used for research and/or teaching purposes, always respecting the agreement of the Declaration of Helsinki of 1994. Fifty-four patients, who sought the clinic to receive treatment with dental implants in an aesthetic area (premaxilla region) and who were able to receive the provisional tooth installation immediately after the implant installation, from May 2018 to April 2020, were included in the

present study. Of the total number of patients who met the conditions proposed for inclusion in the study, thirty-one were female and 23 were male, with aged between 29 and 58 years. A total of 70 sets (implant/abutment) were able to include in the present study.

All implants were installed following the conventional protocols determined for this type of immediate loading procedure. Immediately after implant placement, as a routine protocol for all patients receiving implants, the installation torque, using a manual torquemeter, and the initial stability of the implants, through resonance frequency analysis (RFA) using the Osstell device (Integration Diagnostics AB, Göteborg, Sweden), were measured. The same routine procedure (torque and stability measurement) is applied after installation of the installed prosthetic abutments. All these data are recorded in the clinical history of all patients seen, regardless of the type of treatment received with implants. As a rule of the clinic, only implants that have obtained torque values greater than or equal to 40 Ncm and, which have ISQ values greater than or equal to 70, are subjected to immediate loading.

Only patients who received Morse taper connection implants (DuoCone implant, Implacil De Bortoli, São Paulo, Brazil) and multifunctional abutments (Ideale abutment, Implacil De Bortoli, São Paulo, Brazil) both from the same brand were included in this study; patients who returned from appointments at routinely scheduled times predetermined for their treatment and subsequent evaluations; patients who received an abutment placement that was not removed during the entire treatment; and patients who had all the necessary data recorded in their clinical history. Patients who received treatment with other types of implants and abutments, and patients who missed appointments at predetermined times or who had the abutment removed for some reason were excluded from this study.

Measurements of stability by RFA were performed at the time of implant and prosthetic abutment installation (time T1), after 3 months of implant installation at the time of molding for making the definitive crown (time T2), at 4 months (time T3) at the time of installation of the definitive crown, and after 12 months (time T4) when the crowns are removed for evaluation and replacement of the fixation screw as a routine of the clinic. In all cases, for measurements of the initial implant stability, a type 16 magnetic sensor (Smartpeg™, Integration Diagnostics AB, Göteborg, Sweden) was used, and a type 25 sensor for the abutment stability (Fig. 1a). Only patients who received the Ideale abutment could be included in this study because the main feature of this abutment is the possibility of making screw-retained or cement-retained crowns, as it has internal threads in its upper part similar to a multi-unit abutment (Fig. 1b).

All selected patients received screw-retained crowns, both provisional and permanent crowns. These abutments can be straight and angled at 17 or 30° (Fig. 2), and this variable is used to determine the groups: straight abutment (Abt1 group), 17-degree angled abutment (Abt2 group), and 30-degree angled abutment (Abt3 group).

The abutment model used in each case was duly identified in the clinical history of each patient.

2.2. *In vitro evaluation*

To calculate the difference between the implant stability value and the abutment stability values in the times proposed, an in vitro evaluation was performed to compare the ISQ values obtained of the abutment submitted to different mechanical cycling corresponding with the same times used during the in vivo evaluation. Ten sets (implant and abutments) were used for each group (Abt1, Abt2, and Abt3 group), totalizing 30 sets. Morse taper connection implants (DuoCone implant, Implacil De Bortoli, São Paulo, Brazil) and multifunctional abutments (Ideale abutment, Implacil De Bortoli, São Paulo, Brazil) both from the same brand were included in this test. Firstly, all implants (4 mm in diameter and 11 mm in length) were inserted into an artificial bone block (polyurethane foam) with dimensions of 20 × 20 × 30 mm (1 block per implant). The density of polyurethane foam block at 30 pounds per cubic foot (PCF 30) to simulate high bone density (bone type I) [21], seeking to avoid possible variations in the values of insertion torque and implant stability during the application of cyclic loads. The implants were inserted at a speed of 30 rpm at the crestal bone level using a

