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MEDICINA DENTÁRIA

Assessment of the microbial infiltration at the implant-connection interface in a platform-switching implant system

Pedro Ferrás da Silva Fernandes

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2021

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FACULDADE DE MEDICINA DENTÁRIA

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Thesis presented at the Faculty of Dental Medicine, U. Porto (FMDUP) for the award of the Doctoral Degree in Dental Medicine.

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ACKNOWLEDGEMENTS

To Professor Doutor Sampaio Fernandes, full professor of Faculty of Dental Medicine, U. Porto, who always encouraged and supported me along this path, permanently demonstrating his availability and friendship. I am grateful for his inestimable guidance since I finished my graduation.

To Prof. Doutor Pedro de Sousa Gomes, associate professor of Faculty of Dental Medicine, U. Porto, I would like to state my truly appreciation for the opportunity and support to develop this work. His invaluable scientific guidance and knowledge greatly contributed to the improvement of this work.

To Professora Doutora Helena Fernandes, full professor of Faculty of Dental Medicine, U. Porto, for providing me with the necessary conditions for the laboratory assays of this study, in the BoneLab laboratory that she superiorly directs.

To Prof. Doutora Liliana Grenho, assistant professor of Faculty of Dental Medicine, U. Porto, for the constant availability and invaluable assistance in carrying out the experimental studies.

To Prof. Doutora Andreia Oliveira, assistant professor of Faculty of Medicine, U. Porto, for her help with the statistical analysis.

To D. Emilia Soares, technical assistant of the Department of Mechanical Engineering, FEUP, for her kindness and help in sample preparation.

To my Mother, for the constant encouragement from the beginning to the end of this journey. Her continuous words of support were essential to the completion of this work.

To Sara, my love, who supported and comforted me in the most difficult moments. Thank you for all the valuable advises, love and patience revealed every day.

RESUMO

O *microgap* e a microinfiltração bacteriana na interface implante-pilar protético são preocupações reconhecidas nas restaurações implantossuportadas, pois podem originar inflamação dos tecidos peri-implantares, com consequências deletérias nos níveis ósseos. De relevância adicional, pouco ainda se sabe a respeito da interface estabelecida entre o implante e o pilar de cicatrização ou parafuso de cobertura colocado durante a fase de osteointegração, particularmente em sistemas de implantes com plataforma discrepante.

Nesse sentido, este estudo teve como objetivos: 1) Caracterizar as interfaces implante-parafuso de cobertura e implante-pilar de cicatrização de um sistema de implantes com plataforma discrepante e conexão híbrida, para determinar a microinfiltração bacteriana e a microinfiltração do sistema, avaliando ainda a resposta biológica e a funcionalidade de um agente selante da interface; e 2) Caracterizar o *microgap* e a microinfiltração bacteriana da interface implante-pilar de um sistema com plataforma discrepante e conexão híbrida, aparafusada com valores de torque distintos (ou seja, 25 Ncm, torque recomendado pelo fabricante; e 5 Ncm, um torque reduzido mimetizando uma utilização funcional a longo prazo), na ausência ou presença de um agente selante à base de silicone.

As interfaces implante-pilar de cicatrização e implante-parafuso de cobertura do sistema testado, apesar do baixo *microgap* relatado, permitiram a microinfiltração bacteriana após a colonização interna, particularmente em pontos de tempo iniciais, em ambas as interfaces testadas. Em relação à interface implante-pilar, também foi verificado um *microgap* baixo, independente do torque aplicado. No entanto, enquanto nenhuma microinfiltração bacteriana foi observada no grupo aparafusado com torque de 25 Ncm, foi verificado um aumento da infiltração no grupo dos 5 Ncm. O uso de um selante à base de silicone, com citocompatibilidade e potencial de irritação adequados, foi eficaz na prevenção da infiltração bacteriana nos ambientes experimentais ensaiados.

O sistema de implantes com plataforma discrepante e conexão híbrida que foi testado apresentou um desajuste baixo entre os componentes e uma capacidade de selamento efetiva quando se aplica o torque recomendado pelo fabricante. Apesar do aumento da microinfiltração em condições de baixo torque, a utilização de um agente selante citocompatível e à base de silicone restaurou a eficácia do selamento do sistema.

SUMMARY

Microgap and bacterial microleakage at the implant-prosthetic abutment interface are recognized concerns for implant-supported restorations, leading to inflammation of the peri-implant tissues, with deleterious consequences for crestal bone levels. Of additional relevance, little is still known regarding the interface established between the implant and the healing abutment or cover screw placed for the osseointegration phase, particularly in platform-switched implant systems.

Accordingly, this study aimed to: 1) Characterize the implant-cover screw and implant-healing abutment interfaces of a platform-switched implant system with hybrid connection, to determine the microgap and bacterial microleakage of the system, further evaluating the biological response and functionality of an interface sealing agent; and 2) Characterize the microgap and bacterial microleakage of the implant-abutment interface of a platform-switched system with hybrid connection, screwed at distinct torque values (i.e., 25 Ncm, the manufacturer recommended torque; and 5 Ncm, a reduced torque mimicking the long-term functional use), in the absence or presence of a silicon-based sealing agent.

The implant-healing abutment and implant-cover screw interfaces of the tested system, despite the reported low microgap, allowed for bacterial microleakage after internal colonization, particularly at early time points, at both the assayed interfaces. Regarding the implant-abutment interface, a low microgap was also verified, regardless the applied torque. Notwithstanding, while no bacterial microleakage was reported at 25 Ncm torque, an increased leakage was verified with the 5 Ncm. The use of a silicone-based sealer, with adequate cytocompatibility and irritation potential, was effective on preventing bacterial leakage in the assayed experimental settings.

The assayed platform-switched implant system with hybrid connection presented a low interfacial misfit and an effective sealing capability at manufacturer recommended torque. Despite the increased microleakage at low torque conditions, the application of a cytocompatible silicon-based sealing agent restored the sealing effectiveness of the system.

List of Abbreviations

IAI - Implant-abutment interface

Ncm - Newton centimeter

RTV - Removal torque value

SEM - Scanning electron microscopy

BHI - Brain heart infusion

CFU - Colony-forming units

ATCC - American type culture collection

ISO - International organization for standardization

CPD - Critical point dried

DMSO - Dimethyl sulfoxide

PI - Propidium iodide

SPSS - Statistical package for the social sciences

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CHAPTER 1. General Introduction

The first discovery related to Implantology occurred in 1890 and consisted of a fragment of the jaw of a woman of Mayan origin, thought to date back to 600 AC, with pieces of shell shaped like teeth. In other civilizations, the Phoenicians and the Egyptians used animal teeth as intraosseous implants.[1]

From that time to the present day, Implantology has continuously evolved and what began as an empirical act of replacing a dental piece is, currently, a highly differentiated treatment in Dentistry.

A dental implant is the artificial replacement of a tooth root that is used to support prosthetic restorations that resemble teeth. Nowadays, partially or totally edentulous patients have adequate therapeutic options for replacing the missing teeth by means of implant-supported restorations.[2, 3]

Dental implant-based treatments are a predictable method of oral rehabilitation,[4] as well as implant-supported dentures are an accepted and established therapeutic method[5] that became a successful dentistry procedure for replacing missing teeth.[6] Implants can maintain natural appearance,[7] restore function[8] to fully or partially edentulous patients, and have a good durability,[9] improving the quality of life of many patients.[10] The constant development and research around dental implants allowed clinicians to provide better quality of patient care, achieving complete integration between hard and soft peri-implant tissues.[11]

Oral rehabilitation with osseointegrated implants is currently a routine treatment due to the predictability of implant survival and the preservation of the dental structure of adjacent teeth.[12-14] Osseointegration is highly reliable and with long term results,[15] and has been considered fundamental to the success of the implants.[16, 17] It is required an adequate quantity and quality of bone, and this peri-implant bone has an important role on the longevity of implant supported restorations.[18]

Implant success rates tend to be lower than survival rates and vary greatly depending on the criteria used to assess success.[14] However, there is a consensus of opinion that implants have a success rate of around 95%,[19] a 5-year survival rate of 90 to 98% and a 10-year survival rate of 89 to 95%.[14]

Despite being an excellent treatment option with high success rate, the literature reveals some possible complications, failures and limitations.[20, 21] The most common technical failures are related to screw loosening and fracturing, and micromovement at the implant-abutment connection.[22-25] Regarding its survival and success rates, crestal bone resorption plays an important role and is influenced by biological width establishment,[26] implant-abutment interface (IAI) colonization,[27] peri-implantitis,[28] and overloading.[29]

One major complication of implant-supported restorations that can reduce its longevity is peri-implantitis that is associated with a higher inflammatory cell infiltration and bone loss.[30] It is believed that it can be caused by bacterial leakage at the level of IAI, allowing bacteria to move from the interior of the implant to the peri-implant tissues and vice-versa[31] - bidirectional leakage at the IAI.[32] This is possible due to the existent gap at this interface, also defined as microgap, creating the possibility for the microorganisms to colonize and thrive into the reservoirs inside of the implants.[33] Following, microbial agents can migrate to the peri-implant tissue and cause inflammation and bone loss.[5] The cyclical loading established over the implants through time seems to affect not only the connection between the implant and the abutment, but also the implant-bone contact[5] possibly resulting in loosening or fracture of the screws.[34] Inevitably, the implant and the abutment become less tightened, leading to the increase of the gap in the interface between them, which may allow for fluid infiltration into, and from, the interior of the implant.[35]

The long-term success in implant-supported rehabilitations depends on the maintenance of the peri-implant bone levels. As exposed before, the strategic condition that can potentially modulate this milieu is the IAI, because of the biological and mechanical complications.[20] Regarding this connection, and despite all industry efforts, a perfect connection system is still missing, ultimately leading to a progressive marginal bone loss. Moreover, the direct relationship between the latter and the connection system is not completely clarified, as the bacterial leakage along the implant abutment interface may affect the long-term success of implant-supported restorations due to peri-implant tissues inflammation.[6]

Today, there is a wide variety of implant and abutment geometries and connection systems that were developed in an effort to mitigate the reported complications, as every two-piece implant systems report the need to avoid potential inflammation at the peri-implantar tissues.

As there is not a connection system that totally seals the gap, precluding bacterial contamination of microorganisms through the IAI,[36, 37] the inner portion of the implants can be colonized by bacteria[38] and become a reservoir, in which microbial agents can produce and maintain the inflammation at the peri-implantar tissues.[39]

CHAPTER 2. Literature Review

In 1952, Implantology underwent a major change. In that year, the Swedish Professor Per Ingvar Bränemark, while carrying out animal research on microcirculation in bone repair mechanisms, discovered that it was possible to establish a direct anchorage between the bone and a piece of titanium, provided that certain guidelines were followed.[40, 41]

He carried out several other studies on this phenomenon, using both animal and human models, all of which confirmed this unique property of titanium.

The possibility of bone growth in direct contact with a titanium implant was first published in 1969.[40]

Bränemark *et al*. then defined the term osseointegration – "direct, structural and functional connection between the organized living bone and the surface of a titanium implant, capable of supporting functional loads."[41-43] Through osseointegration, a strong connection can be created between a titanium implant and the bone.[10, 40, 44] The osseointegration mechanism of dental implants is very similar to primary bone healing. First, the inflammatory response promoted by surgical trauma causes circulatory changes and hematoma. Afterwards, regeneration occurs, and the surgical wound is replaced by bone tissue in a remodeling process that leads to the wound maturation. The newly formed bone penetrates the implant, matures and increases in density and hardness.[45] It is then understood that implant osseointegration depends on several factors, such as a correct tissue repair mechanism and an adequate immune response.[46]

Since osseointegration has become a basis for a predictable outcome of implant-based treatments, this rehabilitative option became the method of choice for restoring missing teeth.[44, 47, 48] Implantology presents predictable, reproducible and stable results over time, with success levels close to 90%, when considering all types of treatment with implants.[49]

Regarding soft tissue around the abutments, they should seal and act as a functional barrier between the oral environment and the bone. Initially a coagulum is formed, then shifts to granulation tissue, and afterwards occurs the formation of a barrier epithelium and the maturation of the connective tissue.[50] This mechanic defense is somewhat responsible for the long-term stability of bone height.[10] Albrektsson *et al.*[51] through electronic microscope analysis, and Bränemark *et al.*[44] corroborated the idea of a direct attachment between gingiva and a titanium abutment.

Titanium was first chosen to be the material of the implants because of its mechanical properties,[44] and also because of its low toxicity and resistance to corrosive forces.[51] Dental implants, regarding the method of placement, can be divided in: submerged or two-stage surgery, and non-submerged or one-stage surgery.[52] Both procedures can result in a successful

osseointegration if correctly executed.[53] In the two-stage surgery, the implant is placed under the gingiva for 3 to 6 months, after which a healing abutment is screwed on.[40] With this strategy, loading and exposure to microbiological colonization are absent.[32] After the period of healing - osseointegration phase - a second surgery is performed to uncover the implant and install a healing abutment, to allow for soft tissue healing.[54] After this procedure, and as soon as the prosthetic abutment is screwed, a microgap is formed at the IAI, in a critical anatomical location that is near or below the bone crest.[55]

In a one-stage procedure, or non-submerged, the top of the implant is either within or coronally located in relation to the soft tissue.[54] Nowadays, the healing abutment is placed at the same time as the implant, except in specific cases like the inability to have stability of the implant, or in cases of a concomitant procedure of bone regeneration.[52] In the cases in which implant primary stability is low, clinicians should opt for a two-stage surgery, letting the implant osseointegrate completely in the absence of load, protected by the soft tissue.[52]

Back in 1981, Adell *et al*.[10] stated that during healing period - implant with cover screw - and after abutment connection, a marginal bone loss should be expected. This process would be more extensive in the upper jaw during the healing period, and more intense in the lower jaw, after the placement of the abutment – probably associated with the remodeling capacity of the two bones. They also showed that the accepted bone loss in the first year upon implant placement was 1.5 mm and the annual bone loss after the first year was around 0.1 mm per year, estimating a good long-term prognosis after 1 year.[10]

The concern with defining the success of implants dates back to 1986, when Albrektsson *et al*.[56] created some parameters, which are still accepted today, and which must be observed for an implant to be considered successful:

• An individual free implant must be immobile when clinically tested.

• An undistorted radiograph should show no evidence of peri-implant radiolucency.

• Vertical bone loss must be less than 0.2 mm per year, from the first year of implantation.

• The performance of the individual implant must be characterized by the absence of signs and symptoms of persistent and/or irreversible pain, infections, neuropathies, paresthesia or violation of the mandibular canal.

• Implant longevity must have a minimum success rate of 85% after 5 years of observation, and 80% after 10 years.

In addition to these criteria, it is recommended to measure bone levels, check probing depth, bleeding on probing and address for the presence of exsudation/suppuration.

Strid[57] gave his contribution stating that the implant is osseointegrated when, on radiographic examination, there is no radiolucent areas and when the bone is in direct contact with the implant.

Smith and Zarb,[58] three years later, reviewed the criteria imposed by Albrektsson *et al*.[56] and proposed the following parameters to be evaluated:

- Mobility.
- Peri-implant radiolucent space.
- Vertical bone loss around the implant of less than 0.2 mm per year.
- Patient convenience.
- Depth of the gingival groove to implant.
- Gingival status.
- Injuries to adjacent teeth.
- Proximity to the alveolar nerve.
- Proximity to the maxillary sinus or the floor of the nasal cavities.
- Persistent infection and longevity of implant use.

In Implantology, the simplest definition of failure is an implant that does not meet the success criteria proposed by the aforementioned authors.[56] Briefly, it corresponds to the loss of the implant, with mobility, and/or that presents a peri-implant bone loss greater than 1 mm in the first year and greater than 0.2 mm in the subsequent years. To other authors,[59] failure is clinically characterized by implant mobility, redness and marginal edema, bleeding and/or suppuration on probing, increased probing depth, peri-implant radiolucency, and loss of height of the alveolar bone.

Implant failures are rare, and the long-term predictability of oral implants has been well documented.[60, 61] They can be classified as biological, mechanical, iatrogrenic and functional, being bacteria-related or originated by overloading.[62] The main causes of failure are lack of osseointegration, loss of osseointegration, in the short- and long-term, and fracture of the implant.[63] Lack of osseointegration leads to mobility, which is a pathognomonic sign of loss of an implant.[59, 64]

Osseointegration failure is a complex and multifactorial phenomenon. Depending on the time it occurs, its etiology will be different,[46, 65, 66] so it can be divided into:

Short term – when osseointegration failure occurs before implant loading. It is usually related to factors such as smoking,[67] advanced age,[68] systemic diseases,[61] the quantity and quality of the bone,[69] surgical trauma, early loading, infections, material incompatibility and contamination during the surgical procedure. It results from the implant's inability to osseointegrate, that is, from the inability to establish an intimate contact with bone.[66] In this situation, the mechanisms that would normally lead to a correct healing of the operative wound, through the apposition of bone on the surface of the implant, will not happen and a fibrous tissue scar will be formed between the implant surface and the surrounding bone.[70] The patient frequently feels pain and implant mobility, a sign that insufficient osseointegration has occurred or that it has been largely compromised.[71]

Long term – when osseointegration failure occurs after the implant has received an occlusal load. Is related to peri-implantitis,[72] the biomechanical factors and the patient's poor oral hygiene.[73, 74] This means that even in cases in which the osseointegration has been achieved, it can be lost due to repeated overloads on the implant, or the constant marginal inflammation following plaque accumulation.[59]

It is important to note that peri-implantitis may be due to the bacterial colonization during surgery, or the patient's inability to follow procedures for proper oral hygiene. Bacterial biofilm appears to be involved in peri-implantitis development and it has been described that the composition of the active microbiota in peri-implantitis is similar to that associated with periodontitis,[75] which supports the hypothesis that pathogens present in teeth periodontal pockets can colonize implants, leading to peri-implantitis.[76] Peri-implantitis, like periodontal disease, is generally associated with Gram-negative bacteria, sometimes even with alike bacterial species.[77, 78] Thus, the elimination of these periodontal pathogens from the oral cavity of patients prior to placement of dental implants can inhibit early colonization and reduce the risk of peri-implantitis.

Regarding biomechanical factors, these seem to be responsible for most implant failures as the implant's biomechanical stability is the most important factor for the development of mineralized or non-mineralized tissue at the bone-implant interface.[59] This stability is only achieved if there is occlusal harmony with the antagonist teeth and in the absence of excessive occlusal forces.

Relating the marginal bone loss, several aspects were pointed to explain the etiology of this alteration: surgical trauma, stress distribution by inadequate tightening of the implants during installation, physiological resorption, occlusal mismatches, untreated gingivitis, chronic

inflammation at the IAI, distribution of tensions in the marginal bone crest and presence of micromovements.[11, 79] It must be pointed out that this bone loss may also be found in patients with edentulous jaws with removable dentures.[80]

Adell *et al*.[81] stated the microbial leakage would be of limited clinical relevance, as they showed good long-term results with the Bränemark system studied. Also van Steenberghe[82] considered that the seal provided by osseointegration and the supraosseous connective tissue band would be enough to avoid bacteria into the apical area of the implant. During the period of osseointegration, the IAI is near or below the bone level and can affect healing.[52]

Once osseointegration has taken place, marginal bone loss can be caused by overloading[83] or a long-standing gingivitis.[84]

CRESTAL BONE LEVEL

One way to evaluate the success of the implants is by observing and measuring the marginal or crestal bone levels around them, through radiographs.[54] One of the biggest struggles in implant therapy is to maintain peri-implant marginal bone level through time.[85] Curiously, in the beginning of dental implantology, radiographs were prohibited because it was believed that they could damage bone cells necessary for osseointegration.[86]

At this point, it is important to clarify the differences between success and survival of implants.

Success criteria are based on bone loss that occurs in the first year and during the subsequent time. Normally, the majority of bone loss occurs immediately after the installation of a healing abutment or restauration.[54] It was expectable to occur bone loss after implant placement and uncovering, probably because of the surgical trauma, establishment of biologic width, occlusal loading, response to bacteria and peri-implant infections.[87, 88] The latter seems to be the main factor for bone loss.

Albrektsson *et al.*[56] suggested that the success criteria included up to a mean of 1.5 mm of marginal bone loss in the first year of restoration and less than 0.2 mm of bone loss annually thereafter. One point to be noticed is that it was not known if any bone loss occurred after implant placement, because within early works the radiographs were contraindicated during or after osteotomy.[86]

Bone loss occurred after stage-two surgery, so it was believed that it could be associated with the creation of an interface, and consequently the microgap, at the IAI. An important study from Hermann *et al.*,[89] although completely performed in animals, demonstrated that 1.5 to 2.0 mm of bone loss occurred whenever in presence of a bone-level implant with external hexagon and butt-joint, or platform-match, when the interface was created: after stage-two surgery or immediately in the cases of one stage approach. This also demonstrated that occlusal loading had little to do with bone changes. They also noticed that in the presence of tissue-level implants or one-body implants, there would be no bone loss. Surprisingly, they stated that 1.5 mm of bone loss occurred with implants placed at the bone level, but did not occur around tissue-level implants, supposedly because they did not have an interface at the bone crest. And as far as the interface moved apically, more bone loss occurred - if the interface moved coronally, less bone loss would be found.

After that study, many others[90-92] proved that around butt-joint connections, the amount of bone loss was related to the location of the interface, relatively to the bone crest.

As described around teeth, it was possible to have an equilibrium between the bacteria and their components, and the host response. However, this equilibrium only happens after an inflammatory response that creates bone loss and a 2 mm distance is established from the contaminated interface to the bone.[93]

After an initial marginal bone loss, probably caused by the surgical trauma, it can advance gradually after loading, until biological width is achieved.[94] Many factors can affect the quantity of bone resorption such as the gingival biotype and the bidirectional bacteria leakage in the IAI.[27, 95] Accordingly, many efforts have been made to minimize or eliminate the inflammation around the interface, trying to prevent the microbial leakage that occurs in every contaminated microgap between the implant and abutment connection.[54]

Numerous types of implant-abutment connections are available in the market, resulting in various interfaces at the bone crest, namely external connections, internal connections and morse taper-like.[54] The fundamental goal of these systems is to decrease the size of the gap in the interface, providing an intimate seal at the IAI and excluding the bacteria, hence reducing the bone loss.

Several studies[96, 97] concluded that the size of the butt-joint did not significantly influence bone loss around the IAI, stating that marginal bone loss could not be prevented. This is due to the small size of bacteria, smaller than the IAI, as microorganisms can penetrate through a gap as small as 10 μm.[85]

CONNECTION SYSTEMS

The design of an implant, its geometry and IAI, influence the performance and maintenance of implant supported rehabilitations, right from the start, by determining load transmission at the bone-to-implant and implant-abutment interfaces.[98]

Every implant-abutment connection has a different behavior, as far as resistance to dislodgement and stress dissipation is concerned.[99] When Bränemark start using dental implants, the abutment connection consisted of a 0.7 mm external hexagon. Since that time, several modifications occurred in the external hexagon, such as its height and width and many other implant systems have been developed, differing on its shape, materials, roughness, geometry and type of connection.[99] The ultimate purpose is to find the most efficient implant system that can last longer.

The Bränemark external hexagon was invented to simplify implant insertion and to provide anti-rotational support for the prosthetic rehabilitation.[99] However, when subjected to non-axial forces, the micromovements at the abutment connection were found to be harmful. The need to overcome this problem was one of the motivations for the development of the internal connection, decreasing or eliminating the micromovements.[99, 100]

Moreover, the design of the external hexagon has a higher center of rotation relative to the implant, which means the abutment is practically only secured by the screw.[101] This IAI, external hexagonal, is associated with many clinical complications.[102] It is well documented that in external hexagonal implant systems exists a space between the abutment and the implant, also called microgap.[103, 104] This microgap is wide enough to allow for bacteria penetration, further inciting local inflammation and even bone resorption.[27, 92, 96] The external hexagon was copied to various systems with the justification of its enhanced locking mechanism and anti-rotational element.[105]

After many years, the internal connection appeared with many designs, connection types and terminology trying to overcome some of the limitations of the external hexagonal connection.[106] Manufacturers struggled to innovate better abutments and implant designs in order to achieve an improved method of tightening screws.[107] The internal hexagon appeared as an evolution of the external one, craving an improved load absorption under lateral forces, further reducing mechanical complications, namely screw loosening or fracture.[108]

So, the first osseointegrated implant had an external hexagon,[44] which later evolved to internal connection systems aiming to improve biomechanical and biological parameters, with the ultimate objective of minimizing the microgap and improving occlusal load distribution to

the bone and implant, therefore reducing bacterial colonization and lessening peri-implant bone loss.[20]

When comparing to external connection, internal connection implants provide enhanced esthetics, better connection strength and microbial seal, more stable crestal bone levels and long-term stability of the IAI.[20, 106, 109] Resuming, as we can see on figure 1, implant systems differ for implant morphology and for the IAI geometry:[20]

- External connection – usually with a standard external hexagon.
- Internal connection – internal octagon, internal hexagon or internal trilobe.
- Conical internal connection or morse taper – frequently classified separately because of its particular properties.[110]

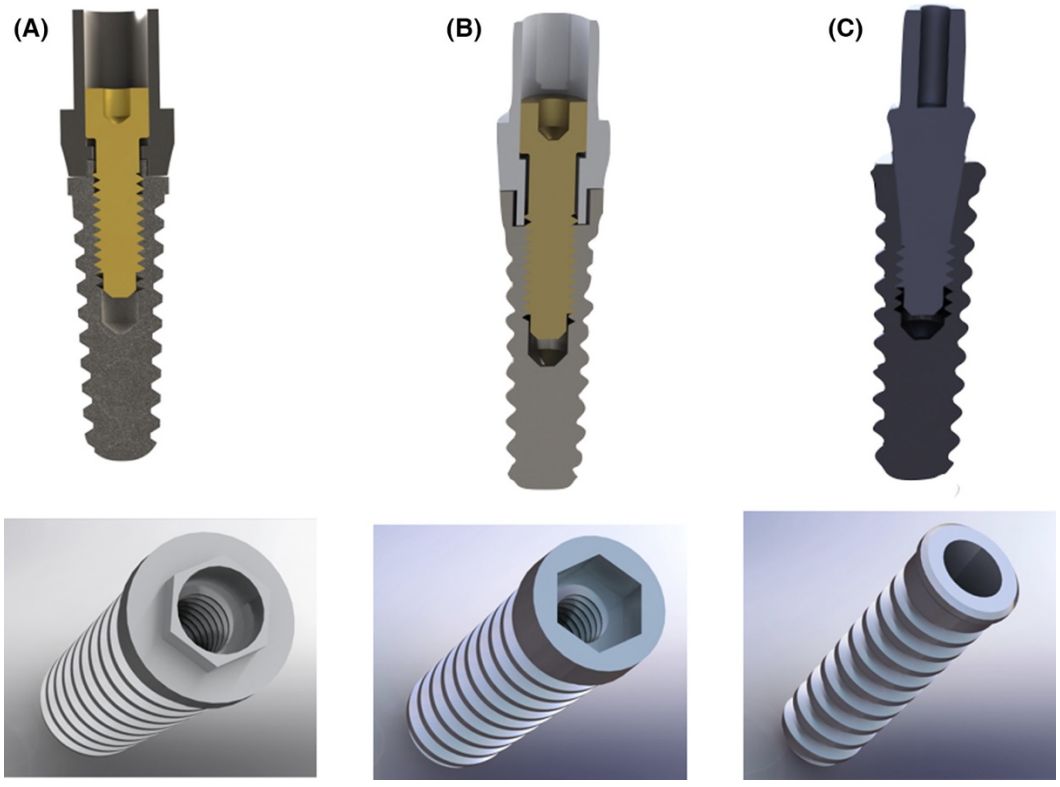

Figure 1. Dental implant connections.

(A) External hexagon. (B) Internal hexagon. (C) Morse taper. Image from the study of Apaza-Bedoya *et al.*.[111]

Tsuruta *et al*.[112] made a different division of the implant-abutment connection into three types: external parallel connection, internal parallel connection and conical connection.

To clarify the connections, it is important to know the differences, advantages and disadvantages between the most common connections. Different connections also behave distinctly during occlusal loading, leading to individual reactions of peri-implant tissues.[105, 113] Furthermore, the design of implant-abutment connection influences the bacteria penetration, and to prevent peri-implantitis it is important to inhibit the passage of bacteria.[6]

External connection

The first connection system, with an external hexagon, has been modified through the years, either regarding diameter or height. Firstly, it was developed to simplify the mechanical matching, but the main advantages are the easiness of the impression procedure and the compatibility with many prosthetic systems. The disadvantages are related to the micromovements under lateral load, leading to the microgap at the IAI.[44, 114]

A study from Nishioka *et al*.[115] recommend avoiding the external hexagon connection in patients with functional overload, regarding the fact that microgap may be related to the strength applied to the abutment.

Internal connection

Theoretically, this connection system presents improved load absorption, greater depth of the connection inside the implant, and more efficient dissipation of the mechanical stresses, as these would not converge into the crestal area but spread alongside the bone that surrounds the implant walls.[108] Both *in vitro* and *in vivo* studies showed improved mechanical resistance at the IAI and less screw loosening of internal connections, when compared to external ones. Nevertheless, the microgap was always present, despite its decreased dimensions.[116]

Conical connection

This connection is a unique kind of internal connection, taking advantage of the internal taper of the implant walls and the external walls of the abutment, locking the components through friction. The taper angle may differ between systems and, even though the attained mechanical friction, most manufacturers still add the screw retention and an anti-rotational system to the implant-abutment constructs.[99]

Regarding mechanical issues, this seems to be the connection with better results, despite the presence of the microgap, although narrower and broadly smaller than 10 μm under static

forces.[110] This connection seems to present also a good resistance to torque loss and screw loosening.[117]

The microgap is reduced and has less microleakage at the IAI, leading to a reduced bacterial colonization, despite the incomplete seal of the interface.[110] Some authors[118, 119] claim the possibility of a cold welding in some conical connections, which is defined as an increase in the loosening torque with respect to tightening torque.[105] However, a study by Norton *et al.*[105] stated that cold welding was only identified in situations in which a tightening torque of 100 Ncm or more were being applied to new components. Such torques are not clinically relevant. They also discovered that differences in the taper (8-degree and 11-degree) had little influence in the loosening torque values. On the other hand, a reduction in the interfacial surface area appeared to significantly influence the loosening torque. Lastly, they concluded that for clinically relevant levels of tightening torque (below 40 Ncm) cold welding did not occur.[105] Nevertheless, it is true that the conical connection has good apparent stability, probably a result of a good support against unfavorable bending moments.[105]

The connection system influences the mechanical and biological outcomes of implant rehabilitations, but other factors also have an important role such as the implant geometry and diameter, connection screw length and thickness, the contact area and the components' material.[100] Increased surface area of the interface between the abutment and the implant results in increased resistance, most likely due to the increased resting friction.[105]

Mechanical factors include static or dynamic occlusal load on the prosthetic component. Micromovement at the IAI increases inflammation at connection location and may lead to bone loss. The interface between implant and abutment is one of the most pointed factors that can lead to implant failure,[120] in part being correlated to bacterial microleakage and subsequent inflammatory cells which may induce bone loss at implant area.[118, 121-124]

Biological factors are also very important for the long-term success,[125] as peri-implant infections can cause discomfort to the patient and accelerate bone loss.[126] Bacterial colonization of the internal part of the implant through the IAI is a well-known problem that remains unsolved.[38] One way to reduce bacterial infiltration is related to the precision of the implant components, as their accuracy will provide a tighter interface.[127]

Additionally, with proper planning and adequate prosthetic treatment, occlusal overload can be prevented.[128] On the other hand, bacterial penetration at the IAI is dependent on the implant system sealing capacity.[92, 129]

After the achievement of the osseointegration of dental implants, one key factor for a long-term success is the stability of the implant-abutment connection.[130] There is a lot of controversy between studies regarding the connection system and the relationship to peri-implant bone loss. Some of these[131, 132] did not find significant differences between external connection and other connections, while different authors[133, 134] found higher bone loss when comparing the referred systems. It seems clear that most studies[134-136] found lower values of marginal bone loss in internal or conical connections when compared to external ones. Usually, studies that involve external connection implants have platform-matching between the implant and the abutment; conical connection obviously has platform-switching at the referred interface. Regardless the connection system being external or internal, Caricasulo *et al*.[20] in their systematic review and meta-analysis demonstrated that implants with platform-switching showed lower peri-implant bone loss values when compared to platform-matching connection. Also concluded that internal, and especially conical connections, appeared to be more prone to maintain the peri-implant bone level. The conical connections seem to be more beneficial biomechanically, due to the reduced microgap and micromovements during loading.[110, 137] Because of an improved distribution of forces at the IAI, there is also a reduced risk of screw-related complications.[117, 119, 138]

PLATFORM-SWITCHED CONNECTIONS

Aiming to minimize the bacterial leakage through the IAI, many manufacturers changed from a butt-joint to an internal cone connection, with a non-matching implant diameter and a smaller-diameter prosthetic component. This is called platform-switching and the reason to do so relies on the potential to prevent marginal bone loss by creating a horizontal offset at the IAI, as the connection shifts the perimeter of the implant-abutment interface inwards, towards the central axis of the implant.(139)

Incredibly, the platform-switching phenomenon was discovered accidentally when a clinician used a regular platform abutment in a wide diameter dental implant because of a commercial unavailability.(140)

Cochran *et al*.,(141) stated that platform-switched implants offer better sealing capability, likely due to the thicker connective tissue that is formed and acts as a barrier, reducing inflammation and marginal bone loss. The bone loss around platform-switched implants was found to be significantly reduced in comparison to that verified around butt-joint connections, also called platform-matching.(54) This lead to many studies about bone loss around implants with different connections. An interesting one from Cochran *et al.*(142) found that only minor bone loss occurred, approximately 1/5 of the expected in butt-joint connection implants, when platform-switched implants were used, whether they had been placed submerged or non-submerged (Figure 2).

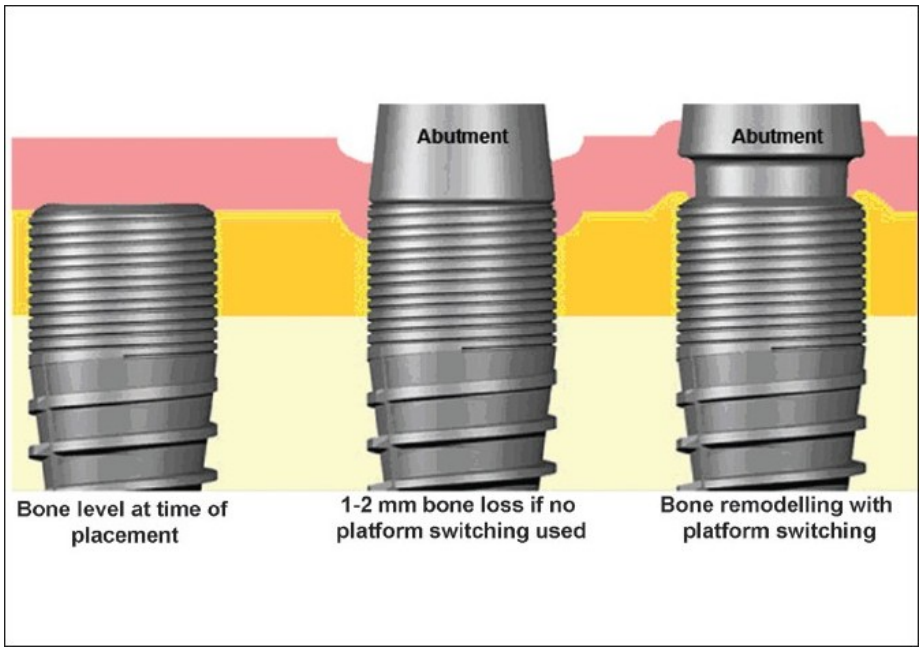

Figure 2. Comparison of crestal bone loss in platform-switched and non-platform-switched implants. Image from the study of Prasad *et al.*.(143)

The exact reason why marginal bone loss around platform-switched implant is much lower than in platform-matched ones, remains broadly unknown.[144, 145] In the past few years, various authors[54, 96, 97, 141, 142] have tried to explain the reasons why bone loss is so reduced in this type of implants, such as:

- With the displacement of the IAI further away from the bone crest, in the case of bacteria penetration, it would be at a greater distance from peri-implant bone;
- The connective tissue covers the IAI, sealing the microgap;
- An increased stability of the components in comparison to an external connection.

The concept of platform-switching is related to the increasing distance of the IAI to the bone surface, keeping the microorganisms that cause bone resorption further away from bone.[36]

The most stable cone connections use morse taper-like connections, having also the advantage of an improved sealing capability of the interface between the abutment and internal wall of the implant.[54] Morse taper is a conical internal connection that creates a friction between the surfaces[146, 147] providing a larger contact area of the IAI, when compared to other connection system. It seems to be somewhat responsible for an improved seal between the components and therefore, originating a reduced microleakage.[106, 112] It is further referred to allow a better joint stability and less bone loss.[110, 147]

However, many implant systems use cone connection without morse, with high tapers. Ranieri *et al.*[148] tested four Morse taper implant systems for bacterial microleakage and found bacterial penetration in all of the systems, which could be explained by the use of a bacteria [S. *sanguinis*] that has an average dimension of 0.8 μm, while the mean of the narrower microgap measured was 4.51 ±1.41 μm.

Morse taper systems were inspired in a concept developed by Stephen A. Morse in 1864, with the basic principle of "a cone within a cone".[149] In this connection system, the IAI is located in the interior of the implant, while in external hexagon connection the union occurs on the implant margins.[92] This was thought to allow higher torques to be applied,[150] which may enhance the connection stability and decrease the micromovements.[151] Higher torques are also considered to enhance sealing capabilities against bacterial contamination in the IAI,[148] but this superior sealing may also be due to the intimate contact between the abutment and internal walls of the implant, acting as a shield at the interface.[105, 127]

Morse taper is defined by 2°50 angle, equaling 5% slope, that the taper surfaces makes relative to the longitudinal axis of the component.[149] When a morse taper connection is present, the force to dislodge the components is higher than the one needed to connect them.[54]

As stated before, and regardless of the improved mechanical behavior and enhanced bacterial seal than other connection due to a better stability of the IAI and decrease of the occurrence of micromovements, morse taper-like connections are unable to fully prevent microbial penetration[54] and avoid the contamination of fluids and microorganisms.[36] Nonetheless, morse taper connections display improved marginal adjustment than external connection, independently of the torque or abutment type.[152]

Taper angle and length of contact can influence the stability of the connection between implant and abutment, also the smaller degree of abutment taper might lead to greater attrition, resulting in a more intimate contact between the implant and the abutment. Likewise, a connection with a long contact might be more stable compared with a short contact length.[148]

To conclude, two systematic reviews, one by Atieh *et al.*[153] and another by Al-Nsour *et al*.[154] must be enunciated, which revealed a significant advantage for the platform-switched systems compared to non-platform-switched ones, as far as the marginal bone loss is concerned. Also Caricasulo *et al*.[20] stated that platform-switching could be a main factor influencing bone response around dental implants, as conical connections with platform-switching had the same performance as other internal connections with platform-switching, but both of them were way better than every connection with platform-matching.

So, it can be inferred that platform-switching itself may be more important than the type of connection.[155] However, Vandeweghe and De Bruyn[156] showed in their study that platform-switching is only effective when there is enough gingiva thickness for the biologic width. A recent study by Linkevicius *et al*.[157] concluded that soft tissue thickness around platform-switched implants affects marginal bone loss, being higher in patients with thin tissues, than in those with thicker tissues.

Lastly, as far as the horizontal offset in the platform-switching is concerned, there isn't a general consensus, and while some studies[144] refer that the greatest platform-abutment mismatch resulted in the least marginal bone loss, other studies[158] found no differences regardless the amount of horizontal offset.

VERTICAL AND HORIZONTAL POSITION

The positioning of the IAI at bone level has been associated with more inflammation and bone loss when compared with one-piece implants without interface, suggesting that the interface may stimulate inflammatory activation culminating in bone loss.[159]

Regarding vertical position of the implant, the bone reaction is different when a bone level implant is placed at bone crest level, 1 mm above or 1 mm below it, either with submerged or non-submerged implants.[142] Enhanced results occurred with bone crest placed implants, however this study didn't took into account the height of the gingiva.

From an esthetic point of view, it has been suggested to place implants a few millimeters below the bone crest, in order to avoid a potentially exposure of the metal of the top of the implant, and also to allow sufficient space to create a good emergence profile.[96]

Whenever the connection between the implant and the prosthetic component is placed apically, hard and soft tissue can become colonized with inflammatory cells and result in bone loss,[89] also leading to gingival recession, and therefore aesthetic failure. Several studies[160-162] showed a higher bone loss in implants placed at bone crest level or below it, when compared to ones positioned supracrestally, revealing that as the connection moves coronally from crestal bone, less bone resorption will occur.[161]

SCREW LOOSENING

As every treatment, implants also have disadvantages. Unfortunately, screw loosening is one of the most common mechanical complications in implant-supported restorations,[163] it can affect the stability of the abutment[164] and lead to bacterial leakage and contribute to peri-implant inflammation and bone loss.[118, 120, 165]

A study by Gunne *et al*.[166] reported that loosening of prosthetic screws was the most frequent mechanical complication.

This problem was so frequent that it led to several investigations, many concluding that the most significant parameters for the connection stability were the design of the screw head, its material and the tightening torque.[167-169] Higher torque usually leads to greater preload values.[170] The greater the preload, the tighter and more secure is the screw joint. However, it shall not exceed the tensile strength of the screw.[170]

It is believed that abutment screw loosening may be due to excessive joint bending and settling effects.[167] Despite the type of connection, implant-supported prosthesis must have an intimate and passive connection of the so-called superstructure to the implant.[170] The two parts, the abutment and the implant, are held together with a prosthetic screw and the clamping or tensile force that keeps them together is called preload.[171]

The main factor for screw loosening is insufficient preload between implant and abutment.[172] Many studies showed a better stability of the abutment by enhancing the preload, which is achieved through the use of a lubricating coating to reduce friction.[173, 174]

Occlusal parafunction and off-axis occlusal loading also create the separating forces.[175, 176]

The frequent thermal changes in the oral environment are also thought to contribute to settling and to the joint-separating forces, because of the thermal expansion and shrinkage of the components.[176, 177] It was even hypothesized that abutment screw length would have impact on screw loosening, but a study by In-Sung Yeo *et al*.[178] concluded that abutment screw length had no significant effect on screw loosening under thermal stress [range between 4ºC and 60ºC]. They also checked the removal torque value (RTV) and found it to be similar pre- and post-thermocycling. Moreover, they stated that daily temperature changes in the oral cavity may have no negative influence on the integrity of the implant-abutment joint if the abutment screw is engaged of a minimum of three and a half threads.[178]

Despite the above information, Mohammed *et al*.[179] also studied the effect of abutment screw length and cyclic loading on RTV in external and internal hexagonal implants and

published different results. The post-loading RTVs were significantly lower than the initial ones, both in external and internal hexagonal connection. Regarding the abutment screws' length, they came to the same conclusion as In-Sun Yeo work,[178] when there is a minimum of 3.5 threads, there would be no significant differences in the RTV in internal or external hexagonal connection.[179]

The connection between the implant and the abutment usually requires a tightening torque of 15 to 35 Ncm, depending on each system.[52] A study by Bousquet *et al*.[52] analyzed the torque values and came to the conclusion that 90% of the subjects, using hand screwdriver, applied a torque of less than 15 Ncm, but it was studied in the end of the surgical procedure and the clinicians might had fear of moving the implants.

During the osseointegration phase, a healing abutment is placed instead of the prosthetic abutment. Usually, manufacturers don't indicate specific torque to be applied in healing abutments, rather suggest manual tightening. It is known that the higher the torque, less is the gap in the connection between implant and the abutment. With healing abutments a similar concept may be envisaged, and a torque equal or superior to 20 Ncm will probably reduce the gap. Despite this fact, higher values do not seem to improve tightness.[52]

When the abutments are tightened with less load than the recommended torque, the risk of screw loosening is higher,[180] as well as the development of screw fractures and deformations.[181]

As low torque values reduce the length of contact between implant and abutment, it can be inferred that is less effectiveness on bacterial seal, thus providing an increased microleakage.[182, 183]

The screw behaves like a spring mechanism that is stretched by preload, and the friction forces maintain that stretch of the threads.[184] Due to external forces, the threads may release some of the stretch and lose some of the preload. Then, as external forces and vibrations act on the joint, the screw ceases to perform its function, fails, and becomes loose.[184] This happens, for example, when the screw is not able to withstand the mastication forces, which induces a combination of vertical and horizontal forces that are transferred through the prosthesis into the implant and then bone.[185]

Screw loosening has a direct impact on the gap between implant components.[159] Mechanically speaking, the loosening of the screw can trigger the instability at the IAI and eventually culminate in screw fracture.[29] Every time a screw is tightened to secure a restoration or abutment to the implant, a certain torque is applied and the screw elongates and produces tension, called preload, which directly influences the clamping force.[186] This preload

depends on the applied torque, on the design of the screw - such as its head, thread design and physical properties of the materials,[186] but also on the settling of the screw after initial application of torque.[184] Component misfit can decrease the preload in a screw joint.[170]

When the separating forces applied to the implant components exceed the generated load, screw loosens.[184] In an ideal scenario, this preload should be able to resist all of the separating forces from the oral environment.[186] On the other hand, care must be taken not to exceed the structural values of the components to prevent permanent distortion or even fracture.[187] If the screw exceeds its yield strength, it may lose its mechanical characteristics and screw fracture can occur.[188] Supposedly, torque suggested by the manufacturers is crucial for having long-term success. Dynamometric manual wrench is recommended to achieve an ideal torque, which should be recommended by each manufacturer.