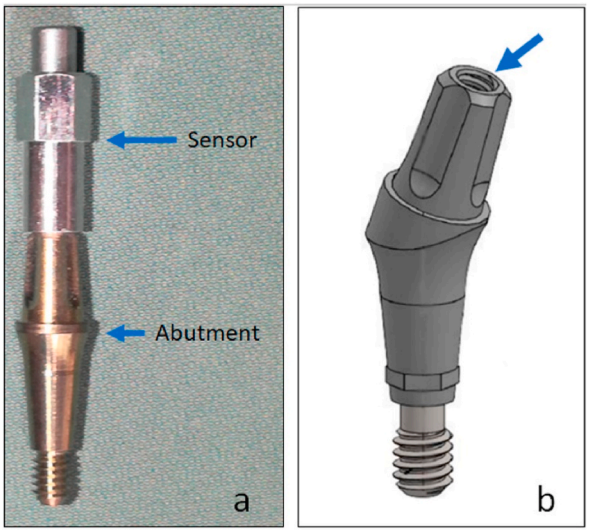

Fig. 1. (a) Schematic image of the abutment showing the portion to screw the crown; (b) Image of the magnetic sensor screwed on the abutment.

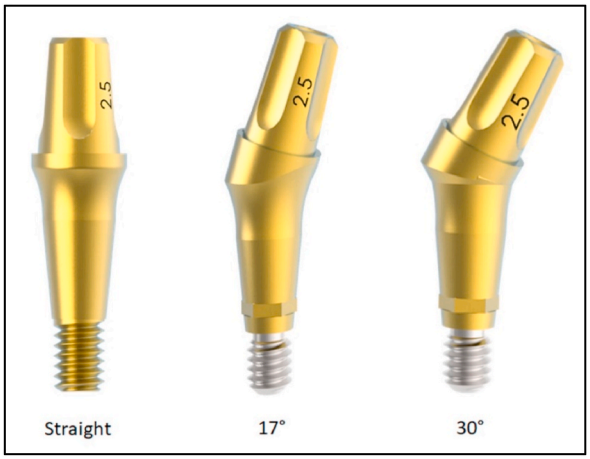

Fig. 2. Image of the 3 abutment models (straight and angled) used in this study.

computerized torque testing machine CME-30nm (Técnica Industrial Oswaldo Filizola, São Paulo, Brazil). Then, the ISQ value was measured for each implant, and the abutment was positioned, torqued and the ISQ was measured.

The samples were subjected to the mechanical cycling test using a Biocycle V2 machine (BioPDI, São Carlos, Brazil), receiving an application of load cycles with a controlled force of 150 N at 4 Hz of frequency. The measurement corresponding between times/cycles applied of abutment ISQ values were: without mechanical cycling - moment of implant and prosthetic abutment installation (time T1); 90,000 cycles – corresponding for 3 months of implant in function (time T2); 120,000 cycles – corresponding for 4 months (time T3); and, 360,000 cycles – corresponding for 12 months (time T4). This calculated quantity of cycles corresponding to the predetermined times was made considering that 360,000 cycles correspond to 12 months of masticatory function, following the standards (load applied, number of cycles, and frequency) of previous studies published [16,17]. Moreover, to apply the load cycles a metallic hemisphere was accoupled on each abutment. During the mechanical cycle, the samples were immersed in water at a controlled temperature of 37 ± 2 °C. In all groups, the load was applied in axial direction to the abutment angulation (Fig. 3).

After application of each predetermined quantity of fatigue cycles, the Smartpeg was attached to the abutment, as shown previously in Fig. 1, and the stability was measured using the Osstell device. Also, at the end of the proposed mechanical cycles, the abutments were carefully removed, and the implant stability was measured again to verify that there was no change in the initial ISQ values.

2.3. Drill sequence used

In both studies (in vivo and in vitro) the drilling sequence used followed exactly the manufacturer's recommendations for each implant diameter and length used. Fig. 4 shows an image with the sequence of drills used to install implants with diameters of 3.5 and 4.0 mm.