Abutment screw loosing is approximately the same for a wet or dry environment, so, saliva contamination does not hinder this matter.[105] On the other hand, repeated placement of screws may cause wear that produces metal debris and affect screw surfaces, decreasing screw resistance to loosening and ultimately hindering preload values.[189, 190] This phenomenon is called the settling effect, and occurs when the applied torque is partially consumed to overcome friction and to flatten rough high spots caused by debris, rather than to elongate the screw.[176] Obviously this will reduce the preload.[191] Debris can also be caused by manipulation in laboratory, and not only on clinical repeated screwing.[189] Whenever there are surface imperfections, they create increased friction and decreased preload. Overall, it has been reported that only 10% of the tightening torque is transformed in preload, while the other 90% is used to overcome friction.[192]

Many studies[176, 186, 190] report a lower torque value for screw loosening, as compared to the one initially applied. This led to the former recommendation of applying a second torque, approximately 10 minutes after the initial, with the same value, in order to try to limit the settling effect.[168, 193] The recommendation is to use new screws at the delivery of final prosthesis, so that screw loosening due to inadequate preload may be prevented. Moreover, a laboratory screw should be used during laboratory procedures, avoiding any screw contamination.

The optimal torque value specified by the manufacturers, usually is designed to develop a preload of 75% of the ultimate tensile strength of the screw.[170] When the friction between the components decreases, the application of torque can increase the preload.[170] With repeated torquing, some of the irregularities of the surfaces become smoother and allow a greater

amount of the torquing force to be converted into preload.[170] So, used prosthetic screws may achieve higher preloads than new ones, if their structural properties are maintained.[170]

There are two ways to tighten the abutment screws, by hand screwdriver or with torque wrenches. Most manufacturers advise to screw healing abutments manually. However, some studies[194-196] have shown that clinicians can generate a wide range of torques when using hand screwdrivers. Depending on the shape of the hand screwdriver, it may be possible to apply more or less torque, thus it may not be adequate for a given procedure.[194]

A study by Alikhasi *et al*.[197] stated the mean torque was 11+/-4 Ncm with minimum 4 Ncm and maximum of 27 Ncm, for a medium handle screwdriver. Complications may derive from over- or under-tightening the screw of the abutment, so it should be emphasized the need to use torque wrenches and apply the recommended torques to diminish the probability of those complications.

In day-to-day practice, manual torque wrench is used, despite its inaccuracy because of the scale printed on the wrench, and because of the calibration itself. Torque variability may be further due to the extreme use of the wrench, which may decrease the accuracy because of sterilization procedures.[195]

Later, since the 1990's the torque driver became an essential device for the application of preload, allowing a clamping force of the abutment against the implant that would prevent screw loosening.[105] Despite all that, complications related to screw loosening continued to appear[23] and led to the development of the internal connection with elevated torque values.[198]

MICROGAP

Implant-based rehabilitations can be set on one-piece or two-piece systems - implant and abutment.

One-piece implant is the only one with an absolute bacterial seal, given the absence of the IAI.[199] The ultimate reason for developing an one-piece implant system relies on eliminating the inflammation and bone loss around the interface created whenever two components are connected.[93] When no interface or gap is present near the bone, there is no bacterial contamination which can lead to host inflammation and consequently marginal bone loss.[54] Both preclinical[54] and clinical[90] trials demonstrated that one-piece implants provide absence of initial bacterial contamination given the exclusion of the interface. If the only concern was bone level maintenance, one-piece implants would always be the first choice. Nevertheless, they have some disadvantages, such as limited options for the finish line, the inability to change the prosthesis angulation after placement and the need for immediate provisionalization in the anterior region.[200]

In every two-piece implants, oral microbiota represents a potential risk, because the gaps and cavities can harbor bacterial species that can easily proliferate.[118] The space between the abutment and the implant is called microgap (Figure 3).[103, 104] It is considered unavoidable and is a consequence of the micro-tolerance between the components, which is required during manufacturing.[201]

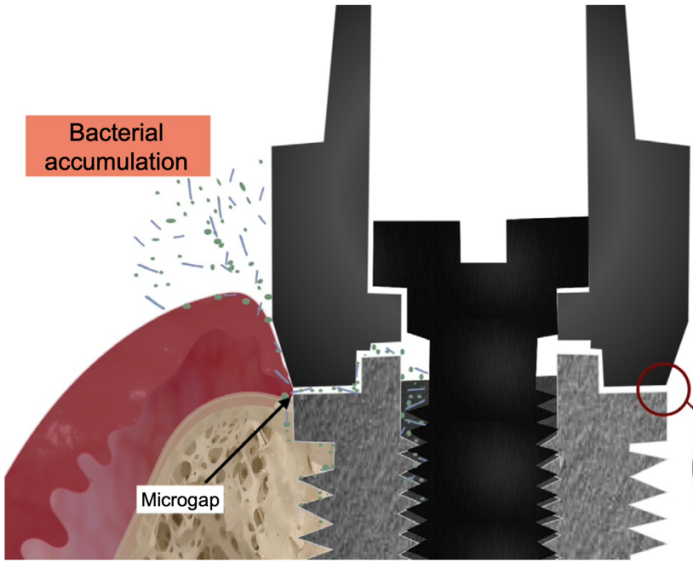

Figure 3. Schematics of peri-implant conditions and identification of the microgap.

Image from the book chapter of Apaza-Bedoya *et al*..[202]

It allows for the passage of fluids, enzymes and other molecules, bacteria and/or their metabolic products, causing biological complications.[125, 159] Microorganisms may grow into the IAI microgap and establish a bacterial reservoir,[55, 203] later causing inflamed soft tissue around the implant-abutment junction.[95]

The closer the microgap is to the alveolar bone crest, the great the bone loss may be.[204]

Well adapted interfaces reduces the microgap, but even a reduced gap of around 5 μm isn't able to prevent infiltration and colonization inside the implants.[125] Microgap depends on various factors, such as the inaccurate components of the connection, poor adaptation and the torque applied.[118, 183, 205] The misfit is directly related to the applied torque, as higher insertion torques provide lower misfit values.[152] It is also related to different materials or production techniques. Titanium has better results than zirconia abutment and in prefabricated abutments.[109, 206] The surface quality of the cone surface, the screw-head, the screw-thread and the tolerance of the cone angle in conical connections, also affects the microgap.[5]

As far as the gap is concerned, the problems can be divided into biological and mechanical.[107, 146, 207] The mechanical ones have been associated with screw-joint instability between the implant and the abutment,[208, 209] normally related to micromovements, loosening or fracturing of the abutment screw,[210] while biological ones are concerned with the bacteria microleakage.[211] One study from Khraisat *et al*.[191] concluded that there was a direct correlation between the implant-abutment misfit and the screw loosening. Curiously, during the 1980s, it was believed that implant components should allow flexibility between them, such as natural teeth.[54]

Scarano *et al*.[107] evaluated the presence of a microgap between abutments and implants and concluded that all implants analyzed had microgap, which allowed for bacterial migration and colonization inside the implant. Moreover, the microgap dimension is unpredictable and variable, and commonly higher than observed in an *in vitro* study.[211]

Whenever there is bacteria colonization inside the implant, it can be either from contamination immediately after implant placement, or after the osteointegration period.

Regarding rehabilitation options, these can be screwed or cemented on an abutment, but either of them have the potential problem of the retaining screw loosening.[212] The critical disadvantage of the cement-retained prosthesis is the retrievability, although they have higher potential of passive fit.[107] The most common rehabilitation procedure consists of an implant and an abutment that are fixed with an abutment screw.[107] The two major setbacks of this system are the gap between the two components - the implant-abutment connection,[32, 213] and

the potential loosening of the abutment screw, which can cause screw fractures or fracture of the framework.[214, 215] Moreover, cyclical movement can cause wear on titanium surfaces, enhancing a posterior microleakage.[216]

Additionally, the abutments can be straight or angulated. Straight ones have a better marginal fit at the interface than the angulated ones, regardless of the type of connection or applied torque load, because the load is different from the axis of the implant-abutment and so is more prone to micromovements.[152]

The sealing of the IAI also depends on the screw tightening torque by creating a preload that connects the implant and the abutment, and the higher it is, the higher of the clamping force between them will be.[5] Nevertheless, there is the limitation of the components, namely the deformation of the screw, abutment or implant, so manufacturer's indication should be followed to prevent eventual damages.

A close relationship was observed between the tightening torque of the abutment screw and the adjustment at interface level.[152] The higher the torque, the lowest the misfit, because when torque increases, there is greater compression between the implant and the abutment, originating better stability in the connection.[208, 217]

Chewing force may cause elastic deformation between the implant and the abutment, resulting in a momentary increase of the microgap.[5] Even when conical connections show no measurable gaps under static loading, under dynamic loading, usually 200 Newton, the gap is noticeable.[5] The 200 Newton force is compared to a normal bite force at posterior implants, while maximum bite forces in patients with bruxism might be higher.[218]

A study by Zipprich *et al*.[5] showed that, under the conditions tested, length and angle of the conical connection didn't affect gap formation. They concluded that when subjected to dynamic loading of 100 Ncm or more, at the IAI, micromovements led to a cyclical opening and closing of gaps between the implant and the abutment, which allowed for microleakage of bacteria and/or liquids from inside of the implant to the peri-implant tissues.[5] This cycling opening and closing of the microgap may even act as a pump, pushing bacterial endotoxins from the inside the implant to the peri-implant tissues.[122, 219, 220] The presence of bacteria at IAI is a risk for peri-implantitis, loss of osteointegration, and possibly implant failure.[221, 222]

The ultimate complication of the microgap is the crestal bone remodeling and is influenced by the location of the microgap between the implant and the abutment.[96, 161] Microgap leads to microleakage and bacterial infiltration which can negatively affect the success of implant restorations.[223] The internal colonization of the implant surface by microorganisms is eventually inevitable. [39]

MICROLEAKAGE

Prevention of microbial leakage at the IAI is of the utmost importance for implant connection system to increase peri-implant bone stability.[77] Microbial microleakage is found in different implant-abutment connections, affecting the long-term success and survival of dental implants,[224] and has been confirmed to occur in both directions, from the interior of the implant to the exterior and on the way around.[225]

Bacterial leakage at the IAI is one of the main causes of peri-implant inflammatory reactions.[124] Peri-implant disease is an infectious disease caused by oral pathogens that initiate host inflammatory responses.[226] At first, there is an inflammation of the peri-implant soft tissues, called peri-implant mucositis. If the bacteria migrate apically, the inflammation may evolve to a destruction of the supporting bone, called peri-implantitis.[227]

Socransky *et al*.[228] reported that, in a universe of approximately 1000 bacterial species present in the oral environment, 10 of them have a strong association with the beginning of the host response that leads to peri-implant disease. *Porphyrormonas gingivalis* and *Prevotella intermedia* are thought to be the ones who orchestrate inflammatory destruction.[229] Along with *F. nucleatum* and *A. actinomycetemcomitans,* which are considered as prime pathogens in producing virulence factors that induce inflammatory destruction of peri-implant tissues – at the mucosal and osseous levels.[230]

It is believed that periodontal pathogens that are adhered to implants and/or abutments can transfer bacteria to the peri-implant tissues, causing inflammation.[230] It can be inferred that it is of major importance to remove biofilms from implant surfaces.

Regarding abutment metal surfaces, a study by Jordan *et al*.[230] indicated that either a smooth chromium-cobalt or a rougher milled titanium surfaces are able to facilitate the formation of bacterial biofilm by *P. gingivalis* and *F. nucleatum*. On the other hand, they stated that *Pr. intermedia* and *A. actinomycetemcomitans* had reduced adherence levels.

The design of implants with polished collars tried to reduce bacterial adhesion, however, studies[230, 231] report that smooth surfaces may also allow for specific bacteria adhesion.

Many studies reported that the IAI became contaminated with bacteria soon after the placement of the abutment over the implant, which can cause inflammation.[77, 123, 207, 211, 213, 220, 232, 233]

The spaces between the abutment and implant body can act as a reservoir of bacteria,[33] and then migrate into peri-implant tissues from the microgap located at the IAI,[182] subsequently causing local inflammation[54] and loss of the supporting bone.[234]

The colonization of the microgap may result in bone resorption.[33] Broggini *at al*.[27] showed that it seems likely to occur a relationship between inflammatory events and bone loss. Harder *et al*.[124] showed that bacterial leakage occurs soon after 5 min of incubation, using an experimental model study, even without cycling loading. A study by Tripodi *et al*.[235] showed that with conical implant-abutment connections, bacterial contamination occurred much lately than in the butt joint connection implants - only after 22 days. It is important to notice that many researches at that time, i.e. 2012, were conducted only until 14 days.

Leakage can be influenced by the connection system, the quality of material and its production, and also the screwing torque applied, as previously discussed.[123, 236] Manufacturers know that by achieving a tighter and precise connection between the implant and the abutment they can reduce joint mobility and ultimately reduce the leakage.[124] However, bacterial penetration depends on many factors such as the precision of fit between the components, the micromovement between them, and the torque applied to connect them.[123] Even the best marginal fit of the implant components wasn't able to prevent bacterial leakage.[118]

Moreover, mastication force induce micromovements, increasing the microgap and inducing a pumping effect from the inside of the implant to the surrounding tissues.[123] In a two-piece implant system, absolute bacterial seal was never achieved in any system.[199] However, the connection that minimize the most the bacterial microleakage is the internal cone implants, also demonstrate an improved stability of the peri-implant soft and hard tissues.[199] Despite that internal connection seems to reflect an improved functional and stable connection than external connection, microleakage is also found in the IAI, either in static or dynamic loading conditions.[237]

SEALING

Many alternatives have been developed to increase the prognosis of implant treatment, and one way is to reduce or avoid the presence of microorganisms inside of the implants, aiming to decrease peri-implant bone loss, because none implant connection system is able to completely seal its interior.[39]

The use of sealants and decontamination of the implant cavities are some of the measures used to prevent or avoid bacterial contamination of the IAI,[33, 238] as they act as physical barrier that fill any misfit at the IAI, reducing micromovements and microleakage.[239]

Many materials were used to establish an infection barrier, for example sealing the implant abutment screw hole with gutta percha, vinyl polysiloxane, silicone membrane and polytetrafluoroethylene tape,[240] being the latter the one that exhibits the best characteristics for sealing implant-supported prostheses.[241]

A study from Pimentel *et al*.[242] used a silicone membrane to seal the IAI and concluded that although the sealing agent decreased the penetration of the pathogens, it could not fully prevent it.

Metallic nanoparticles, such as silver nanoparticles, are one of the most promising nanomaterials with antibacterial properties.[39] These nanoparticles are widely used in food preservation and in several fields of medicine[243] because of its high antimicrobial and bactericidal activity against both Gram-positive and Gram-negative bacteria, and also for its antifungal properties.[243, 244]

Keedi *et al*. [94] studied the efficacy of a polyglycol dimethacrylate-based adhesive to seal the IAI. The material was applied only on the inside of the indexing area of the implant, preventing a possibly adverse reaction of the material on the oral environment. The number of DNA copies was found to be significantly higher in the control group.

However, the use of sealants may affect the mechanical stability of the connection. A study by Biscoping *et al*.[245] concluded that sealing materials do affect the torque necessary for tightening or loosening the abutment screws. Quirynen and van Steenberghe[32] stated that an effective hermetic sealing would kill the microorganisms. Podhorsky *et al*.[246] concluded that disinfectants or sealing materials studied can reduce bacterial colonization, however, they cannot totally prevent it.

CHAPTER 3. Objectives

Microgap and bacterial microleakage at the implant-prosthetic abutment interface are recognized concerns for implant-supported restorations, leading to inflammation of the peri-implant tissues, with deleterious consequences for crestal bone levels. However, little is known regarding the interface established between the implant and the healing abutment or cover screw placed for the osseointegration phase, particularly in platform-switched implant systems.

Having that in mind, the following objectives were outlined:

- Characterization of the implant-cover screw and implant-healing abutment interfaces of a platform-switched implant system with hybrid connection, to determine the microgap and bacterial microleakage of the system, in the absence or presence of a silicon-based sealing agent;
- Characterization of the microgap and bacterial microleakage of the implant-abutment interface of a platform-switched system with hybrid connection, screwed at distinct torque values, in the absence or presence of a silicon-based sealing agent;
- Evaluation of the biological response and functionality of a silicon-based interfacial sealing agent.

CHAPTER 4.

Microgap and bacterial microleakage in temporary fixtures

Fernandes PF, Grenho L, Fernandes MH, Sampaio-Fernandes JC, Gomes PS. Microgap and bacterial microleakage during the osseointegration period: An in vitro assessment of the cover screw and healing abutment in a platform-switched implant system. J Prosthet Dent. 2021 Oct 25:S0022-3913(21)00501-1. doi: 10.1016/j.prosdent.2021.07.030. (Appendix 1).

INTRODUCTION

Dental implants are reliable therapeutic options for the oral rehabilitation of partially and completely edentulous patients, with documented long-term predictability, effectiveness, and high success rates.[247] The long-term survival of dental implants has been reported to be closely related to the structural and biological functionality of the peri-implant crestal bone, with resorption leading to implant failure, before or after osseointegration.[248] Crestal bone loss has a multifactorial etiology and has been considered an indicator of bone loss progression and the initiation of peri-implantitis.[20] Factors associated with crestal bone loss include bone and soft tissue features, implant placement location and formation of the biological width, occlusal and surgical trauma, implant structure and design, and the characteristics of the implant-abutment interface.[20, 155]

Implant-supported restorations rely on either a 2-stage or 1-stage surgical procedure, both with high success rates.[249] In the 2-stage approach, the implant is initially inserted and submerged into the bone and soft tissues and sealed with a cover screw. The implant is load-free and protected from the oral environment during osseointegration. Subsequently, and after the bone healing process is completed, a healing abutment is placed for soft tissue stabilization, and a transmucosal prosthetic abutment is then attached to support the restoration. In the 1-stage approach, the implant and a transmucosal healing abutment are placed in a single procedure, with the implant remaining exposed to the oral environment throughout the osseointegration period and before the connection of the prosthetic abutment.[249]

In either approach, the connection of the prosthetic abutment with the implant involves an interface—the implant-abutment interface (IAI)—characterized by a microgap between components.[250] This microgap may initiate bacterial leakage, with the accumulation of microorganisms at the interface,[251] eventually resulting in direct and/or indirect inflammation of the peri-implant tissues with deleterious consequences for the bone level.[252-254] The connection design and its influence on the microgap and bacterial microleakage outcomes have been evaluated,[255-258] and it is generally concluded that internal and combination connection systems outperform external connection designs, with the most positive outcomes being associated with platform-switching approaches. Other factors have been further addressed, especially related to the screw-tightening torque.[259-263]

However, research on the interface between the implant and the covering screws and abutments used during the osseointegration phase is sparse. This interface is of clinical significance given the recent evidence of early contamination of the implant with periodontal

pathogens that may occur before the placement of the healing abutment.[264, 265] This contamination suggests that a pathogenic biofilm may develop that hinders the sealing of epithelial tissues at the implant surface, triggering and sustaining peri-implant inflammation, promoting corrosion and structural damage, and leading to complications at both the early and late healing stage.[266]

Therefore, the aim of this study was to characterize the *in vitro* microgap and bacterial microleakage of *E. faecalis*[267-269] to the implant-cover screw and implant-healing abutment interfaces in a platform-switched implant system and to determine the efficacy of an antibacterial silicone-based sealer (Gapseal; Hager & Werken)[270-272] for preventing microleakage within these interfaces. The null hypothesis was that no significant difference would be found in the microleakage of the implant-healing abutment and implant-cover screw interfaces in the presence or absence of the silicone sealer.

MATERIAL AND METHODS

Implants with a combination of two internal connection systems and a platform-switched design (4.0x12 mm – Vega; Klockner), titanium healing abutments (height of 5mm, Vega; Klockner), and cover screws (Sealing cap; Klockner) were supplied by the manufacturer (Figure 4).

Figure 4. Macroscopic image and respective X-rays of implant and healing abutment and cover screw fixtures (Klockner®).
Scale bar corresponds to 2 mm.

To determine the microgap between the implant and the healing abutments or cover screws, 20 assemblies were prepared with healing abutments (n=10) or cover screws (n=10). These were tightened at a 10 Ncm torque. The sample size calculation assumed a mean difference of 0.5 μm between groups, a power of 90%, and a significance level of 5%, indicating a sample size of 9 in each comparison group. The assemblies were embedded in an autopolymerizing acrylic resin (Vertex Orthoplast; Vertex-dental) and then longitudinally sectioned with a diamond saw (Struers Accutrom-5; Struers Inc) at a feed speed of 0.150 mm/s. The sections were polished, washed, and dried and then observed by scanning electron microscopy (SEM) (Quanta 400 FEG ESEM/EDAX Genesis X4M; FEI Co) for microgap determination. The assessment was performed in 6 positions for each assembly, 3 on each side, corresponding to the highest values spaced at least by 2.5 μm. Measurements were determined and averaged. Data analysis was conducted independently by 2 researchers (L.G., P.S.G.) and repeated 3 times.

Enterococcus faecalis (ATCC 29212) was used for the assessment of bacterial microleakage. A standard bacterial suspension of *E. faecalis* was prepared in brain heart infusion (BHI; Liofilchem) broth at a density of 10^8 colony forming units (CFU)/mL. Under sterile conditions, the implants were inoculated with 0.2 μL of bacterial suspension, previously determined as the ideal volume for inoculation in both assembled systems. After bacterial inoculation, healing abutments (n=10) and cover screws (n=10) were installed and tightened to 10 Ncm. To prevent external contamination, the assemblies were immersed for 30 seconds in tubes containing sterile BHI broth, which were incubated at 37 °C for 48 hours. If bacterial growth was identified by cloudiness, the assembly was excluded.

The assemblies were incubated for 30 days in tubes with sterile BHI broth at 37 °C and 100 rpm. As a positive control, untightened assemblies were used (n=5) and, as a negative control, assemblies not subjected to bacterial inoculation were tested (n=5) and maintained under the defined experimental conditions. Every 24 hours, bacterial microleakage was evaluated and recorded by the presence of turbidity in the broth or deposits at the bottom of the tubes – a visual macroscopic indication of bacterial leakage. After 30 days, if no bacterial leakage had been observed, the assemblies were carefully unscrewed, and the bacterial viability was assessed by sampling with sterile paper-points. Any specimens without viable bacteria were excluded from the assay. At defined periods, randomly selected assemblies (Research Randomizer; https://www.randomizer.org/) were characterized by SEM imaging. Briefly, the specimens were washed with phosphate-buffered saline, fixed for 30 minutes with 1.5%

glutaraldehyde, and dehydrated in an ascending series of alcohols. Assemblies were critical point dried (CPD 7501; Polaron Range), sputter-coated with a thin film of gold and palladium, and imaged by SEM (Quanta 400 FEG ESEM/EDAX Genesis X4M; FEI Co).

The microleakage of the assemblies was further assessed after applying a commercially available sealer (Gapseal; Hager & Werken). Twenty implants were inoculated with *E. faecalis* suspension as previously described, filled with the sealer according to the manufacturer's instructions, and immediately connected to the healing abutments (n=10) or the cover screws (n=10), which were tightened to 10 Ncm (Figure 5).

Figure 5. Application of sealing agent inside implant, followed by installation of healing abutment.

The assemblies were incubated up to 30 days as previously described, and bacterial microleakage was assessed as previously described. To address the possible influence of the sealer in the removal torque of the abutments or cover screws, another set of assemblies were tightened according to the manufacturers' recommendations, with or without the sealer. After 48 hours, the assemblies were unscrewed, and the removal torque recorded, which was not significantly different.

The irritation potential of the sealant, as supplied, was tested with the chorioallantoic membrane (CAM) assay according to the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) guidelines.[273-276]

In short, fertilized chicken eggs were incubated with hourly rotation at 37 °C in a 60% humidified atmosphere in an incubator (Octagon Advance; Brinsea Products, Inc). At day 9

after fertilization, an eggshell window was created to access the CAM. Subsequently, 300 μL of the sealer was distributed into the CAM silicone O-rings used to retain the sealer and delimitate the area of analysis. For a contact period of up to 5 minutes, the CAM was observed and imaged by using a stereomicroscope (Stemi 305; Zeiss) and an attached imaging system (Axiocam 208 color; Zeiss), and the time taken for injury to occur was recorded. Specimens were tested in quintuplicates. The irritation Index (II) was scored according to the severity and speed of damage regarding hemorrhage, vascular lysis, and coagulation from:

$II = \frac{(301-H)}{300} \times 5 + \frac{(301-L)}{300} \times 7 + \frac{(301-C)}{300} \times 9$, where H, L, and C correspond to the time in seconds required to initiate hemorrhage, lysis, and coagulation, respectively. The Irritation Index is classified in accordance with the following score: 0–0.9, no irritation; 1–4.9 weak irritation; 5–8.9, moderate irritation; and 9–21, severe irritation.

For quantitative assays, data were presented as the mean ±standard deviation of replicates. Normality was evaluated by the Shapiro-Wilk test, and as variables were normally distributed, a 2-sample *t*-test was performed to compare means between the 2 groups under study. Regarding microleakage, data were presented by using the Kaplan-Meier method and compared statistically by using the log-rank test (α=.05). Statistical analysis was performed with a statistical software program (IBM SPSS Statistics, v26.0; IBM Corp).

RESULTS

The microgap of the healing abutment-implant interface and cover screw-implant interface were determined by SEM evaluation of the sectioned assemblies (Figure 6). The quantitative assessment revealed a mean ±standard deviation value of 1.65 ±0.34 µm at the healing abutment-implant interface and a significantly greater value of 2.18 ±0.29 µm at the cover screw-implant interface (*P*=.002) (Figure 7).

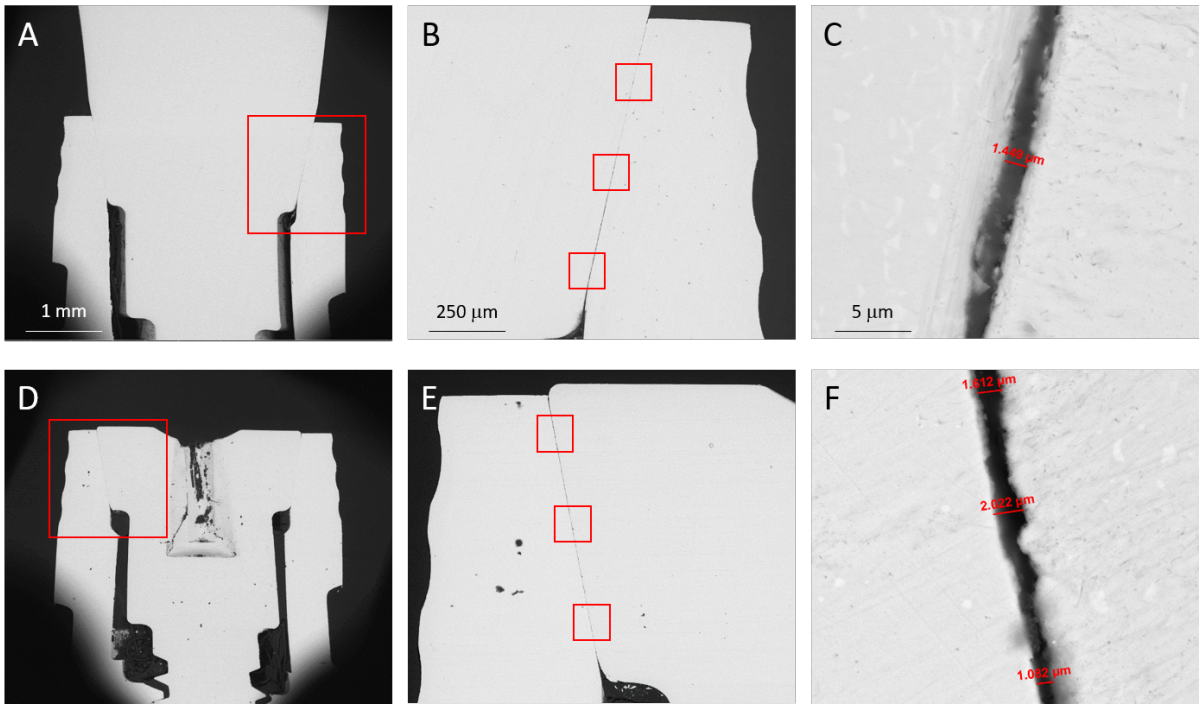

Figure 6. Representative scanning electron micrographs of implant-healing abutment and implant-cover screw interfaces and assessment of interfacial microgap.
A-C, Healing abutment. D-F, Cover screw. Original magnification: A,D, ×50, B,E, ×200, C,F, ×10 000.

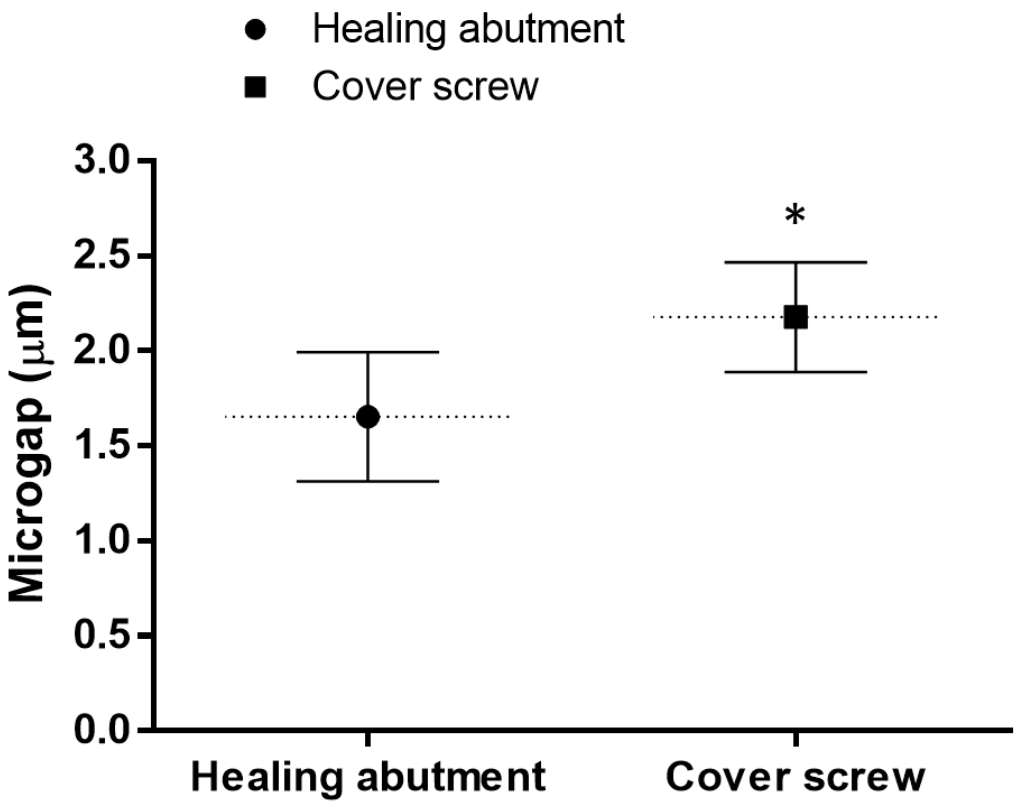

Figure 7. Mean of highest microgap values of system interfaces tightened to 10 Ncm.
*significantly different from healing abutment (t test=3.713, P=.002).

Both assemblies exhibited bacterial leakage in the first days, broadly up to day 9 of the incubation period (Figure 8). For the remaining assemblies, 44% of the healing abutments and 56% of the cover screws showed no bacterial leakage up to 30 days despite the confirmed viability of the bacteria inside the implants. No significant differences were found between the conditions (*P*=.142). SEM imaging of selected assemblies, collected 24 hours after bacterial leakage, revealed the infiltration of *E. faecalis* through the microgap for both the healing abutment-implant and cover screw-implant systems, with a progressive organization of biofilm (Figure 9).

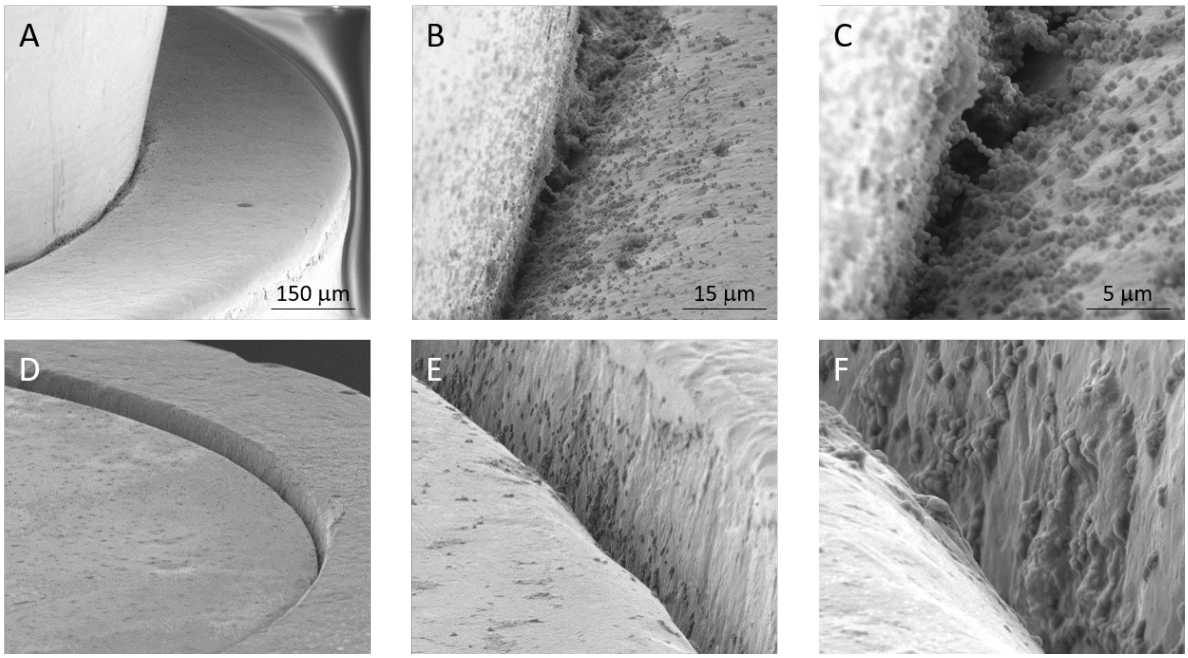

Figure 8. Representative scanning electron micrographs of healing abutment/implant and cover screw/implant interfaces *E. faecalis* leakage.
A-C, Healing abutment. D-F, Cover screw. Original magnification: A,D, ×400, B,E, ×4000, C,F, ×10000.

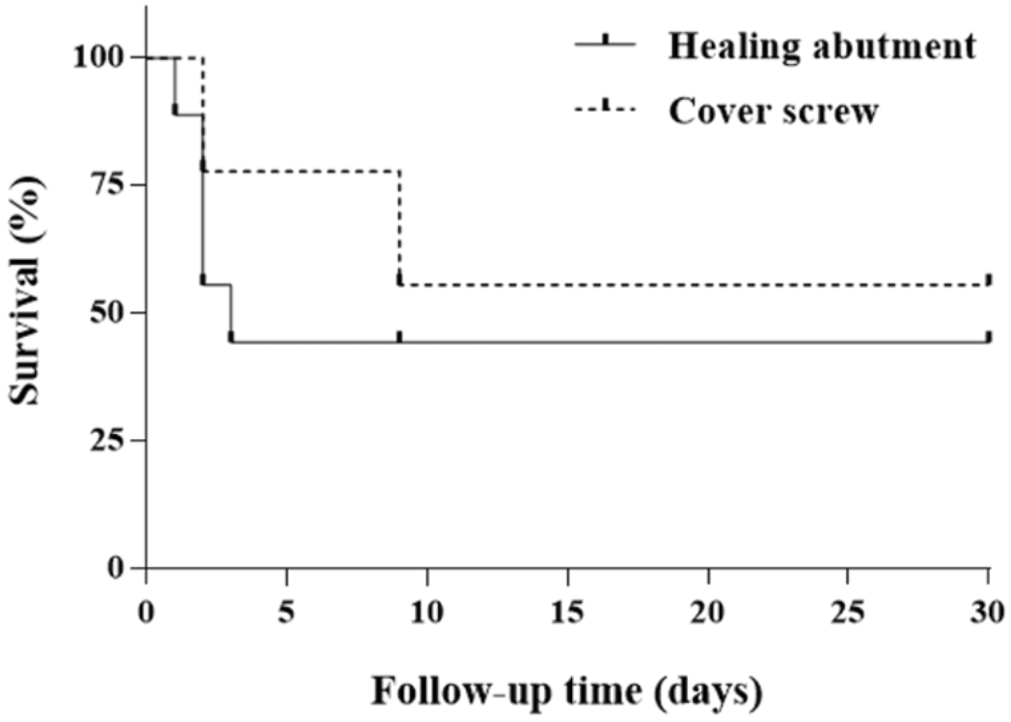

Figure 9. Bacterial microleakage assessment of implant-healing abutment and implant-cover screw interfaces upon *E. faecalis* inoculation and incubation for up to 30 days.

As shown in Figure 10, the application of the sealer homogenously filled the interface and spread to the neighboring surfaces. The sealer completely prevented bacteria leakage throughout the assayed period of 30 days, for both interfaces (Figure 11).

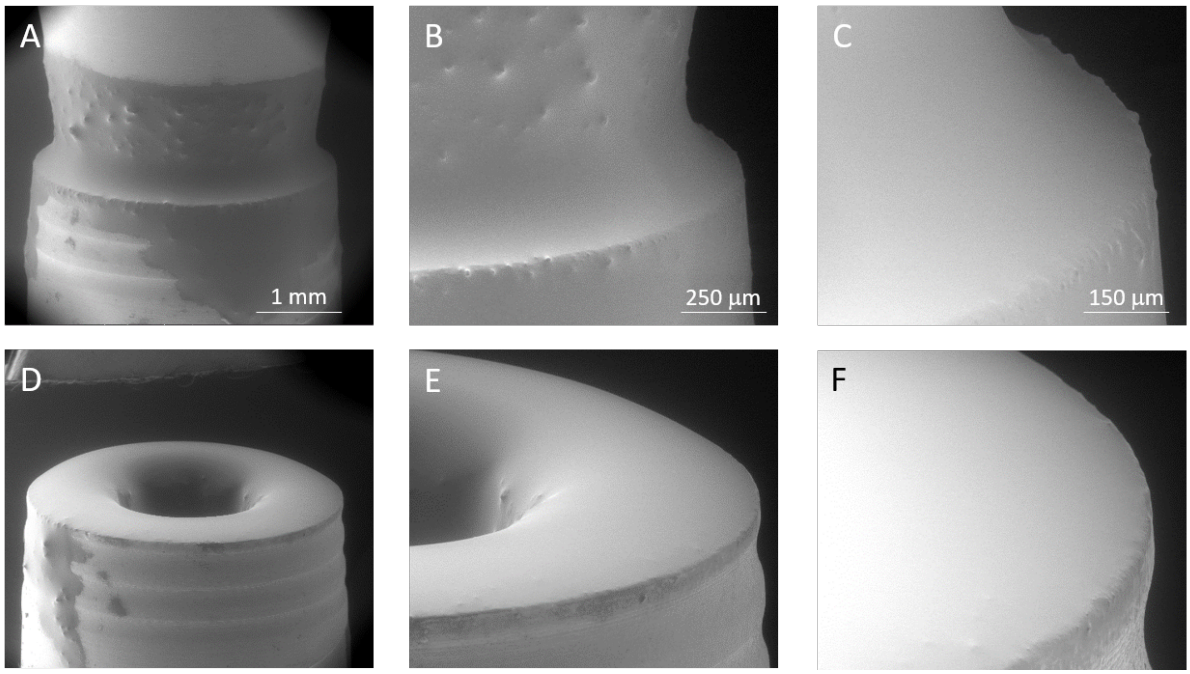

Figure 10. Representative scanning electron micrographs of implant-healing abutment and implant-cover screw interfaces with silicone sealant.
A-C, Healing abutment. D-F, Cover screw. Original magnification: A,D, ×60, B,E, ×150, C,F, ×400.

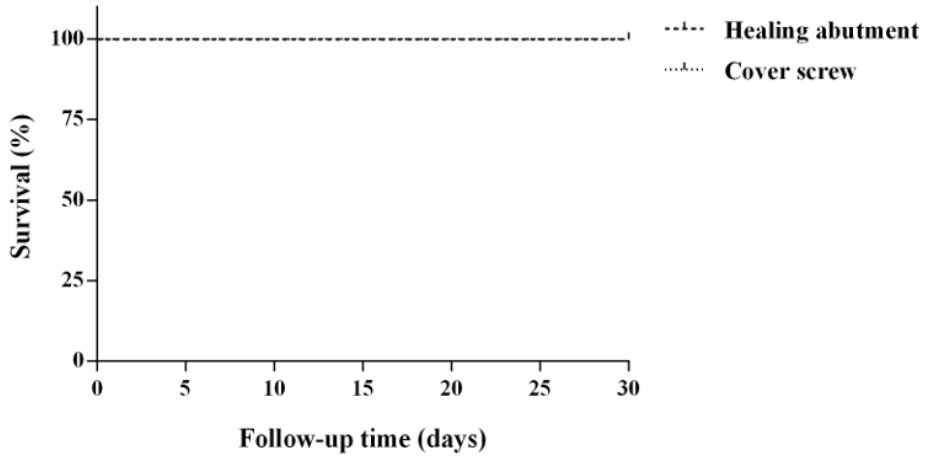

Figure 11. Bacterial microleakage assessment of implant-healing abutment and implant-cover screw interfaces upon E. faecalis inoculation and application of sealing agent up to 30 days.

From the HET-CAM irritation assay, the direct application of the sealer on the CAM induced no significant adverse vascular alterations, hemorrhage, coagulation, or vascular lysis at the 5 minute observation period, similar to the negative control. The irritation index for the sealer and the negative control were both 0.07, corresponding to a no irritation score. The positive control induced an irritation index of 12.80, corresponding to severe irritation. Representative macrographs of the CAM assay are presented in Figure 12.

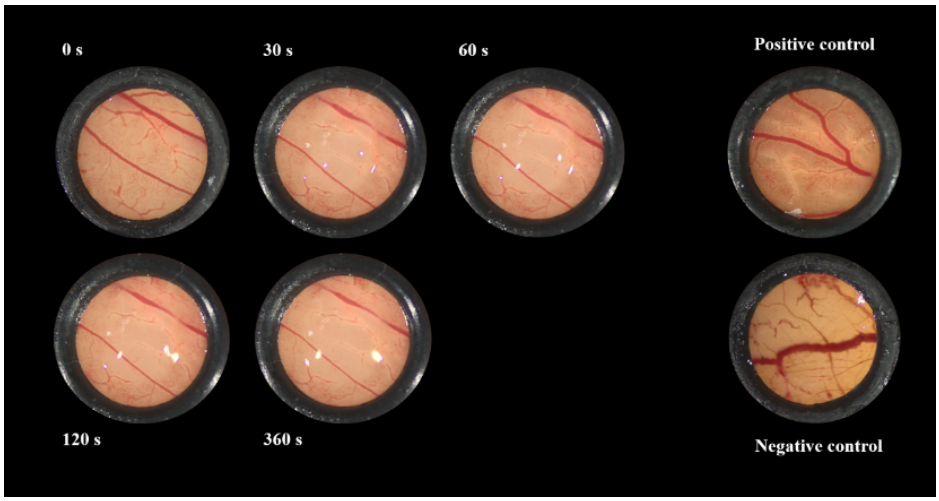

Figure 12. Representative images of HET-CAM assay of sealant at different time points indicating no significant vascular alterations.

DISCUSSION

This *in vitro* study was designed to address the microgap and bacterial microleakage of the implant-cover screw and implant-healing abutment interfaces of a platform-switched implant system, further characterizing the functionality of an interface sealing agent. Based on the results, the null hypothesis that no significant difference would be found in the bacterial microleakage of the assayed system in the presence or absence of the silicone sealer was rejected.

Despite the high success rate of implant-supported restorations,[247] early and late failures of the osseointegration process have been reported and have been correlated with the colonization of pathogenic microorganisms.[251] During the bone remodeling phase, the microgap at the implant-prosthetic abutment interface allows the leakage of bacteria and bacteria-derived products. These may trigger and sustain local immuno-inflammatory activation, possibly culminating in peri-implant bone loss.[252, 253] Nevertheless, information on the role of the interface between the implant and the cover screw or healing abutment, in terms of bacterial microleakage, is sparse.

The interfacial microgap was accessed by SEM, and maximal values were found to be within the 1.5 to 2.0μm range for both interfaces. These values were lower than reported values for the microgap of prosthetic abutment-implant interfaces, (0.1 to 10 μm, before loading).[250] The formation of space at the implant-fixture interface seems to be inevitable because of the technical precision limit on the components' fabrication[258] and may further increase with the cyclic loading during oral function. Space at the interface may allow the movement of bacteria and related metabolic products, promoting the colonization and biofilm development within the internal implant surfaces.

Bacterial microleakage was assayed with *E. faecalis*, which has been used in *in vitro* bacterial microleakage studies addressing implant fixture interfaces.[256] *E. faecalis* are Gram-positive cocci involved in the pathogenesis of oral infections, associated with periodontitis[25] and are frequent colonizers of oral implant surfaces.[267] The role of *E. faecalis* in the pathogenesis of peri-implantitis has been reported.[268] In the present experimental setting, bacterial microleakage was ≈50% of the conditions, with most of the leakage occurring early for both interfaces.