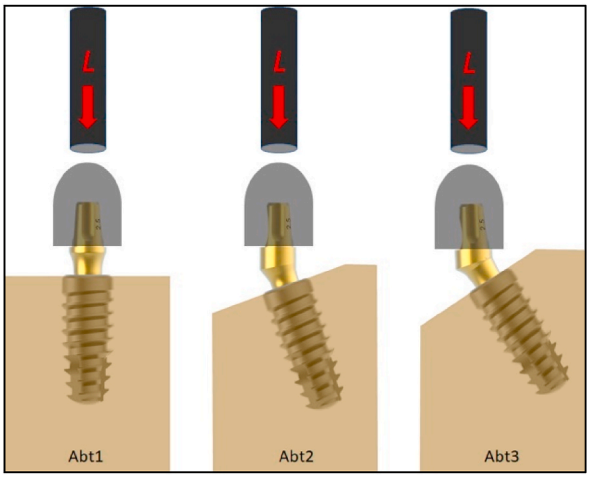

Fig. 3. Schematic image of the load (*L*) direction application during the mechanical cycling on the samples of each group.

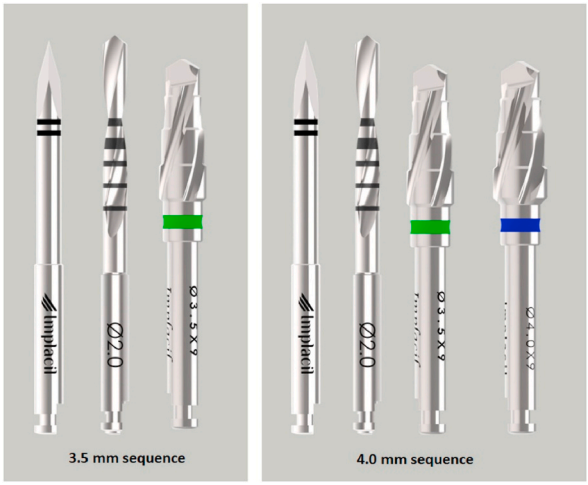

Fig. 4. Representative image showing the sequence of drills used to install implants with diameters of 3.5 and 4.0 mm.

2.4. Statistical analysis

All data collected were statistically evaluated using the GraphPad Prism 5.01 software (GraphPad Software Inc., San Diego, USA), considering in all cases the value of $p < 0.05$ as statistically significant. First, The ANOVA test was used to detect ISQ differences between all considered times (T1, T2, T3, ant T4). Furthermore, the ISQ values between the 3 different abutment models used at the same time were compared using the Bonferroni's multiple comparison test. The ISQ values obtained in vivo and in vitro of the abutment were compared at the same time using the *t*-test. Possible correlation between implant insertion torque values and ISQ values, abutment angulation and ISQ values, time and ISQ values, and in vivo and in vitro analysis were analyzed using the Pearson correlation test.

3. Results

3.1. In vivo results

Seventy IA sets of forty-four patients (31 women and 23 men, from 29 to 58-year ages) were evaluated. During the evaluation periods, all implants used for this analysis presented osseointegration. The overall average and standard deviation of insertion torque was 52.1 ± 8.12 Ncm, and for the ISQ was 74.4 ± 3.17. Regarding the insertion torque using the dimension parameters, no statistical difference was detected between both implant diameter (*t*-test: *p*-value = 0.0796), and at the 3 implants length (ANOVA test: *p*-value = 0.4186). As for the ISQ values of the implants, comparing the data between both diameters, there was a significant difference (*t*-test: *p*-value = 0.0207), and between the 3 length no difference was detected (ANOVA test: *p*-value = 0.9001). Table 1 presents the distribution of implants in terms of diameter, length, mean value of insertion torque, and ISQ values of the implant.

The number of samples analyzed in each group was: 35 abutments for the Abt1 group (straight), 26 abutments for the Abt2 group (17°), and 9 abutments for the Abt3 group (30°). Fig. 5 shows the details of implant and abutment distribution and quantity in each position.

The abutment ISQ values collected during the retrospective in vivo analysis were statistically compared between the 3 proposed groups at the same time, and no differences were observed between them (Table 2).

Table 1
Demographic implant data.

Implantation details			
Number of patients (total)	54		
Number of implants (total)	70		
Implant details	Number of implants	IT (in Ncm)	ISQ
Ø 3.5 mm	14	48.8 ± 7.70	74.0 ± 3.21
Ø 4.0 mm	56	53.0 ± 8.01	76.1 ± 2.38
L 11 mm	20	51.3 ± 8.06	74.5 ± 3.35
L 13 mm	46	52.0 ± 8.32	74.4 ± 3.19
L 15 mm	4	56.5 ± 8.02	75.3 ± 2.50

Ø: Diameter; *L*: length; ISQ: Implant stability quotient; IT: Insertion torque.

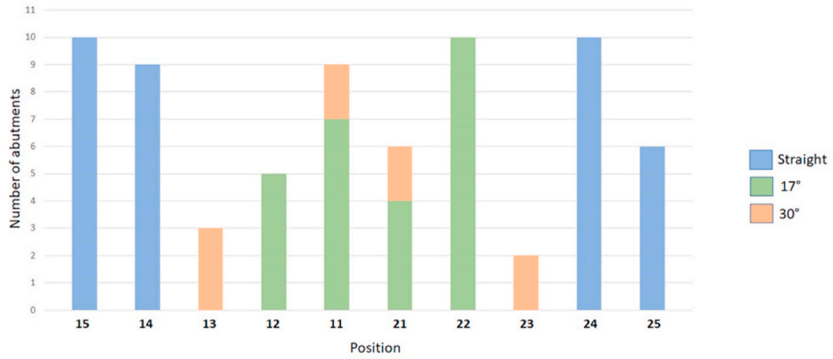

Fig. 5. Details of implant and abutment distribution in each tooth position.

Then, an overall average made between the groups was used for the comparisons. The overall data of implant stability quotient values obtained for all groups in each evaluation time were 61.5 ± 3.94 (95% CI: [60–63]) at T1, 62.8 ± 3.73 (95% CI, [61–64]) at T2, 63.4 ± 3.08 (95% CI: [61–64]) at T3, and 66.5 ± 4.33 (95% CI: [63–68]) at T4. The statistical analysis comparing these data between the 4 evaluation times showed a significant difference ($p < 0.0001$). Fig. 6 shows a box plots graph with the data distribution.

3.2. *In vitro results*

All ISQ values collected during the in vitro test were statistically compared between the 3 groups proposed at the same time, and no differences were observed between them ($p > 0.05$). Then, an overall average made between the groups was used for the comparisons and for the percent difference in relation to the implant ISQ, which are shown in Table 3.

The initial and final implant ISQ values (after mechanical cycling) remained practically the same in all samples, without statistical difference. The percent difference evolution calculated of the means between the times of the abutment ISQ value was: 2.8% for T1 to T2, 1.1% for T2 to T3, and 4.0% for T3 to T4. The statistical analysis comparing these measured values between the 4 evaluation moments (T1-T4) showed a significant difference ($p < 0.0001$) among them. Whereas, comparing the abutment ISQ values collected during the in vitro analysis, between the 3 proposed groups at the same time, and no differences were observed between them (Table 4).

Comparing the evolution between the two analyzes performed (in vivo and in vitro) at the same time, no significant differences were detected between the ISQ values. The graph in Fig. 7 shows the evolution and statistical comparisons.

3.3. *Correlation analysis*

No correlation was detected between the implant insertion torque values and ISQ values, abutment angulation and ISQ values, and time and ISQ values tested. However, between in vivo and in vitro ISQ values a strong correlation was detected ($r = 0.7093$).

4. Discussion

The technique of micromovement evaluation by resonance frequency analysis (RFA) allows the observation of implants and abutments stability in a non-invasive way and without harm to the patient, can be considered as a normal event without changing the routine practice [22,23]. Obtaining these data (micromovement of abutments) was only possible in tests performed in vitro with the use of 3D microtomography images or radiographic images [11,17,18]. This RFA technique allows the analysis of the same set (AI) at different times, that is, it is possible to evaluate the same sample at different times, allowing to evaluate its behavior and/or the changes that have occurred. Other in vitro techniques were used to verify the interface between the implant and the abutment, most of them through scanning electron microscopy and with destruction of the samples by the cuts made for these evaluations [5,10,19]. In these in vitro studies, it was shown that IA sets with conical connection present spaces (gaps) in the cervical region of the connection and contact in the most apical region before the application of loads. However, after the application of loads, these gaps disappeared along the entire length of contact between the abutment and the implant. The results obtained in the present study, by measuring the stability of the abutments installed immediately after placement of the implants and in the in vitro test, showed a similar behavior, that

Table 2
Statistical analysis of the in vivo data comparing the groups at the 4 proposal times using the Bonferroni's multiple comparison test.