The similar microleakage results for both fixtures might be related to the attained microgap levels—found to be similar for both systems—given the correlation between microgap and interfacial microleakage.[250]

Another factor that may influence microgap and microleakage is the tightening torque of the system's components.[263] In the present experiment, and despite the manufacturers' recommendations to conduct manual tightening without a specific torque value, the insertion torque was adjusted to 10 Ncm for reproducibility. This value was based on previous studies reporting a typical manual tightening torque within a 7 to 14 Ncm range[259] and an average of approximately 10 Ncm (range 7.2 to 10.9 Ncm).[260] A reduction in the interfacial gap with increasing torque values has been reported,[257] resulting from the increased clamping force between the implant and the interfacial fixture, increasing the surface contact area and thus decreasing the interfacial gap.[250] Additionally, higher tightening torques seem to minimize biomechanically related complications, reducing screw loosening and micromotion. Micromotion may interfere with the stability of the soft tissue seal that protects the underlying bone tissue and the bone-implant interface[261] and further contribute to a micropumping effect at the microgap, intensifying the leakage of bacteria and related by-products, as well as increasing the movement of saliva, blood, and crevicular fluid.[262]

A silicone sealing agent was assessed for functionality and biological response. This agent is composed of a viscous silicone matrix and thymol, a monoterpene phenol with established antifungal and antibacterial activity.[270] The product is bioactive and provides a physical and chemical barrier against pathogen colonization of the interfacial microgap. Given its antibacterial activity and ability to fill in the gap spaces, the sealing agent has been used for implant-prosthetic abutment interfaces, with documented *in vitro*[271] and clinical[272] effectiveness. However, the authors are unaware of studies that evaluated its efficacy on healing abutments and implant cover screws. In the present study, the application of the sealing agent was found to prevent bacterial leakage throughout the assessed 30-day period of follow-up. The biological response to the sealant was further addressed in the HET-CAM irritation assay. This characterization system allows the assessment of irritation and inflammation of implanted substances[274, 276] and has been reported to be a reliable alternative to *in vivo* assessment given its suitable reproducibility and sensitivity, which is based on the alteration of vascular endpoints.[275] No significant vascular alterations were detected, with the silicone sealer inducing a response similar to that of the negative control, thus supporting its nonirritation potential.

Limitations of the present study included that the microleakage was evaluated in the absence of mechanical loading. Future studies should evaluate microleakage upon the combined application of dynamic mechanical testing and hydrothermal cycling, modeling for short- and long-term clinical use. Future studies may also include an extended biological characterization of the sealing agent for further endpoints such as cytotoxicity, genotoxicity, and hemocompatibility.

CONCLUSIONS

Based on the findings of this *in vitro* study, the following conclusions were drawn:

1. Despite the low microgap values for both interfaces, bacterial microleakage was found in around 50% of the assayed specimens in a 30-day follow-up period.
2. The silicone sealer tested had no irritation potential according to the HET-CAM assay and provided effective sealing for the systems tested.

CHAPTER 5.

Microgap and bacterial microleakage in prosthetic abutments

Fernandes PF, Grenho L, Fernandes MH, Sampaio-Fernandes JC, Sousa Gomes P. Microgap and microleakage of a hybrid connection platform-switched implant system in the absence or presence of a silicone-based sealing agent. Odontology. 2021 Aug 27. doi: 10.1007/s10266-021-00652-x. (Appendix 2).

INTRODUCTION

In recent years, there has been a great evolution in the field of implantology - both in terms of materials and techniques – and what started as an empirical act of teeth replacement became a well-differentiated therapeutic approach with high success rates.[277]

Osseointegration – the direct apposition of bone within a mechanically-stable implant surface – became a fundamental requirement for the predictable outcome of implant-based treatments, in spite of different factors that seem to hinder the predictability and success of oral rehabilitations.[47] Marginal bone loss seems to be one of the most common complications throughout the distinct bone healing phases, potentially compromising the long-term stability and success of oral rehabilitations. Diverse factors, including peri-implantitis, occlusal overload, surgical trauma, implant/abutment design and physic-chemical properties, as well as microleakage, have been associated with the loss of marginal bone.[278-280]

Currently, the vast majority of implant-based rehabilitations rely on the use of two-piece components - the implant and the abutment, given the system capability to protect the implant from undesirable forces during loading, as well as the ability to orientate the prosthetic angle of the rehabilitation.[281] Regardless of the connection type, the interface between both pieces cannot be precisely matched due to the technical precision limits of production, which originates a microgap at the implant-abutment interface (IAI).[107] In the vast majority of the systems, the microgap ranges from 1 μm to 50 μm upon connection and prior to loading, whether it may increase with cyclic loading under function.[32, 282]

Oral bacteria are within the range of 0.2–1.5μm (width) and 2–10μm (length), and though can easily penetrate the microgap and access the internal cavity of the implant, promoting a product exchange between the implant interior and the oral environment.[283] The movement of bacteria, as well as of bacteria-derived metabolic products and other small molecules and fluids, into and out the internal cavity of the implants, is defined as the IAI microleakage.[100] The accumulation and perpetuation of pathobionts within the gap and cavity of the IAI, neighboring the peri-implant tissues, seems to induce bone resorption, either directly – via the release of cytotoxic byproducts, or indirectly – via the activation of the immune-inflammatory response.[27, 118, 146, 284] Microleakage is further increased by micromovements that elapse from the microabrasion, microshift and/or microrotation that occur between the implant and abutment components during function.[285]

In order to lessen microleakage-related complications, the design of the implant systems has been improved. Platform-switching – relying on a connection platform smaller than the diameter of the implant collar, aim an inward reposition of the IAI, thus distancing microleakage and micromotion events away from the alveolar ridge.[140, 153] Different meta-analytical reviews and clinical trials have reported favorable outcomes in the use of platform-switching, as compared to platform-matching, regarding marginal bone level and bacterial load at the peri-implant tissues, within conical, internal and external polygonal connections.[286, 287] Notwithstanding, no data has been reported on the most recent platform-switched systems with hybrid connections – conjoining a conical interface with an internal polygonal connection, despite the fact that literature supports that all implant/abutment connections present an amount of microgap and associated microleakage, that needs to be addressed.[288, 289]

Accordingly, different strategies have been developed to minimize/prevent bacterial leakage, ranging from the use of disinfectants and antibacterial drugs (e.g., chlorhexidine, doxycycline – aiming to reduce bacterial colonization) to sealing agents (e.g., silicones, o-rings – aiming to physically close the microgap, preventing microleakage).[290, 291] Whether some data has been published for platform-matching systems,[205, 292, 293] to the best of the authors' knowledge, the efficacy of sealing agents has not been assayed in platform-switched interfaces.

Hence, the present study aims to, for the first time: 1) evaluate, *in vitro*, the microgap and microleakage of a platform-switched implant system with hybrid connection, screwed at distinct torque values; and 2) address the efficacy and biological response of a commercial sealing agent, applied within the referred platform-switched implant system with hybrid connection.

MATERIAL AND METHODS

Experimental groups

Platform-switched hybrid connection implants (4 mm in diameter and 11 mm in length – KLOCKNER Vega®, Barcelona, Spain) and titanium straight abutments (total length of 8 mm – KLOCKNER®, Barcelona, Spain), directly supplied by the manufacturer, were selected and assigned to two equal groups according to the torque applied to the assemblies, i.e., 25 Ncm and 5 Ncm (Figure 13). In order to minimize the effect of sedimentation on the screws, which may lead to preload reduction, components were retightened after 10 minutes, with the initial torque value. Before every adjustment, the torquemeter was checked for correct calibration.

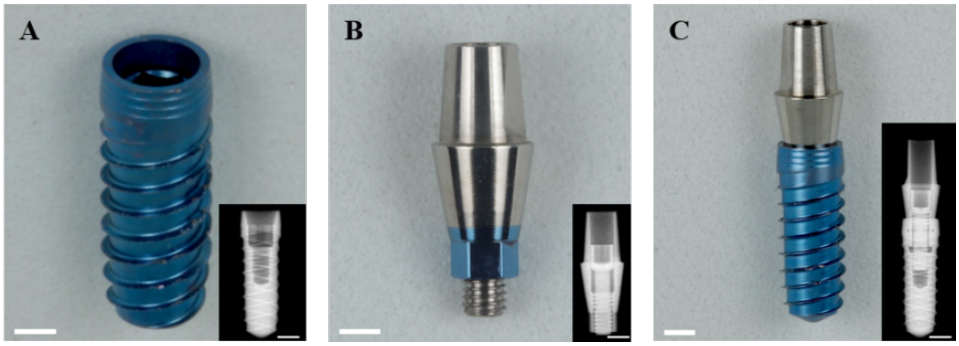

Figure 13. A – Implant Klockner Vega RV 4 x 11 mm; B – Klockner Vega RV 8 mm straight abutment; and C – Assembly implant and straight abutment. Inset images: respective X-ray micrography.
Scale bar 2 mm.

Microgap determination

To determine the width of the gap between the implant and abutment, 20 abutments were connected to the implants and tightened at 25 Ncm (n=10) and 5 Ncm (n=10). The assemblies were following included in a self-polymerizable acrylic resin (Vertex Orthoplast, Vertex-dental, Netherlands) and cut along their longitudinal axis with a diamond wheel mounted on a precision cut-off and grinding machine, with a feed speed of 0.150 mm/s (Struers Accutrom-5, Struers Inc, United States). Sections were then polished, washed and dried. Images of the IAI were obtained under scanning electron microscopy (SEM, Quanta 400 FEG ESEM/EDAX Genesis X4M). Data collection was performed in six positions, 3 on each side

of the IAI (Figure 2A, red squares on center micrographs). Three measures, corresponding to the highest values spaced at least 2.5 µm, were determined and averaged. Data analysis was conducted independently by two calibrated researchers and repeated three times. Calibration consisted of determining measurements in 5 images, in a 2-day interval. Calibration was validated if measurements were similar beyond an agreement level of 90%. The reliability of the intra-examiner measurement was addressed through the intraclass correlation coefficient (ICC) and found to be of high reliability (<0.90).

Microleakage assessment

To evaluate the bacterial microleakage, 20 implants were removed from their packaging under sterile conditions and inoculated with 0.2 µL (an optimized volume regarding the internal capacity of the implant system) of *Enterococcus faecalis* (ATCC 29212) suspension in BHI (Brain Heart Infusion, Liofilchem) broth, at 10^8 colony-forming units (CFU)/mL. After inoculation, the abutments were carefully screwed to the implants at 25 Ncm (n=10) and 5 Ncm (n=10), under sterile conditions. To avoid false positives and discard potential external contaminations, all assemblies were pre-incubated in tubes containing sterile BHI broth for 30 seconds, that were further incubated at 37 °C for 48 hours. Tubes with a cloudy broth (evidencing bacterial growth) led to the exclusion of the respective assembly from further observations. The assemblies, transferred to new tubes with sterile BHI broth, were incubated at 37 °C and 100 rpm up to 30 days. For negative control, abutments were laid on but not tightened to implants free of bacterial inoculation. For positive control, the abutments were laid on but not tightened to implants inoculated with bacterial suspension, as the experimental groups. Visual inspection of the tubes was performed daily. A turbid broth indicated bacterial leakage. If no turbidity was detected after 30 days of incubation, the abutments were carefully unscrewed, and the inner surface of the implants were sampled using sterile paper points to assess the viability of the bacteria. Additionally, bacterial microleakage was qualitatively evaluated by SEM. At selected time-points of incubation, implant/abutment assemblies were washed twice with warm phosphate buffered saline (PBS), fixed for 30 minutes with 1.5% glutaraldehyde in sodium cacodylate (0.14 M) and dehydrated gradually with a series of ethanol solutions. Assemblies were critically point dried (CPD 7501, Polaron Range), sputter-coated with gold/palladium (Au/Pd) thin film and visualized by SEM (FEI Quanta 400FEG ESEM/EDAX Genesis X4M).

Microleakage assessment after implant-abutment sealing

For the efficacy assessment of the sealing strategy, implant-abutment assemblies were inoculated with *E. faecalis*, as aforementioned, loaded with Gapseal® (Hager Werken, Germany) according to the manufacturer's instructions and carefully screwed at 5 Ncm (n=10). Gapseal® is a silicone matrix sealer with thymol antiseptic. The sealed assemblies were characterized by SEM (FEI Quanta 400FEG ESEM/EDAX Genesis X4M). Bacterial microleakage was assessed, as previously detailed, up to 30 days.

To evaluate whether the use of sealer could change the removal torque of the abutments, another set of implant-abutment assemblies were sealed with Gapseal® and screwed as previously described. After 48 hours, all abutments were unscrewed, and the torque of removal recorded. No significant differences were attained regarding torque removal, in the presence or absence of Gapseal® application.

Biological characterization of the sealing agent - *In vitro* cytotoxicity assessment

The biological response of Gapseal® was addressed *in vitro*, with a fibroblastic cell line, in accordance with ISO 10993-5 standards - Biological evaluation of medical devices — Part 5: Tests for *in vitro* cytotoxicity.

Sealer' extracts were prepared on 24-well plates, upon incubation in complete cell culture medium (α-minimum essential medium (α-MEM) supplemented with 10% (v/v) foetal bovine serum, 100 IU/mL penicillin and 100 µg/mL streptomycin (all from Gibco®)), for 24 hours, at 37 °C and a 5 % CO_2 humidified atmosphere.

L929 mouse fibroblast cells (NCTC clone 929, ATCC) were grown in complete cell culture medium (supplemented α-MEM, as described above). Cells were seeded at a density of 10^5 cells/mL into 96-well plates and incubated for 24 hours, at 37 °C, under a 5 % CO_2 humidified atmosphere. After incubation, the cells were exposed to undiluted and diluted (30, 10, 3, 1 and 0.1 %) extracts, in cell culture medium. As negative control, cells were incubated with fresh cell culture medium and as positive control, cells were incubated with dimethyl sulfoxide (DMSO, Panreac®). The plates were incubated for an additional period of 24 hours. Cell viability and cell metabolic activity were evaluated by live/dead staining and MTT assay, respectively. For live/dead staining, the extracts were removed, and cells were incubated with a Calcein AM (1 µM, BioLegend®) and propidium iodide (PI, 50 µL/mL, BD Biosciences®)

solution for 15 minutes at 37 °C, protected from light. Fluorescent cells were visualized and recorded using a Celena S digital imaging system (Logos Biosystems®), equipped with green (488 nm) and red (594 nm) filters. Concerning MTT assay, at the end of the culture period, cells were incubated with 10 % MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium, Sigma-Aldrich), for 3 hours, at 37 °C. The formazan precipitates formed were solubilized in DMSO and the absorbance was measured at 550 nm on a microplate reader (Synergy HT, BioTek®). The results were expressed as percentage of control culture (negative control).

Statistical analysis

Quantitative data are presented as mean ± standard deviation of replicates. Normality was evaluated by the Shapiro-Wilk test and as variables were normally-distributed, a two-sample *t*-test was performed to compare means between the two groups under study. Regarding microleakage, data was presented using the Kaplan-Meier method and compared statistically using the log rank test. The significance level was set at 5% (p values ≤ 0.05 were considered significant). Statistical analysis was performed in the IBM SPSS statistics 26.0 software.

RESULTS

The microgap at the implant-abutment assemblies was determined upon SEM imaging, on 25 Ncm and 5 Ncm torque groups. Representative SEM micrographs and defined areas of evaluation are depicted on Figure 14A. Briefly, a reduced microgap length was identified on the 25 Ncm group (within the 0.25 µm range), as compared to the 5 Ncm group (within the 1.25 µm range), as shown on representative SEM images (Figure 14A) and calculated averaged maximal values (Figure 14B).

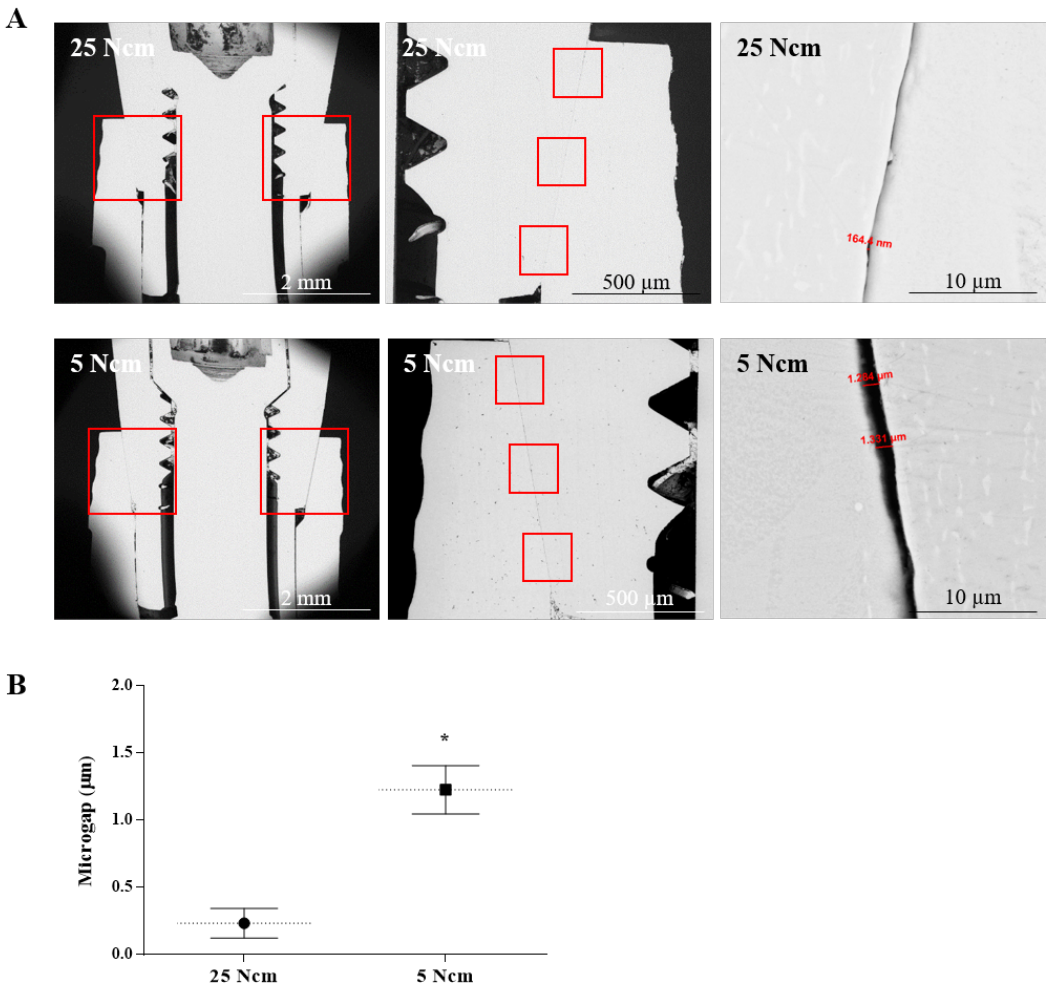

Figure 14. A – SEM micrographs of the implant/abutment interface at different magnifications, and B – Mean of the highest microgap values in the implant/abutment assemblies, tightened at 25 Ncm and 5 Ncm.

Bacterial microleakage was assessed for 30 days, following implant inoculation with *E. faecalis* and abutment assembly, at either 25 Ncm or 5 Ncm torques. Assemblies of the 25

Ncm group showed no leakage during the experimental period. On the other hand, assemblies tightened at 5 Ncm allowed bacteria microleakage. Until day 3, 38% of the assemblies presented turbidity in the culture medium, as consequence of microbial leakage, while the remaining implants showed no signs of leakage over the 30 days of follow-up (Figure 15A). SEM imaging revealed no bacterial leakage for 25 Ncm condition and the gradual infiltration of *E. faecalis* trough the IAI, for assemblies tightened at 5 Ncm (Figure 15B). Higher magnifications images showed the presence of *E. faecalis* organized in biofilm and extensively colonizing implant and abutment's surfaces.

Given the absence of bacterial microleakage at the 25 Ncm group, the assessment of the efficacy of the sealing agent (Gapseal®) was conducted exclusively at 5 Ncm torque. SEM imaging revealed a complete filling/covering of the IAI (Figure 16A) and a complete efficacy on preventing the microleakage of bacterial suspension throughout the experimental period of 30 days (Figure 16B).

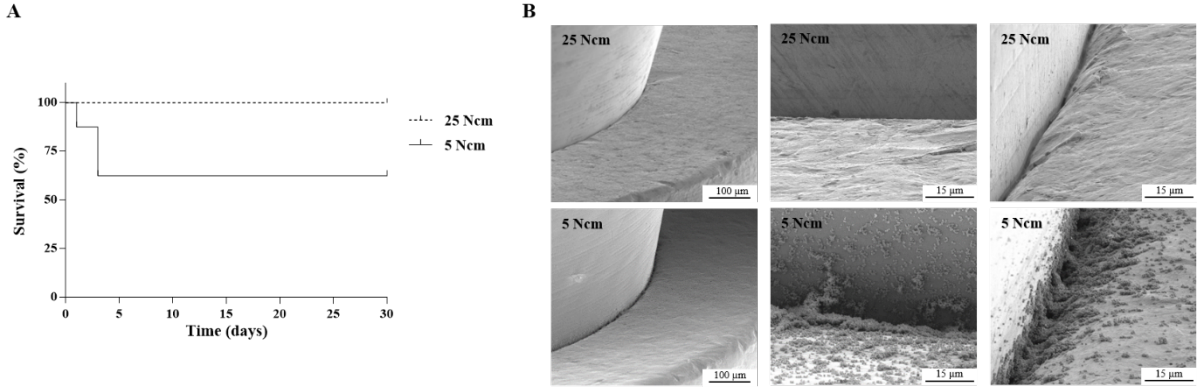

Figure 15. A – Bacterial microleakage assessment for implant-abutments assemblies tightened at 25 Ncm or 5 Ncm, after *E. faecalis* inoculation, up to 30 days of follow-up. B – SEM imaging of the IAI of 25 Ncm and 5 Ncm conditions.

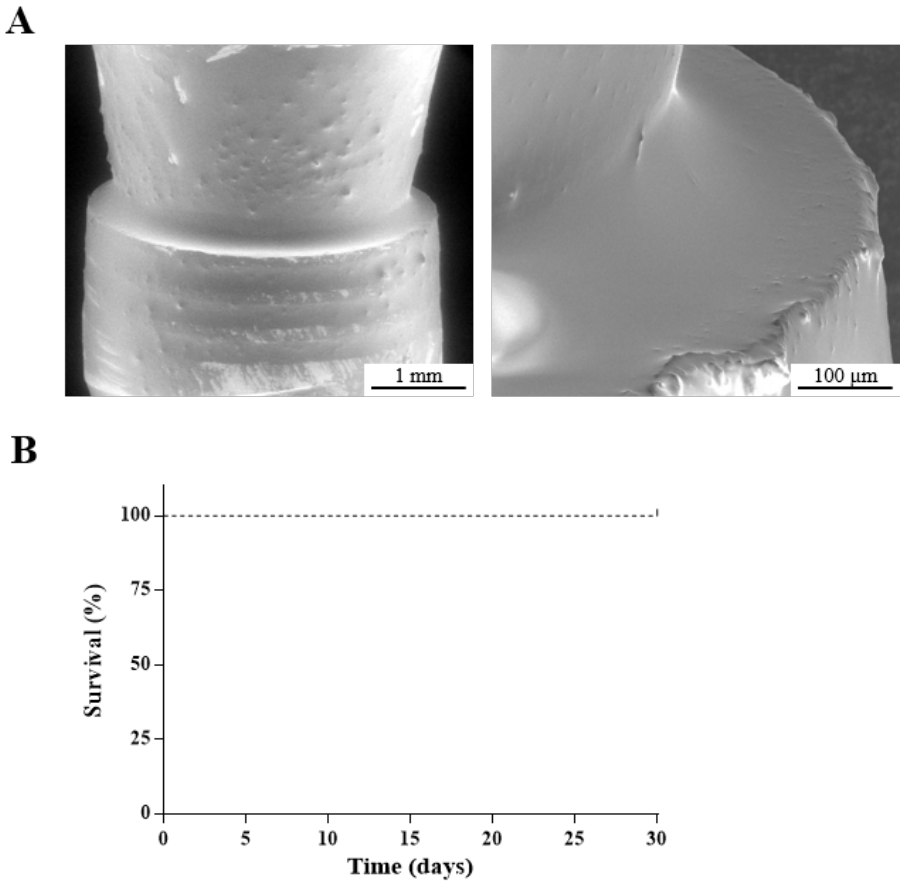

Figure 16. A – SEM imaging of the implant/abutment interface following the application of the sealing agent, at two different magnifications. B – Bacterial microleakage assessment for implant-abutments assemblies tightened at 5 Ncm, after *E. faecalis* inoculation and application of Gapseal®, up to 30 days of follow-up.

The biological characterization of the sealer was further assessed *in vitro*, with fibroblastic cells grown in the presence of sealer' extract at distinct concentrations (ranging from the undiluted extract - 100%, to a 0.3% dilution), in accordance with ISO 10993-5 standards. Undiluted sealer' extract significantly decrease the metabolic activity of the cultures, as compared to control. On the other hand, diluted Gapseal® extracts, within the 0.3-30 % range, presented no significant differences to control (Figure 17A). For these dilutions and through live/dead fluorescent imaging, cultures revealed a high viability evidenced by the predominant green fluorescence, with cells presenting an elongated morphology and significant cytoplasmic spreading, similar to negative control (Figure 17B).

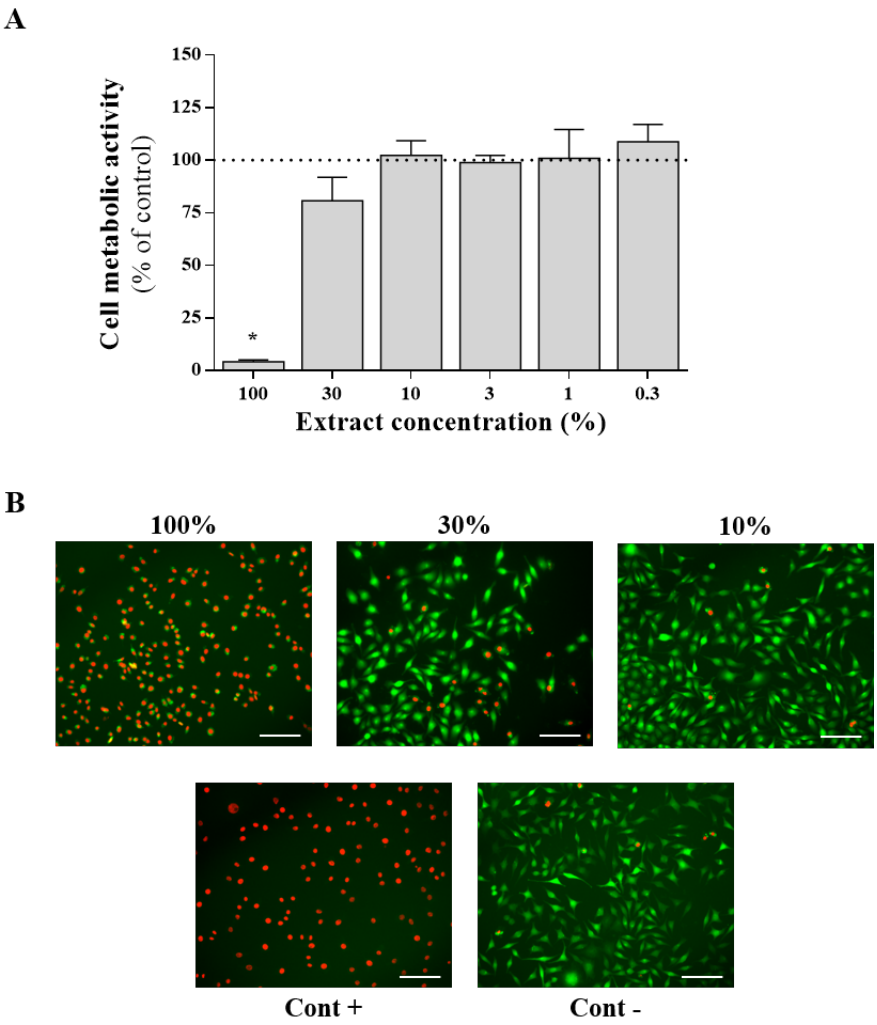

Figure 17. *In vitro* biological evaluation of Gapseal® extracts following culture with L929 cells, for 24 hours, as determined by (A) cell metabolic activity and (B) fluorescence live/dead cell staining.
Live cells stain green and dead cells stain red. Negative control: cells exposed to culture medium and positive control: cells exposed to DMSO. (scale bar: 100 μm). *Significantly different from negative control (dotted line), $p < 0.05$.

DISCUSSION

This study aimed to characterize, for the first time, the microgap and microleakage of a platform-switched implant system with a hybrid connection, with distinct torques at the implant/abutment assembly (i.e., 25 Ncm and 5 Ncm), further addressing the efficacy and biological response of a commercially available sealing agent applied at the implant/abutment interface (IAI).

Platform-switched systems, based on the inward shifting of the abutment contour over the implants, reduce the stress concentration at the crestal bone level and improve soft tissue stabilization, allowing an increased preservation of the peri-implant bone level.[153, 294] Biologically, platform-switching allows more surface area for the establishment of the biological width and, by distancing leakage – and IAI motion-related events away from the crestal ridge, further prevents bacterial contamination and inflammatory cell infiltration that were associated with the marginal bone loss.[140] Additionally, platform-switched systems have been combined with conical connections, further reducing microleakage by the cold-welding effect, thus restricting bacterial penetration and pumping effect inwards and outwards of the implant chamber.[282] Conical connections demonstrated an improved sealing performance and abutment stability, as well as long-term clinical functionality and resistance to bending forces and fatigue, as compared to external and internal polygonal connections.[110, 112, 295]

Recent modifications on implant systems introduced hybrid connections, as the one evaluated within this study, elapsing from the combination of a conic and an internal polygonal connection, claiming an increased component fit and antirotational characteristics.[100] Whether these connections seem to display improved clinical outcomes given the lower rotation center and improved mechanic stability,[106, 296, 297] no data is available regarding bacterial penetration and microleakage of these systems, as addressed in this research.

Furthermore, and despite the influence of the implant/abutment connection on the system microleakage,[282] the torque of the IAI also seems to be a major determinant.[152] In the present study, distinct torque values were used to mimic the clinical application – the 25 Ncm torque reflects the recommendations of the manufacturer, while the 5 Ncm torque simulates a decreased stability at the IAI, elapsing from a long-term function under loading. Screw loosening, rotational forces and the settling effect decrease the screw preload and interface tolerance of the implant-abutment assembly,[116] with a decreased torque level being selected to mimic this clinical situation.

In this study microgap was evaluated by SEM imaging revealing very low IAI misfit regardless of the applied torque load – maximal values ranged around 0.25 μm and 1.25 μm, for 25 Ncm and 5 Ncm torques, respectively. Hybrid implant/abutment connections are expected to display a reduced misfit, given the improved surface area of contact, load distribution and anti-rotational fit of the components, performing superiorly to external and internal connections.[288, 297] Additionally, a close relationship between the adjustment of the IAI and the torque of the prosthetic abutment screw has been established, as presently demonstrated, with the highest gap values being attained at decreased torque levels.[222, 298] Mechanistically, the reduction of the strengthening torque decreases the stress responsible for the elongation of the screw, thus lessening the compressive force that approximates the implant and abutment surfaces, diminishing the linear area of contact and increasing misfit.[299]

The gap at the IAI seems to be directly related to the microleakage of the system, potentially associated with the passage of bacteria, bacteria-derived products, molecules and fluids through the IAI, contributing to the inflammation of peri-implant tissues and subsequent bone loss that may compromise the success of implant-based rehabilitations.[100] In the present work, microleakage assessment was evaluated for 30 days upon colonization of the implant chamber with *E. faecalis* suspension. *E. faecalis* is a Gram-positive coccus, commonly associated with a wide range of infections in the oral cavity, such as primary and secondary endodontic infections, dental caries, peri-radicular abscesses, periodontitis and peri-implantitis, being considered a keystone pathogen within the peri-implantitis-related bone loss [300, 301]. *E. faecalis* has been routinely used on microleakage studies at the IAI.[282] In the present study, bacterial leakage was not identified on the 25 Ncm group, while for the 5 Ncm group, leakage was established on 38% of the samples up to 3 days of the experimental period. This data supports the high efficacy of the system on precluding microleakage when recommended torque values were used, i.e., 25 Ncm. Notwithstanding, low torque conditions allowed microleakage, supporting the need of further therapeutic interventions to prevent bacterial penetration into the IAI.

Whether distinct approaches have been assayed to physically close the IAI, and consequently the microgap, the use of sealing agents seems to be effective on minimizing bacterial leakage, as recently reviewed in a meta-analysis.[302] Sealing gels improved the fastening and long-term stability of the IAI and decreased microleakage in both conical/internal and external connection implant systems.[293] Nonetheless, no data is available regarding the sealing efficacy for hybrid connection platform-switched systems. In this research, even in low

torque conditions (5 Ncm), the application of the sealing agent was effective on preventing microleakage for the 30-day experimental period. The used sealer is composed of a highly viscous silicone matrix with thymol – a natural phenolic monoterpene with effective antibacterial activity,[303] and seems to occupy the spaces within the IAI, creating a physic and chemical barrier for microorganisms' colonization. The biological response of the sealer was further addressed *in vitro*, within a fibroblastic cell line, in accordance with the ISO 10993-5 standards. An adequate cytocompatible profile was attained, with no significant differences being established regarding cultures grown in the presence of diluted sealer extracts and the control. Silicone-based materials report an adequate cytocompatibility and biocompatibility, being commonly used within medical-related applications.[304] Additionally, thymol, apart from the effective antibacterial activity, seems to induce an adequate biological response, thus modulating the inflammation and enhancing the wound healing environment.[303, 305]

Within the present experimental design, care was given to address the major methodologic limitations acknowledged to influence the *in vitro* microleakage assessment of the implant-abutment interface, namely the critical selection of the bacterial species, optimization of the volume and concentration of the bacterial inoculum, standardization of implant-abutment manipulation, and validation of the bacterial viability at selected end-points.[306] Nonetheless, future studies may consider the conjoined application of cyclic loading and hydrothermal cycling, further mimicking the clinical application of the system.

CONCLUSIONS

This research addresses, for the first time, the assessment of the microgap and the bacterial microleakage of a hybrid connection platform-switched implant system, further evaluating the efficacy of a sealing agent at low torque conditions. Overall, the implant system presented a very low misfit at the IAI, even in low torque conditions. The use of recommended torque values (25 Ncm) was able to prevent bacterial microleakage, while reduced torque levels (5 Ncm) allowed for bacterial microleakage in 38% of the cases. Notwithstanding, the use of a sealing agent, reporting an adequate cytocompatibility profile, was found to restore the sealing effectiveness of the system.

CHAPTER 6. Concluding remarks

Nowadays, most of the available dental implant systems are two-piece systems and although the effort of manufacturers, developed systems are not able to completely prevent microleakage at the implant-abutment interface, leaving the potential risk of inflammation of the surrounding tissues. This microbial colonization can be hindered or enhanced by the marginal fit of the implant-abutment connection, thus a tight seal at the implant-abutment interface is of major importance so that bacterial colonization may be prevented.

Regarding the microgap between implants and healing abutments and/or cover screws, it has been proved to tolerate bacterial microleakage, possibly fostering a pathogenic biofilm during the early healing stages that may impair the sealing of epithelial tissues at the implant surface and promote peri-implant inflammation.

Preventing bacterial access to the microgap with a sealing agent should improve the outcome of implant-supported restorations, reducing the probability of early- and late-stage complications.

Overall, the implant system used in this study presented a very low misfit at the implant-abutment interface, even in low torque conditions, revealing an excellent behavior when using recommended torque values, even preventing bacterial microleakage. Although reduced torque levels allowed for bacterial microleakage, the use of a sealing agent could restore the sealing effectiveness of the system, within the assayed experimental model.

In vitro studies poorly mimic the reality of the oral environment, nevertheless they can be useful to understand the dynamics of the implant-abutment interface. On the other hand, studying non-loading conditions grant the possibility to independently evaluate the microbial penetration in the implant-abutment interface as a consequence to the precision fit between the implant and the abutment, and the torque forces applied.

Within the present experimental design, care was given to address the major methodologic limitations like the critical selection of the bacterial species, optimization of the volume inoculated, and validation of the bacterial viability.

It is important to notice that the presence of microorganisms in the implant-abutment interface doesn’t always lead to implant failure or compromise all rehabilitations in a short period of time. However, if the rehabilitation is in an esthetic area, the presence of microorganisms may be fundamental as minimal bone loss can affect peri-implant tissues compromising the shape and contour.

To overcome the deleterious effect of the microgap, it is recommended to use implant-abutment interfaces with internal connection and platform-switching as well as respecting

manufacturers' recommended torque values, as these procedures aim to minimize microgaps and the eventual adverse effects of microleakage.

The use of sealing agents seems to help preventing bacterial contamination, as they could act as a lubricant, preload values can be higher, reducing microgap and subsequent microleakage, ultimately decreasing the incidence of prosthetic complications.

Further studies are needed to evaluate long-term stability of the sealing agent through time and aging and may consider the conjoined application of cyclic loading and hydrothermal cycling to mimic the clinical application of the system.

Future studies may also include an extended biological characterization of the sealing agent for further endpoints such as cytotoxicity, genotoxicity, and hemocompatibility.

REFERENCES

1. Ring ME. A thousand years of dental implants: a definitive history--part 1. Compend Contin Educ Dent. 1995;16(10):1060, 2, 4 passim.
2. Astrand P, Ahlqvist J, Gunne J, Nilson H. Implant treatment of patients with edentulous jaws: a 20-year follow-up. Clin Implant Dent Relat Res. 2008;10(4):207-17.
3. Jung RE, Zembic A, Pjetursson BE, Zwahlen M, Thoma DS. Systematic review of the survival rate and the incidence of biological, technical, and aesthetic complications of single crowns on implants reported in longitudinal studies with a mean follow-up of 5 years. Clin Oral Implants Res. 2012;23 Suppl 6:2-21.
4. Qian J, Wennerberg A, Albrektsson T. Reasons for marginal bone loss around oral implants. Clin Implant Dent Relat Res. 2012;14(6):792-807.
5. Zipprich H, Weigl P, Ratka C, Lange B, Lauer HC. The micromechanical behavior of implant-abutment connections under a dynamic load protocol. Clin Implant Dent Relat Res. 2018;20(5):814-23.
6. Grecchi F, M DIG, Cura F, Candotto V, Carinci F. A new system of implant abutment connection: how to improve a two piece implant system sealing. Oral Implantol (Rome). 2017;10(3):234-40.
7. Hartog L, Meijer HJ, Santing HJ, Vissink A, Raghoebar GM. Patient satisfaction with single-tooth implant therapy in the esthetic zone. Int J Prosthodont. 2014;27(3):226-8.
8. Quaresma SE, Cury PR, Sendyk WR, Sendyk C. A finite element analysis of two different dental implants: stress distribution in the prosthesis, abutment, implant, and supporting bone. J Oral Implantol. 2008;34(1):1-6.
9. Zembic A, Kim S, Zwahlen M, Kelly JR. Systematic review of the survival rate and incidence of biologic, technical, and esthetic complications of single implant abutments supporting fixed prostheses. Int J Oral Maxillofac Implants. 2014;29 Suppl:99-116.
10. Adell R, Lekholm U, Rockler B, Branemark PI. A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. Int J Oral Surg. 1981;10(6):387-416.
11. Hunt PR, Gartner JL, Norkin FJ. Choice of a dental implant system. Compend Contin Educ Dent. 2005;26(4):239-40, 42, 45-8 passim; quiz 51, 71.
12. Bornstein MM, Halbritter S, Harnisch H, Weber HP, Buser D. A retrospective analysis of patients referred for implant placement to a specialty clinic: indications, surgical procedures, and early failures. The International journal of oral & maxillofacial implants. 2008;23(6):1109-16.
13. Meraw SJ, Eckert SE, Yacyshyn CE, Wollan PC. Analysis of surgical referral patterns for endosseous dental implants. The International journal of oral & maxillofacial implants. 1999;14(2):265-70.
14. Klokkevold PR, Han TJ. How do smoking, diabetes, and periodontitis affect outcomes of implant treatment? The International journal of oral & maxillofacial implants. 2007;22 Suppl:173-202.
15. Krebs M, Schmenger K, Neumann K, Weigl P, Moser W, Nentwig GH. Long-term evaluation of ANKYLOS(R) dental implants, part i: 20-year life table analysis of a longitudinal study of more than 12,500 implants. Clin Implant Dent Relat Res. 2015;17 Suppl 1:e275-86.

16. Trullenque-Eriksson A, Guisado-Moya B. Retrospective long-term evaluation of dental implants in totally and partially edentulous patients. Part I: survival and marginal bone loss. Implant Dent. 2014;23(6):732-7.
17. Trullenque-Eriksson A, Guisado Moya B. Retrospective long-term evaluation of dental implants in totally and partially edentulous patients: part II: peri-implant disease. Implant Dent. 2015;24(2):217-21.
18. Tettamanti L, Bassi MA, Trapella G, Candotto V, Tagliabue A. Applications of biomaterials for bone augmentation of jaws: clinical outcomes and in vitro studies. Oral Implantol (Rome). 2017;10(1):37-44.
19. Esposito M, Grusovin MG, Willings M, Coulthard P, Worthington HV. The effectiveness of immediate, early, and conventional loading of dental implants: a Cochrane systematic review of randomized controlled clinical trials. Int J Oral Maxillofac Implants. 2007;22(6):893-904.
20. Caricasulo R, Malchiodi L, Ghensi P, Fantozzi G, Cucchi A. The influence of implant-abutment connection to peri-implant bone loss: A systematic review and meta-analysis. Clin Implant Dent Relat Res. 2018;20(4):653-64.
21. Pita MS, Anchieta RB, Barao VA, Garcia IR, Jr., Pedrazzi V, Assuncao WG. Prosthetic platforms in implant dentistry. J Craniofac Surg. 2011;22(6):2327-31.
22. Jemt T, Laney WR, Harris D, Henry PJ, Krogh PH, Jr., Polizzi G, et al. Osseointegrated implants for single tooth replacement: a 1-year report from a multicenter prospective study. Int J Oral Maxillofac Implants. 1991;6(1):29-36.
23. Naert I, Quirynen M, van Steenberghe D, Darius P. A study of 589 consecutive implants supporting complete fixed prostheses. Part II: Prosthetic aspects. J Prosthet Dent. 1992;68(6):949-56.
24. Becker W, Becker BE. Replacement of maxillary and mandibular molars with single endosseous implant restorations: a retrospective study. J Prosthet Dent. 1995;74(1):51-5.
25. Sahin S, Cehreli MC. The significance of passive framework fit in implant prosthodontics: current status. Implant Dent. 2001;10(2):85-92.
26. Berglundh T, Lindhe J. Dimension of the peri-implant mucosa. Biological width revisited. J Clin Periodontol. 1996;23(10):971-3.
27. Broggini N, McManus LM, Hermann JS, Medina R, Schenk RK, Buser D, et al. Peri-implant inflammation defined by the implant-abutment interface. J Dent Res. 2006;85(5):473-8.
28. Lang NP, Berglundh T, Working Group 4 of Seventh European Workshop on P. Peri-implant diseases: where are we now?--Consensus of the Seventh European Workshop on Periodontology. J Clin Periodontol. 2011;38 Suppl 11:178-81.
29. Fu JH, Hsu YT, Wang HL. Identifying occlusal overload and how to deal with it to avoid marginal bone loss around implants. Eur J Oral Implantol. 2012;5 Suppl:S91-103.
30. do Nascimento C, Miani PK, Watanabe E, Pedrazzi V, de Albuqerque RF, Jr. In vitro evaluation of bacterial leakage along the implant-abutment interface of an external-hex implant after saliva incubation. Int J Oral Maxillofac Implants. 2011;26(4):782-7.
31. do Nascimento C, Miani PK, Pedrazzi V, Goncalves RB, Ribeiro RF, Faria AC, et al. Leakage of saliva through the implant-abutment interface: in vitro evaluation of three different implant connections under unloaded and loaded conditions. Int J Oral Maxillofac Implants. 2012;27(3):551-60.

32. Quirynen M, van Steenberghe D. Bacterial colonization of the internal part of two-stage implants. An in vivo study. Clin Oral Implants Res. 1993;4(3):158-61.
33. Aloise JP, Curcio R, Laporta MZ, Rossi L, da Silva AM, Rapoport A. Microbial leakage through the implant-abutment interface of Morse taper implants in vitro. Clin Oral Implants Res. 2010;21(3):328-35.
34. Tey VHS, Phillips R, Tan K. Five-year retrospective study on success, survival and incidence of complications of single crowns supported by dental implants. Clin Oral Implants Res. 2017;28(5):620-5.
35. Blum K, Wiest W, Fella C, Balles A, Dittmann J, Rack A, et al. Fatigue induced changes in conical implant-abutment connections. Dent Mater. 2015;31(11):1415-26.
36. Ricomini Filho AP, Fernandes FS, Straioto FG, da Silva WJ, Del Bel Cury AA. Preload loss and bacterial penetration on different implant-abutment connection systems. Braz Dent J. 2010;21(2):123-9.
37. Duarte AR, Rossetti PH, Rossetti LM, Torres SA, Bonachela WC. In vitro sealing ability of two materials at five different implant-abutment surfaces. J Periodontol. 2006;77(11):1828-32.
38. da Silva-Neto JP, Nobilo MA, Penatti MP, Simamoto PC, Jr., das Neves FD. Influence of methodologic aspects on the results of implant-abutment interface microleakage tests: a critical review of in vitro studies. Int J Oral Maxillofac Implants. 2012;27(4):793-800.
39. Matsubara VH, Igai F, Tamaki R, Tortamano Neto P, Nakamae AE, Mori M. Use of Silver Nanoparticles Reduces Internal Contamination of External Hexagon Implants by Candida albicans. Braz Dent J. 2015;26(5):458-62.
40. Branemark PI, Adell R, Breine U, Hansson BO, Lindstrom J, Ohlsson A. Intra-osseous anchorage of dental prostheses. I. Experimental studies. Scand J Plast Reconstr Surg. 1969;3(2):81-100.
41. Branemark PI. Osseointegration and its experimental background. J Prosthet Dent. 1983;50(3):399-410.
42. Francischone CE VL, Bränemark P. Osseointegration and esthetics in single tooth rehabilitation. 1 ed: Quintessence Publishing Co, Inc; 2000.
43. Lindhe J KT, Lang NP. Clinical periodontology and implant dentistry. Fourth ed. Munksgaard: Blackwell Publishing Co; 2003.
44. Branemark PI, Hansson BO, Adell R, Breine U, Lindstrom J, Hallen O, et al. Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. Scand J Plast Reconstr Surg Suppl. 1977;16:1-132.
45. Alvim-Pereira F, Montes CC, Mira MT, Trevilatto PC. Genetic susceptibility to dental implant failure: a critical review. The International journal of oral & maxillofacial implants. 2008;23(3):409-16.
46. Kronstrom M, Svenson B, Hellman M, Persson GR. Early implant failures in patients treated with Branemark System titanium dental implants: a retrospective study. The International journal of oral & maxillofacial implants. 2001;16(2):201-7.
47. Misch CE. Endosteal implants for posterior single tooth replacement: alternatives, indications, contraindications, and limitations. J Oral Implantol. 1999;25(2):80-94.
48. Dula K, Mini R, van der Stelt PF, Buser D. The radiographic assessment of implant patients: decision-making criteria. The International journal of oral & maxillofacial implants. 2001;16(1):80-9.