Bonferroni's Multiple Comparison Test	T1	T2	T3	T4
Abt1 vs Abt2	$p = 0.0564$	$p = 0.2253$	$p = 0.1846$	$p = 0.0728$
Abt1 vs Abt3	$p = 0.7917$	$p = 0.0567$	$p = 0.0858$	$p = 0.7364$
Abt2 vs Abt3	$p = 0.3717$	$p = 0.0772$	$p = 0.1554$	$p = 0.0769$

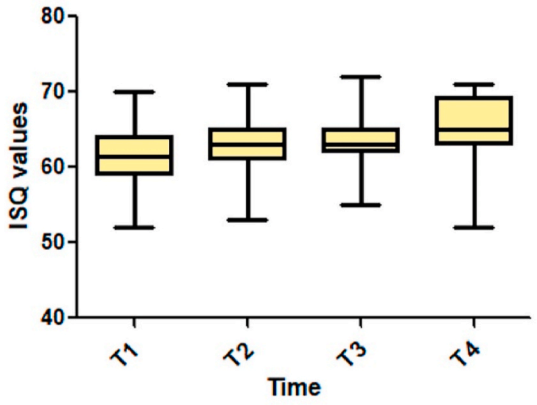

Fig. 6. A box plots graph showing the abutment ISQ mean values distribution in the four times.

Table 3
Data obtained in the in vitro analysis.

Parameter	T1	T2	T3	T4
IT	45.5 ± 2.63	–	–	–
Imp ISQ	73.9 ± 1.64	–	–	73.7 ± 1.57
Abt ISQ	61.5 ± 2.66	63.2 ± 3.02	63.9 ± 2.55	66.5 ± 2.97

Table 4
Statistical analysis of in vitro data comparing the groups at the 4 proposal times.

Bonferroni's Multiple Comparison Test	T1	T2	T3	T4
Abt1 vs Abt2	$p = 0.6149$	$p = 0.8193$	$p = 0.7894$	$p = 0.7322$
Abt1 vs Abt3	$p = 0.7894$	$p = 0.9696$	$p = 0.8475$	$p = 0.5406$
Abt2 vs Abt3	$p = 0.9695$	$p = 0.8193$	$p = 0.8161$	$p = 0.8489$

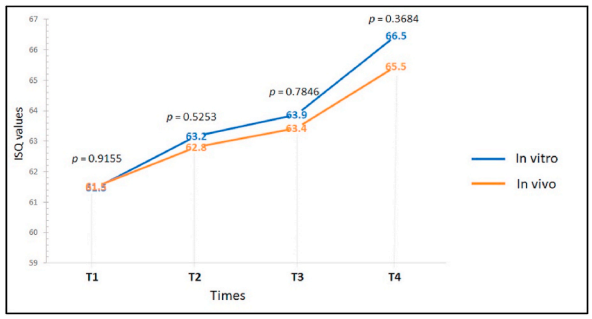

Fig. 7. Line graph comparing the ISQ evolution between the two analyzes performed (in vivo and in vitro) at the same time showing the statistical calculated values. No significant differences were detected between the ISQ values at the same time.

is, there was an increase in ISQ values measured from the value initially obtained (baseline).

Micromovements in the IA sets can produce changes to the implant surface as well as the abutment fixation screw and common interfaces [24]. These micromovements can cause loss of preload, which results in reduced contact forces between the abutment cylinder and the implant body and, consequently, loosening of the fixation screw [25]. In addition, the micromovements existing in the IA sets allow the passage of bacteria and their generated fluids, which can cause inflammation of different intensities in the peri-implant tissues [26]. For these reasons, our study sought to clinically evaluate the stability of abutments with Morse taper connection at different times, as there were no similar publications in the literature so far. With the development of the multifunctional abutment Ideale, which allows the manufacture of cemented and/or screw-retained crowns [27], it was possible to develop this study showing the behavior of this type of connection.