49. Renouard F RB. Risk factors in implant dentistry: simplified clinical analysis for predictable treatment. Illinois: Quintessence Publishing Co; 1999.
50. Berglundh T, Abrahamsson I, Welander M, Lang NP, Lindhe J. Morphogenesis of the peri-implant mucosa: an experimental study in dogs. Clin Oral Implants Res. 2007;18(1):1-8.
51. Albrektsson T, Branemark PI, Hansson HA, Lindstrom J. Osseointegrated titanium implants. Requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. Acta Orthop Scand. 1981;52(2):155-70.
52. Bousquet P, Bennasar IC, Tramini P, Jacquemot M, Cuisinier F. Tightening of healing abutments: influence of torque on bacterial proliferation risk, an in vitro investigation. Biomed Tech (Berl). 2014;59(6):495-500.
53. Weber HP, Buser D, Donath K, Fiorellini JP, Doppalapudi V, Paquette DW, et al. Comparison of healed tissues adjacent to submerged and non-submerged unloaded titanium dental implants. A histometric study in beagle dogs. Clin Oral Implants Res. 1996;7(1):11-9.
54. Sasada Y, Cochran DL. Implant-Abutment Connections: A Review of Biologic Consequences and Peri-implantitis Implications. Int J Oral Maxillofac Implants. 2017;32(6):1296-307.
55. Callan DP, Cobb CM, Williams KB. DNA probe identification of bacteria colonizing internal surfaces of the implant-abutment interface: a preliminary study. J Periodontol. 2005;76(1):115-20.
56. Albrektsson T, Zarb G, Worthington P, Eriksson AR. The long-term efficacy of currently used dental implants: a review and proposed criteria of success. Int J Oral Maxillofac Implants. 1986;1(1):11-25.
57. Bränemark P, Zarb G, Albrektsson T. Prótesis tejido-integradas: la osseointegración e la odontologia clinica. Berlim.: Quintessence Publishing Co, Inc; 1987.
58. Smith DE, Zarb GA. Criteria for success of osseointegrated endosseous implants. The Journal of prosthetic dentistry. 1989;62(5):567-72.
59. Piattelli A, Scarano A, Piattelli M. Microscopical aspects of failure in osseointegrated dental implants: a report of five cases. Biomaterials. 1996;17(12):1235-41.
60. Mellonig JT, Griffiths G, Mathys E, Spitznagel J, Jr. Treatment of the failing implant: case reports. Int J Periodontics Restorative Dent. 1995;15(4):384-95.
61. Esposito M, Hirsch J, Lekholm U, Thomsen P. Differential diagnosis and treatment strategies for biologic complications and failing oral implants: a review of the literature. Int J Oral Maxillofac Implants. 1999;14(4):473-90.
62. Esposito M, Thomsen P, Molne J, Gretzer C, Ericson LE, Lekholm U. Immunohistochemistry of soft tissues surrounding late failures of Branemark implants. Clin Oral Implants Res. 1997;8(5):352-66.
63. Lekholm U, Gunne J, Henry P, Higuchi K, Linden U, Bergstrom C, et al. Survival of the Branemark implant in partially edentulous jaws: a 10-year prospective multicenter study. The International journal of oral & maxillofacial implants. 1999;14(5):639-45.
64. Ashley ET, Covington LL, Bishop BG, Breault LG. Ailing and failing endosseous dental implants: a literature review. The journal of contemporary dental practice. 2003;4(2):35-50.
65. Hutton JE, Heath MR, Chai JY, Harnett J, Jemt T, Johns RB, et al. Factors related to success and failure rates at 3-year follow-up in a multicenter study of overdentures

supported by Branemark implants. The International journal of oral & maxillofacial implants. 1995;10(1):33-42.
66. Esposito M, Hirsch JM, Lekholm U, Thomsen P. Biological factors contributing to failures of osseointegrated oral implants. (II). Etiopathogenesis. European journal of oral sciences. 1998;106(3):721-64.
67. Fugazzotto PA. Success and failure rates of osseointegrated implants in function in regenerated bone for 72 to 133 months. The International journal of oral & maxillofacial implants. 2005;20(1):77-83.
68. Moy PK, Medina D, Shetty V, Aghaloo TL. Dental implant failure rates and associated risk factors. The International journal of oral & maxillofacial implants. 2005;20(4):569-77.
69. Quirynen M, Teughels W. Microbiologically compromised patients and impact on oral implants. Periodontology 2000. 2003;33:119-28.
70. Esposito M, Thomsen P, Ericson LE, Lekholm U. Histopathologic observations on early oral implant failures. The International journal of oral & maxillofacial implants. 1999;14(6):798-810.
71. Friberg B, Jemt T, Lekholm U. Early failures in 4,641 consecutively placed Branemark dental implants: a study from stage 1 surgery to the connection of completed prostheses. The International journal of oral & maxillofacial implants. 1991;6(2):142-6.
72. Rosenberg ES, Cho SC, Elian N, Jalbout ZN, Froum S, Evian CI. A comparison of characteristics of implant failure and survival in periodontally compromised and periodontally healthy patients: a clinical report. The International journal of oral & maxillofacial implants. 2004;19(6):873-9.
73. Weyant RJ, Burt BA. An assessment of survival rates and within-patient clustering of failures for endosseous oral implants. Journal of dental research. 1993;72(1):2-8.
74. Bert M. Complicaciones y fracassos en implantoes osteointegrados: causas, tratamientos e prevención.. Barcelona: Masson; 1995.
75. Sumida S, Ishihara K, Kishi M, Okuda K. Transmission of periodontal disease-associated bacteria from teeth to osseointegrated implant regions. The International journal of oral & maxillofacial implants. 2002;17(5):696-702.
76. Leonhardt A, Adolfsson B, Lekholm U, Wikstrom M, Dahlen G. A longitudinal microbiological study on osseointegrated titanium implants in partially edentulous patients. Clinical oral implants research. 1993;4(3):113-20.
77. 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(4):432-7.
78. Barbosa RE, do Nascimento C, Issa JP, Watanabe E, Ito IY, de Albuquerque RF, Jr. Bacterial culture and DNA Checkerboard for the detection of internal contamination in dental implants. J Prosthodont. 2009;18(5):376-81.
79. Haraldson T. [Functional evaluation of bridges on intraosseous implants in the edentulous jaw after use of 6 months to 6 1/2 years]. Tandlakartidningen. 1980;72(20):1106-7.
80. Tallgren A. The continuing reduction of the residual alveolar ridges in complete denture wearers: a mixed-longitudinal study covering 25 years. J Prosthet Dent. 1972;27(2):120-32.

81. Adell R, Eriksson B, Lekholm U, Branemark PI, Jemt T. Long-term follow-up study of osseointegrated implants in the treatment of totally edentulous jaws. Int J Oral Maxillofac Implants. 1990;5(4):347-59.
82. van Steenberghe D. Periodontal aspects of osseointegrated oral implants modum Branemark. Dent Clin North Am. 1988;32(2):355-70.
83. Quirynen M, Naert I, van Steenberghe D. Fixture design and overload influence marginal bone loss and fixture success in the Branemark system. Clin Oral Implants Res. 1992;3(3):104-11.
84. Lindquist LW, Rockler B, Carlsson GE. Bone resorption around fixtures in edentulous patients treated with mandibular fixed tissue-integrated prostheses. J Prosthet Dent. 1988;59(1):59-63.
85. Alqutaibi AY, Aboalrejal AN. Microgap and Micromotion at the Implant Abutment Interface Cause Marginal Bone Loss Around Dental Implant but More Evidence is Needed. J Evid Based Dent Pract. 2018;18(2):171-2.
86. Adell R. Tissue integrated prostheses in clinical dentistry. Int Dent J. 1985;35(4):259-65.
87. Oh TJ, Yoon J, Misch CE, Wang HL. The causes of early implant bone loss: myth or science? J Periodontol. 2002;73(3):322-33.
88. Tatarakis N, Bashutski J, Wang HL, Oh TJ. Early implant bone loss: preventable or inevitable? Implant Dent. 2012;21(5):379-86.
89. Hermann JS, Cochran DL, Nummikoski PV, Buser D. Crestal bone changes around titanium implants. A radiographic evaluation of unloaded nonsubmerged and submerged implants in the canine mandible. J Periodontol. 1997;68(11):1117-30.
90. Buser D, Mericske-Stern R, Dula K, Lang NP. Clinical experience with one-stage, non-submerged dental implants. Adv Dent Res. 1999;13:153-61.
91. Hartman GA, Cochran DL. Initial implant position determines the magnitude of crestal bone remodeling. J Periodontol. 2004;75(4):572-7.
92. Weng D, Nagata MJ, Bosco AF, de Melo LG. Influence of microgap location and configuration on radiographic bone loss around submerged implants: an experimental study in dogs. Int J Oral Maxillofac Implants. 2011;26(5):941-6.
93. Graves DT, Li J, Cochran DL. Inflammation and uncoupling as mechanisms of periodontal bone loss. J Dent Res. 2011;90(2):143-53.
94. Bosque Keedi C, Azevedo Marques AD, Arenas Rodrigues VA, Avila-Campos MJ, Tortamano P. Efficacy of a Polyglycol Dimethacrylate-Based Adhesive in Sealing the Implant-Abutment Interface. Implant Dent. 2019;28(3):265-71.
95. Ericsson I, Persson LG, Berglundh T, Marinello CP, Lindhe J, Klinge B. Different types of inflammatory reactions in peri-implant soft tissues. J Clin Periodontol. 1995;22(3):255-61.
96. Hermann JS, Schoolfield JD, Schenk RK, Buser D, Cochran DL. Influence of the size of the microgap on crestal bone changes around titanium implants. A histometric evaluation of unloaded non-submerged implants in the canine mandible. J Periodontol. 2001;72(10):1372-83.
97. King GN, Hermann JS, Schoolfield JD, Buser D, Cochran DL. Influence of the size of the microgap on crestal bone levels in non-submerged dental implants: a radiographic study in the canine mandible. J Periodontol. 2002;73(10):1111-7.

98. Bozkaya D, Muftu S, Muftu A. Evaluation of load transfer characteristics of five different implants in compact bone at different load levels by finite elements analysis. J Prosthet Dent. 2004;92(6):523-30.
99. Ceruso FM, Barnaba P, Mazzoleni S, Ottria L, Gargari M, Zuccon A, et al. Implant-abutment connections on single crowns: a systematic review. Oral Implantol (Rome). 2017;10(4):349-53.
100. Liu Y, Wang J. Influences of microgap and micromotion of implant-abutment interface on marginal bone loss around implant neck. Arch Oral Biol. 2017;83:153-60.
101. Gonzalo E, Vizoso B, Lopez-Suarez C, Diaz P, Pelaez J, Suarez MJ. Evaluation of Milled Titanium versus Laser Sintered Co-Cr Abutments on the Marginal Misfit in Internal Implant-Abutment Connection. Materials (Basel). 2020;13(21).
102. Jemt T. Failures and complications in 391 consecutively inserted fixed prostheses supported by Branemark implants in edentulous jaws: a study of treatment from the time of prosthesis placement to the first annual checkup. Int J Oral Maxillofac Implants. 1991;6(3):270-6.
103. Silva-Neto JP, Prudente MS, Carneiro Tde A, Nobilo MA, Penatti MP, Neves FD. Microleakage at the implant-abutment interface with different tightening torques in vitro. J Appl Oral Sci. 2012;20(5):581-7.
104. Dias EC, Bisognin ED, Harari ND, Machado SJ, da Silva CP, Soares GD, et al. Evaluation of implant-abutment microgap and bacterial leakage in five external-hex implant systems: an in vitro study. Int J Oral Maxillofac Implants. 2012;27(2):346-51.
105. Norton MR. Assessment of cold welding properties of the internal conical interface of two commercially available implant systems. J Prosthet Dent. 1999;81(2):159-66.
106. Kofron MD, Carstens M, Fu C, Wen HB. In vitro assessment of connection strength and stability of internal implant-abutment connections. Clin Biomech (Bristol, Avon). 2019;65:92-9.
107. Scarano A, Assenza B, Piattelli M, Iezzi G, Leghissa GC, Quaranta A, et al. A 16-year study of the microgap between 272 human titanium implants and their abutments. J Oral Implantol. 2005;31(6):269-75.
108. Schwarz F, Hegewald A, Becker J. Impact of implant-abutment connection and positioning of the machined collar/microgap on crestal bone level changes: a systematic review. Clin Oral Implants Res. 2014;25(4):417-25.
109. Siadat H, Beyabanaki E, Mousavi N, Alikhasi M. Comparison of fit accuracy and torque maintenance of zirconia and titanium abutments for internal tri-channel and external-hex implant connections. J Adv Prosthodont. 2017;9(4):271-7.
110. Schmitt CM, Nogueira-Filho G, Tenenbaum HC, Lai JY, Brito C, Doring H, et al. Performance of conical abutment (Morse Taper) connection implants: a systematic review. J Biomed Mater Res A. 2014;102(2):552-74.
111. Apaza-Bedoya K, Tarce M, Benfatti CAM, Henriques B, Mathew MT, Teughels W, et al. Synergistic interactions between corrosion and wear at titanium-based dental implant connections: A scoping review. J Periodontal Res. 2017;52(6):946-54.
112. Tsuruta K, Ayukawa Y, Matsuzaki T, Kihara M, Koyano K. The influence of implant-abutment connection on the screw loosening and microleakage. Int J Implant Dent. 2018;4(1):11.

113. Freitas AC, Jr., Bonfante EA, Rocha EP, Silva NR, Marotta L, Coelho PG. Effect of implant connection and restoration design (screwed vs. cemented) in reliability and failure modes of anterior crowns. Eur J Oral Sci. 2011;119(4):323-30.
114. Cooper LF, Tarnow D, Froum S, Moriarty J, De Kok IJ. Comparison of Marginal Bone Changes with Internal Conus and External Hexagon Design Implant Systems: A Prospective, Randomized Study. Int J Periodontics Restorative Dent. 2016;36(5):631-42.
115. Nishioka RS, de Vasconcellos LG, de Melo Nishioka LN. External hexagon and internal hexagon in straight and offset implant placement: strain gauge analysis. Implant Dent. 2009;18(6):512-20.
116. Gehrke SA, Delgado-Ruiz RA, Prados Frutos JC, Prados-Privado M, Dedavid BA, Granero Marin JM, et al. Misfit of Three Different Implant-Abutment Connections Before and After Cyclic Load Application: An In Vitro Study. Int J Oral Maxillofac Implants. 2017;32(4):822-9.
117. Coppede AR, Bersani E, de Mattos Mda G, Rodrigues RC, Sartori IA, Ribeiro RF. Fracture resistance of the implant-abutment connection in implants with internal hex and internal conical connections under oblique compressive loading: an in vitro study. Int J Prosthodont. 2009;22(3):283-6.
118. Jansen VK, Conrads G, Richter EJ. Microbial leakage and marginal fit of the implant-abutment interface. Int J Oral Maxillofac Implants. 1997;12(4):527-40.
119. Hansson S. Implant-abutment interface: biomechanical study of flat top versus conical. Clin Implant Dent Relat Res. 2000;2(1):33-41.
120. Goodacre CJ, Bernal G, Rungcharassaeng K, Kan JY. Clinical complications with implants and implant prostheses. J Prosthet Dent. 2003;90(2):121-32.
121. Gross M, Abramovich I, Weiss EI. Microleakage at the abutment-implant interface of osseointegrated implants: a comparative study. Int J Oral Maxillofac Implants. 1999;14(1):94-100.
122. 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(10):1168-72.
123. Steinebrunner L, Wolfart S, Bossmann K, Kern M. In vitro evaluation of bacterial leakage along the implant-abutment interface of different implant systems. Int J Oral Maxillofac Implants. 2005;20(6):875-81.
124. Harder S, Dimaczek B, Acil Y, Terheyden H, Freitag-Wolf S, Kern M. Molecular leakage at implant-abutment connection--in vitro investigation of tightness of internal conical implant-abutment connections against endotoxin penetration. Clin Oral Investig. 2010;14(4):427-32.
125. Passos SP, Gressler May L, Faria R, Ozcan M, Bottino MA. Implant-abutment gap versus microbial colonization: Clinical significance based on a literature review. J Biomed Mater Res B Appl Biomater. 2013;101(7):1321-8.
126. Vazquez Alvarez R, Perez Sayans M, Gayoso Diz P, Garcia Garcia A. Factors affecting peri-implant bone loss: a post-five-year retrospective study. Clin Oral Implants Res. 2015;26(9):1006-14.
127. Dibart S, Warbington M, Su MF, Skobe Z. In vitro evaluation of the implant-abutment bacterial seal: the locking taper system. Int J Oral Maxillofac Implants. 2005;20(5):732-7.

128. Saba S. Occlusal stability in implant prosthodontics -- clinical factors to consider before implant placement. J Can Dent Assoc. 2001;67(9):522-6.
129. Proff P, Steinmetz I, Bayerlein T, Dietze S, Fanghanel J, Gedrange T. Bacterial colonisation of interior implant threads with and without sealing. Folia Morphol (Warsz). 2006;65(1):75-7.
130. Almeida EO, Freitas AC, Jr., Bonfante EA, Marotta L, Silva NR, Coelho PG. Mechanical testing of implant-supported anterior crowns with different implant/abutment connections. Int J Oral Maxillofac Implants. 2013;28(1):103-8.
131. Arnhart C, Kielbassa AM, Martinez-de Fuentes R, Goldstein M, Jackowski J, Lorenzoni M, et al. Comparison of variable-thread tapered implant designs to a standard tapered implant design after immediate loading. A 3-year multicentre randomised controlled trial. Eur J Oral Implantol. 2012;5(2):123-36.
132. Esposito M, Maghaireh H, Pistilli R, Grusovin MG, Lee ST, Trullenque-Eriksson A, et al. Dental implants with internal versus external connections: 5-year post-loading results from a pragmatic multicenter randomised controlled trial. Eur J Oral Implantol. 2016;9 Suppl 1(2):129-41.
133. Koo KT, Lee EJ, Kim JY, Seol YJ, Han JS, Kim TI, et al. The effect of internal versus external abutment connection modes on crestal bone changes around dental implants: a radiographic analysis. J Periodontol. 2012;83(9):1104-9.
134. Pozzi A, Agliardi E, Tallarico M, Barlattani A. Clinical and radiological outcomes of two implants with different prosthetic interfaces and neck configurations: randomized, controlled, split-mouth clinical trial. Clin Implant Dent Relat Res. 2014;16(1):96-106.
135. Pieri F, Aldini NN, Marchetti C, Corinaldesi G. Influence of implant-abutment interface design on bone and soft tissue levels around immediately placed and restored single-tooth implants: a randomized controlled clinical trial. Int J Oral Maxillofac Implants. 2011;26(1):169-78.
136. Pessoa RS, Vaz LG, Marcantonio E, Jr., Vander Sloten J, Duyck J, Jaecques SV. Biomechanical evaluation of platform switching in different implant protocols: computed tomography-based three-dimensional finite element analysis. Int J Oral Maxillofac Implants. 2010;25(5):911-9.
137. Nascimento C, Ikeda LN, Pita MS, Pedroso e Silva RC, Pedrazzi V, Albuquerque RF, et al. Marginal fit and microbial leakage along the implant-abutment interface of fixed partial prostheses: An in vitro analysis using Checkerboard DNA-DNA hybridization. J Prosthet Dent. 2015;114(6):831-8.
138. Koutouzis T, Wallet S, Calderon N, Lundgren T. Bacterial colonization of the implant-abutment interface using an in vitro dynamic loading model. J Periodontol. 2011;82(4):613-8.
139. Al-Qutaib AY. There is No Clear Evidence of Superiority Concerning the Platform-switching in the Preservation of the Marginal Bone Level. J Evid Based Dent Pract. 2015;15(3):118-20.
140. Lazzara RJ, Porter SS. Platform switching: a new concept in implant dentistry for controlling postrestorative crestal bone levels. Int J Periodontics Restorative Dent. 2006;26(1):9-17.
141. Cochran DL, Mau LP, Higginbottom FL, Wilson TG, Bosshardt DD, Schoolfield J, et al. Soft and hard tissue histologic dimensions around dental implants in the canine restored with smaller-diameter abutments: a paradigm shift in peri-implant biology. Int J Oral Maxillofac Implants. 2013;28(2):494-502.