Regarding the 3 types of abutment angulation tested in the present study, straight and angled abutments (17 and 30°), recent

studies have shown a decrease in untorque values in indexed abutments in relation to their inclination after mechanical cycling, and the prosthetic abutments with 30° of angulation had the lowest values [28]. Similar conclusions were described in another study, which suggested that screw loosening increases with increasing abutment angulations and collar lengths after dynamic cyclic loading [29]. However, Hsu and Collaborates showed that the clinical performance of angled abutments is comparable to that of straight abutments regarding both soft tissue responses and overall survival rates [30]. However, in vitro stress/strain analysis studies of angled abutments have shown that stress/strain levels increase as abutment angulation increases. Moreover, studies comparing micromotion level between a straight abutment, a 15°–25° abutment angulation, an increase in the micromotion level by 30% was observed [29,31].

In the mechanical cycling test, where a synthetic bone was used to fix the implants, they had the same ISQ value at all predetermined measurement times, that is, unlike the clinical scenario, where the ISQ values of the implant must have undergone variations during the osseointegration of the implants, the ISQ values of the abutments showed a very similar evolution to the in vitro test. These results suggest that, in this model of tested conical connection sets, the ISQ values of the abutments were independent of the ISQ values of the implants. However, clinical studies that evaluated the evolution of ISQ in implants showed that implants with high ISQ (>70), 3 months later, the values did not show much difference [32].

Clinically, in our present study, the implant insertion torque did not show statistically significant differences regardless of the dimensions used (diameter and/or length), corroborating studies published by other authors [33,34]. As for the ISQ values evaluated according to the dimensions of the implants immediately after the implantation, only between the 2 diameters (3.5 and 4.0 mm) were statistically significant differences detected, similar to other published studies [33,34]. As demonstrated by the results, mainly the data obtained in vitro, it was evident that the stability values measured corresponded to the stability of the abutment, since as the ISQ value of the implant inserted in the synthetic bone did not change, the variation found corresponds to the abutment. Thus, as the measurements were independent, we can suggest that the size of the implant does not affect the behavior of the abutment. Although we should always be cautious with the results obtained in vitro, in the present study it was necessary to obtain this information to corroborate the data obtained clinically.

The introduction of the concept called one abutment one time has been widely used in implant dentistry due to the advantages it brings to patients in relation to possible discomfort [35]. However, studies have shown that this concept does not present differences in clinical results regarding the use of abutments that are connected and disconnected to the implant [36]. Initially, the proposition of this concept had as main objective to improve the condition of peri-implant tissues and reduce crestal bone predation around the implants, which was not confirmed in the results obtained in clinical studies [37]. In the present study, all evaluated abutments were installed and torqued at the time of implant installation, and were no longer removed, thus making it impossible to assess the possible effects generated by the maneuver of connecting and disconnecting the abutment. As previously described, the results showed an important evolution in the stability measured by RFA over time, which would probably be different if the abutments were disconnected during the evaluation period. However, data from situations in which the abutments had to be removed for some reason were not included, which could be a good topic for future studies.

As limitations of the present study, we can mention the lack of comparison of the tested abutment with other types of abutments and other models of connections between abutment and implant [38]. In addition, the difference in the homogeneous number of abutments models analyzed in the in vivo study, a fact that is inherent to the will of the researchers.

5. Conclusions

The use of resonance frequency analysis to measure the stability of the abutments used in this study showed that there was a progressive increase in stability between the predetermined times for the measurements. Furthermore, the results showed that with the application of loads on the abutments, the union between abutment and implant is increased in this type of system tested. A strong correlation was found between the ISQ values of the abutments obtained in vitro and in vivo.

Institutional Review Board Statement: The present clinical study was carried out at the Postgraduate Center of Bioface/PgO/UCAM (Uruguay), in full compliance with ethical principles, including the World Medical Association Declaration of Helsinki and the additional requirements of Uruguayan law. The ethics committee responsible for the evaluation of studies involving patients classified the study as exempt from ethical review, as all procedures performed were routine, and involve the use of existing data that contain only non-identifiable data about human beings. The patients signed a written informed consent form.

Informed Consent Statement: All selected participants were informed about the conditions and the type of study that would be carried out, and they completed and signed the informed consent according to the agreement of the Declaration of Helsinki of 1994.

Author contribution statement

Sergio Gehrke: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Guillermo Castro Cortellari: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Piedad N. De Aza & José Henrique Cavalcanti de Lima: Conceived and designed the experiments; Wrote the paper.

Juan Carlos Prados Frutos: Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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