142. Cochran DL, Bosshardt DD, Grize L, Higginbottom FL, Jones AA, Jung RE, et al. Bone response to loaded implants with non-matching implant-abutment diameters in the canine mandible. J Periodontol. 2009;80(4):609-17.
143. D KP, Shetty M, Bansal N, Hegde C. Platform switching: An answer to crestal bone loss. Journal of Dental Implants. 2021;1(1):13-7.
144. Canullo L, Fedele GR, Iannello G, Jepsen S. Platform switching and marginal bone-level alterations: the results of a randomized-controlled trial. Clin Oral Implants Res. 2010;21(1):115-21.
145. Heitz-Mayfield LJ, Darby I, Heitz F, Chen S. Preservation of crestal bone by implant design. A comparative study in minipigs. Clin Oral Implants Res. 2013;24(3):243-9.
146. 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(4):426-34.
147. Macedo JP, Pereira J, Vahey BR, Henriques B, Benfatti CAM, Magini RS, et al. Morse taper dental implants and platform switching: The new paradigm in oral implantology. Eur J Dent. 2016;10(1):148-54.
148. Ranieri R, Ferreira A, Souza E, Arcoverde J, Dametto F, Gade-Neto C, et al. The bacterial sealing capacity of morse taper implant-abutment systems in vitro. J Periodontol. 2015;86(5):696-702.
149. Hernigou P, Queinnec S, Flouzat Lachaniette CH. One hundred and fifty years of history of the Morse taper: from Stephen A. Morse in 1864 to complications related to modularity in hip arthroplasty. Int Orthop. 2013;37(10):2081-8.
150. Nishioka RS, de Vasconcellos LG, de Melo Nishioka GN. Comparative strain gauge analysis of external and internal hexagon, Morse taper, and influence of straight and offset implant configuration. Implant Dent. 2011;20(2):e24-32.
151. Gratton DG, Aquilino SA, Stanford CM. Micromotion and dynamic fatigue properties of the dental implant-abutment interface. J Prosthet Dent. 2001;85(1):47-52.
152. Velez J, Pelaez J, Lopez-Suarez C, Agustin-Panadero R, Tobar C, Suarez MJ. Influence of Implant Connection, Abutment Design and Screw Insertion Torque on Implant-Abutment Misfit. J Clin Med. 2020;9(8).
153. Atieh MA, Ibrahim HM, Atieh AH. Platform switching for marginal bone preservation around dental implants: a systematic review and meta-analysis. J Periodontol. 2010;81(10):1350-66.
154. Al-Nsour MM, Chan HL, Wang HL. Effect of the platform-switching technique on preservation of peri-implant marginal bone: a systematic review. Int J Oral Maxillofac Implants. 2012;27(1):138-45.
155. de Medeiros RA, Pellizzer EP, Vechiato Filho AJ, Dos Santos DM, da Silva EV, Goiato MC. Evaluation of marginal bone loss of dental implants with internal or external connections and its association with other variables: A systematic review. J Prosthet Dent. 2016;116(4):501-6 e5.
156. Vandeweghe S, De Bruyn H. A within-implant comparison to evaluate the concept of platform switching: a randomised controlled trial. Eur J Oral Implantol. 2012;5(3):253-62.
157. Linkevicius T, Puisys A, Steigmann M, Vindasiute E, Linkeviciene L. Influence of Vertical Soft Tissue Thickness on Crestal Bone Changes Around Implants with

Platform Switching: A Comparative Clinical Study. Clin Implant Dent Relat Res. 2015;17(6):1228-36.
158. Galindo-Moreno P, Leon-Cano A, Monje A, Ortega-Oller I, O'Valle F, Catena A. Abutment height influences the effect of platform switching on peri-implant marginal bone loss. Clin Oral Implants Res. 2016;27(2):167-73.
159. Broggini N, McManus LM, Hermann JS, Medina RU, Oates TW, Schenk RK, et al. Persistent acute inflammation at the implant-abutment interface. J Dent Res. 2003;82(3):232-7.
160. Todescan FF, Pustiglioni FE, Imbronito AV, Albrektsson T, Gioso M. Influence of the microgap in the peri-implant hard and soft tissues: a histomorphometric study in dogs. Int J Oral Maxillofac Implants. 2002;17(4):467-72.
161. Piattelli A, Vrespa G, Petrone G, Iezzi G, Annibali S, Scarano A. Role of the microgap between implant and abutment: a retrospective histologic evaluation in monkeys. J Periodontol. 2003;74(3):346-52.
162. Siar CH, Toh CG, Romanos G, Swaminathan D, Ong AH, Yaacob H, et al. Peri-implant soft tissue integration of immediately loaded implants in the posterior macaque mandible: a histomorphometric study. J Periodontol. 2003;74(5):571-8.
163. Ekelund JA, Lindquist LW, Carlsson GE, Jemt T. Implant treatment in the edentulous mandible: a prospective study on Branemark system implants over more than 20 years. Int J Prosthodont. 2003;16(6):602-8.
164. Weiss EI, Kozak D, Gross MD. Effect of repeated closures on opening torque values in seven abutment-implant systems. J Prosthet Dent. 2000;84(2):194-9.
165. Wolfinger GJ. Implant prosthodontic and restorative complications. Int J Oral Maxillofac Implants. 2003;18(5):766-7.
166. Gunne J, Jemt T, Linden B. Implant treatment in partially edentulous patients: a report on prostheses after 3 years. Int J Prosthodont. 1994;7(2):143-8.
167. Jorneus L, Jemt T, Carlsson L. Loads and designs of screw joints for single crowns supported by osseointegrated implants. Int J Oral Maxillofac Implants. 1992;7(3):353-9.
168. Breeding LC, Dixon DL, Nelson EW, Tietge JD. Torque required to loosen single-tooth implant abutment screws before and after simulated function. Int J Prosthodont. 1993;6(5):435-9.
169. Patterson EA, Johns RB. Theoretical analysis of the fatigue life of fixture screws in osseointegrated dental implants. Int J Oral Maxillofac Implants. 1992;7(1):26-33.
170. Tzenakis GK, Nagy WW, Fournelle RA, Dhuru VB. The effect of repeated torque and salivary contamination on the preload of slotted gold implant prosthetic screws. J Prosthet Dent. 2002;88(2):183-91.
171. Brunski JB, Puleo DA, Nanci A. Biomaterials and biomechanics of oral and maxillofacial implants: current status and future developments. Int J Oral Maxillofac Implants. 2000;15(1):15-46.
172. Diez JS, Brigagao VC, Cunha L, Neves AC, da Silva-Concilio LR. Influence of diamondlike carbon-coated screws on the implant-abutment interface. Int J Oral Maxillofac Implants. 2012;27(5):1055-60.
173. Jorn D, Kohorst P, Besdo S, Rucker M, Stiesch M, Borchers L. Influence of lubricant on screw preload and stresses in a finite element model for a dental implant. J Prosthet Dent. 2014;112(2):340-8.

174. Kim SK, Lee JB, Koak JY, Heo SJ, Lee KR, Cho LR, et al. An abutment screw loosening study of a Diamond Like Carbon-coated CP titanium implant. J Oral Rehabil. 2005;32(5):346-50.
175. Eckert SE, Meraw SJ, Cal E, Ow RK. Analysis of incidence and associated factors with fractured implants: a retrospective study. Int J Oral Maxillofac Implants. 2000;15(5):662-7.
176. Winkler S, Ring K, Ring JD, Boberick KG. Implant screw mechanics and the settling effect: overview. J Oral Implantol. 2003;29(5):242-5.
177. Hoyer SA, Stanford CM, Buranadham S, Fridrich T, Wagner J, Gratton D. Dynamic fatigue properties of the dental implant-abutment interface: joint opening in wide-diameter versus standard-diameter hex-type implants. J Prosthet Dent. 2001;85(6):599-607.
178. Yeo IS, Lee JH, Kang TJ, Kim SK, Heo SJ, Koak JY, et al. The effect of abutment screw length on screw loosening in dental implants with external abutment connections after thermocycling. Int J Oral Maxillofac Implants. 2014;29(1):59-62.
179. Mohammed HH, Lee JH, Bae JM, Cho HW. Effect of abutment screw length and cyclic loading on removal torque in external and internal hex implants. J Adv Prosthodont. 2016;8(1):62-9.
180. Shin HM, Huh JB, Yun MJ, Jeon YC, Chang BM, Jeong CM. Influence of the implant-abutment connection design and diameter on the screw joint stability. J Adv Prosthodont. 2014;6(2):126-32.
181. Dincer Kose O, Karatasli B, Demircan S, Kose TE, Cene E, Aya SA, et al. In Vitro Evaluation of Manual Torque Values Applied to Implant-Abutment Complex by Different Clinicians and Abutment Screw Loosening. Biomed Res Int. 2017;2017:7376261.
182. Tallarico M, Canullo L, Caneva M, Ozcan M. Microbial colonization at the implant-abutment interface and its possible influence on peri-implantitis: A systematic review and meta-analysis. J Prosthodont Res. 2017;61(3):233-41.
183. Gehrke SA, Shibli JA, Aramburu 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.
184. Burguete RL, Johns RB, King T, Patterson EA. Tightening characteristics for screwed joints in osseointegrated dental implants. J Prosthet Dent. 1994;71(6):592-9.
185. Rangert B, Jemt T, Jorneus L. Forces and moments on Branemark implants. Int J Oral Maxillofac Implants. 1989;4(3):241-7.
186. Byrne D, Jacobs S, O'Connell B, Houston F, Claffey N. Preloads generated with repeated tightening in three types of screws used in dental implant assemblies. J Prosthodont. 2006;15(3):164-71.
187. Haack JE, Sakaguchi RL, Sun T, Coffey JP. Elongation and preload stress in dental implant abutment screws. Int J Oral Maxillofac Implants. 1995;10(5):529-36.
188. Rodrigues EDS, Benetti P, Carli JP, Paranhos LR, Santos PL, Linden MS. A Comparison of Torque Stress on Abutment Screw of External Hexagon and Morse Taper Implant. J Contemp Dent Pract. 2018;19(11):1306-11.
189. Lee HW, Alkumru H, Ganss B, Lai JY, Ramp LC, Liu PR. The Effect of Contamination of Implant Screws on Reverse Torque. Int J Oral Maxillofac Implants. 2015;30(5):1054-60.

190. Siamos G, Winkler S, Boberick KG. Relationship between implant preload and screw loosening on implant-supported prostheses. J Oral Implantol. 2002;28(2):67-73.
191. Khraisat A, Abu-Hammad O, Al-Kayed AM, Dar-Odeh N. Stability of the implant/abutment joint in a single-tooth external-hexagon implant system: clinical and mechanical review. Clin Implant Dent Relat Res. 2004;6(4):222-9.
192. Cao X, Cai X, Chen R, Zhang H, Jiang T, Wang Y. A thermosensitive chitosan-based hydrogel for sealing and lubricating purposes in dental implant system. Clin Implant Dent Relat Res. 2019;21(2):324-35.
193. Dixon DL, Breeding LC, Sadler JP, McKay ML. Comparison of screw loosening, rotation, and deflection among three implant designs. J Prosthet Dent. 1995;74(3):270-8.
194. Hill EE, Phillips SM, Breeding LC. Implant abutment screw torque generated by general dentists using a hand driver in a limited access space simulating the mouth. J Oral Implantol. 2007;33(5):277-9.
195. Stroosnijder E, Gresnigt MM, Meisberger EW, Cune MS. Loss of Accuracy of Torque Wrenches Due to Clinical Use and Cleaning Procedure: Short Communication. Int J Prosthodont. 2016;29(3):253-5.
196. Goheen KL, Vermilyea SG, Vossoughi J, Agar JR. Torque generated by handheld screwdrivers and mechanical torquing devices for osseointegrated implants. Int J Oral Maxillofac Implants. 1994;9(2):149-55.
197. Alikhasi M, Kazemi M, Jalali H, Hashemzadeh S, Dodangeh H, Yilmaz B. Clinician-generated torque on abutment screws using different hand screwdrivers. J Prosthet Dent. 2017;118(4):488-92.
198. Arvidson K, Bystedt H, Frykholm A, von Konow L, Lothigius E. A 3-year clinical study of Astra dental implants in the treatment of edentulous mandibles. Int J Oral Maxillofac Implants. 1992;7(3):321-9.
199. Baj A, Bolzoni A, Russillo A, Lauritano D, Palmieri A, Cura F, et al. Cone-morse implant connection system significantly reduces bacterial leakage between implant and abutment: an in vitro study. J Biol Regul Homeost Agents. 2017;31(2 Suppl 1):203-8.
200. Prithviraj DR, Gupta V, Muley N, Sandhu P. One-piece implants: placement timing, surgical technique, loading protocol, and marginal bone loss. J Prosthodont. 2013;22(3):237-44.
201. Tallarico M, Fiorellini J, Nakajima Y, Omori Y, Takahisa I, Canullo L. Mechanical Outcomes, Microleakage, and Marginal Accuracy at the Implant-Abutment Interface of Original versus Nonoriginal Implant Abutments: A Systematic Review of In Vitro Studies. Biomed Res Int. 2018;2018:2958982.
202. Apaza-Bedoya K, Bijukumar D, Benfatti CAM, Mathew MT, Silva JSPd, Souza JCM. Adverse local and systemic effect of nanoparticles released from oral and cranio-maxillofacial implants. Nanostructured Biomaterials for Cranio-Maxillofacial and Oral Applications2018. p. 6am 3-79.
203. Quirynen M, Vogels R, Peeters W, van Steenberghe D, Naert I, Haffajee A. Dynamics of initial subgingival colonization of 'pristine' peri-implant pockets. Clin Oral Implants Res. 2006;17(1):25-37.
204. Weng D, Nagata MJ, Bell M, Bosco AF, de Melo LG, Richter EJ. Influence of microgap location and configuration on the peri-implant bone morphology in submerged implants. An experimental study in dogs. Clin Oral Implants Res. 2008;19(11):1141-7.

205. Coelho PG, Sudack P, Suzuki M, Kurtz KS, Romanos GE, Silva NR. In vitro evaluation of the implant abutment connection sealing capability of different implant systems. J Oral Rehabil. 2008;35(12):917-24.
206. Alonso-Perez R, Bartolome JF, Ferreiroa A, Salido MP, Pradies G. Original vs. non-original abutments for screw-retained single implant crowns: An in vitro evaluation of internal fit, mechanical behaviour and screw loosening. Clin Oral Implants Res. 2018;29(12):1230-8.
207. Koutouzis T, Gadalla H, Lundgren T. Bacterial Colonization of the Implant-Abutment Interface (IAI) of Dental Implants with a Sloped Marginal Design: An in-vitro Study. Clin Implant Dent Relat Res. 2016;18(1):161-7.
208. Cibirka RM, Nelson SK, Lang BR, Rueggeberg FA. Examination of the implant-abutment interface after fatigue testing. J Prosthet Dent. 2001;85(3):268-75.
209. Steinebrunner L, Wolfart S, Ludwig K, Kern M. Implant-abutment interface design affects fatigue and fracture strength of implants. Clin Oral Implants Res. 2008;19(12):1276-84.
210. Yokoyama K, Ichikawa T, Murakami H, Miyamoto Y, Asaoka K. Fracture mechanisms of retrieved titanium screw thread in dental implant. Biomaterials. 2002;23(12):2459-65.
211. Piattelli A, Scarano A, Paolantonio M, Assenza B, Leghissa GC, Di Bonaventura G, et al. Fluids and microbial penetration in the internal part of cement-retained versus screw-retained implant-abutment connections. J Periodontol. 2001;72(9):1146-50.
212. . !!! INVALID CITATION !!! .
213. Quirynen M, Bollen CM, Eyssen H, van Steenberghe D. Microbial penetration along the implant components of the Branemark system. An in vitro study. Clin Oral Implants Res. 1994;5(4):239-44.
214. Cho SC, Small PN, Elian N, Tarnow D. Screw loosening for standard and wide diameter implants in partially edentulous cases: 3- to 7-year longitudinal data. Implant Dent. 2004;13(3):245-50.
215. Smedberg JI, Nilner K, Frykholm A. A six-year follow-up study of maxillary overdentures on osseointegrated implants. Eur J Prosthodont Restor Dent. 1999;7(2):51-6.
216. Fretwurst T, Buzanich G, Nahles S, Woelber JP, Riesemeier H, Nelson K. Metal elements in tissue with dental peri-implantitis: a pilot study. Clin Oral Implants Res. 2016;27(9):1178-86.
217. Larrucea Verdugo C, Jaramillo Nunez G, Acevedo Avila A, Larrucea San Martin C. Microleakage of the prosthetic abutment/implant interface with internal and external connection: in vitro study. Clin Oral Implants Res. 2014;25(9):1078-83.
218. Kim HK, Heo SJ, Koak JY, Kim SK. In vivo comparison of force development with various materials of implant-supported prostheses. J Oral Rehabil. 2009;36(8):616-25.
219. do Nascimento C, Pedrazzi V, Miani PK, Moreira LD, de Albuquerque RF, Jr. Influence of repeated screw tightening on bacterial leakage along the implant-abutment interface. Clin Oral Implants Res. 2009;20(12):1394-7.
220. Assenza B, Tripodi D, Scarano A, Perrotti V, Piattelli A, Iezzi G, et al. Bacterial leakage in implants with different implant-abutment connections: an in vitro study. J Periodontol. 2012;83(4):491-7.

221. 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(6):677-85.
222. 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(4):344-50.
223. Vigolo P, Gracis S, Carboncini F, Mutinelli S, Group ACR. Internal- vs External-Connection Single Implants: A Retrospective Study in an Italian Population Treated by Certified Prosthodontists. Int J Oral Maxillofac Implants. 2016;31(6):1385-96.
224. Wachtel A, Zimmermann T, Spintig T, Beuer F, Muller WD, Schwitalla AD. A Novel Approach to Prove Bacterial Leakage of Implant-Abutment Connections In Vitro. J Oral Implantol. 2016;42(6):452-7.
225. do Nascimento C, Barbosa RE, Issa JP, Watanabe E, Ito IY, Albuquerque RF, Jr. Bacterial leakage along the implant-abutment interface of premachined or cast components. Int J Oral Maxillofac Surg. 2008;37(2):177-80.
226. Schminke B, Vom Orde F, Gruber R, Schliephake H, Burgers R, Miosge N. The pathology of bone tissue during peri-implantitis. J Dent Res. 2015;94(2):354-61.
227. Rosen PS, Froum SJ. Emerging Issues Associated With Peri-implant Disease. Compend Contin Educ Dent. 2016;37(7):440-7;quiz8.
228. Socransky SS, Haffajee AD. The bacterial etiology of destructive periodontal disease: current concepts. J Periodontol. 1992;63(4 Suppl):322-31.
229. Kamaguchi A, Baba H, Hoshi M, Inomata K. Coaggregation between Porphyromonas gingivalis and mutans streptococci. Microbiol Immunol. 1994;38(6):457-60.
230. Jordan RP, Marsh L, Ayre WN, Jones Q, Parkes M, Austin B, et al. An assessment of early colonisation of implant-abutment metal surfaces by single species and co-cultured bacterial periodontal pathogens. J Dent. 2016;53:64-72.
231. Koseki H, Yonekura A, Shida T, Yoda I, Horiuchi H, Morinaga Y, et al. Early staphylococcal biofilm formation on solid orthopaedic implant materials: in vitro study. PLoS One. 2014;9(10):e107588.
232. Tesmer M, Wallet S, Koutouzis T, Lundgren T. Bacterial colonization of the dental implant fixture-abutment interface: an in vitro study. J Periodontol. 2009;80(12):1991-7.
233. Jaworski ME, Melo AC, Picheth CM, Sartori IA. Analysis of the bacterial seal at the implant-abutment interface in external-hexagon and Morse taper-connection implants: an in vitro study using a new methodology. Int J Oral Maxillofac Implants. 2012;27(5):1091-5.
234. Covani U, Marconcini S, Crespi R, Barone A. Bacterial plaque colonization around dental implant surfaces. Implant Dent. 2006;15(3):298-304.
235. Tripodi D, Vantaggiato G, Scarano A, Perrotti V, Piattelli A, Iezzi G, et al. An in vitro investigation concerning the bacterial leakage at implants with internal hexagon and Morse taper implant-abutment connections. Implant Dent. 2012;21(4):335-9.
236. Kano SC, Binon PP, Curtis DA. A classification system to measure the implant-abutment microgap. Int J Oral Maxillofac Implants. 2007;22(6):879-85.
237. Da Silva EF, Pellizzer EP, Quinelli Mazaro JV, Garcia Junior IR. Influence of the connector and implant design on the implant-tooth-connected prostheses. Clin Implant Dent Relat Res. 2010;12(3):254-62.

238. Pautke C, Kolk A, Brokate M, Wehrstedt JC, Kneissl F, Miethke T, et al. Development of novel implant abutments using the shape memory alloy nitinol: preliminary results. Int J Oral Maxillofac Implants. 2009;24(3):477-83.
239. Seloto CB, Strazzi Sahyon HB, Dos Santos PH, Delben JA, Assuncao WG. Efficacy of Sealing Agents on Preload Maintenance of Screw-Retained Implant-Supported Prostheses. Int J Oral Maxillofac Implants. 2018;33(1):123-6.
240. Alshehri M, Albaqiah H. Antimicrobial Efficacy of Materials Used for Sealing the Implant Abutment Screw Hole: An In Vitro Evaluation. Implant Dent. 2017;26(6):911-4.
241. Maluly-Proni AT, Anchieta RB, Suzuki TY, Garcia de Oliveira F, Guedes AP, Rocha EP, et al. Sealing the Screw Access Channel with Polytetrafluoroethylene Tape: Advantages of the Technique. Int J Oral Maxillofac Implants. 2017;32(5):1132-4.
242. Pimentel AC, Manzi MR, Sartori SG, da Graca Naclerio-Homem M, Sendyk WR. In vivo effectiveness of silicone gel sheets as barriers at the inner microgap between a prosthetic abutment and an external-hexagon implant platform. Int J Oral Maxillofac Implants. 2014;29(1):121-6.
243. Percival SL, Bowler PG, Russell D. Bacterial resistance to silver in wound care. J Hosp Infect. 2005;60(1):1-7.
244. Zarei M, Jamnejad A, Khajehali E. Antibacterial effect of silver nanoparticles against four foodborne pathogens. Jundishapur J Microbiol. 2014;7(1):e8720.
245. Biscoping S, Ruttmann E, Rehmann P, Wostmann B. Do Sealing Materials Influence Superstructure Attachment in Implants? Int J Prosthodont. 2018;31(2):163-5.
246. Podhorsky A, Putzier S, Rehmann P, Streckbein P, Domann E, Wostmann B. Bacterial Contamination of the Internal Cavity of Dental Implants After Application of Disinfectant or Sealant Agents Under Cyclic Loading In Vitro. Int J Prosthodont. 2016;29(5):493-5.
247. Howe MS, Keys W, Richards D. Long-term (10-year) dental implant survival: A systematic review and sensitivity meta-analysis. J Dent. 2019;84:9-21.
248. Albrektsson T, Buser D, Sennerby L. Crestal bone loss and oral implants. Clin Implant Dent Relat Res. 2012;14(6):783-91.
249. Esposito M, Grusovin M, Chew Y, Coulthard P, Worthington H. One-stage versus two-stage implant placement. A Cochrane systematic review of randomised controlled clinical trials. Eur J Oral Implantol. 2009;2:91-9.
250. Liu Y, Wang J. Influences of microgap and micromotion of implant–abutment interface on marginal bone loss around implant neck. Arch Oral Biol. 2017;83:153-60.
251. Dias E, Sperandio M, Napimoga M. Association between implant-abutment microgap and implant circularity to bacterial leakage: an in vitro study using tapered connection implants. Int J Oral Maxillofac Implants. 2018;33:505-11.
252. Tallarico M, Canullo L, Caneva M, Özcan M. Microbial colonization at the implant-abutment interface and its possible influence on peri-implantitis: a systematic review and meta-analysis. J Prosthet Res. 2017;61:233-41.
253. Koutouzis T. Implant-abutment connection as contributing factor to peri-implant diseases. Periodontol 2000. 2019;81:152-66.
254. Sasada Y, Cochran D. Implant-abutment connections: a review of biologic consequences and peri-implantitis implications. Int J Oral Maxillofac Implants 2017;32:1296-307.

255. 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. 2020;13:1131.
256. Mishra S, Chowdhary R, Kumari S. Microleakage at the different implant abutment interface: a systematic review. J Clin Diagn Res. 2017;11:10-5.
257. Verdugo C, Núñez G, Avila A, San Martín C. Microleakage of the prosthetic abutment/implant interface with internal and external connection: in vitro study. Clin Oral Implants Res. 2014;9:1078-83.
258. Alves D, de Carvalho P, Elias C, Vedovatto E, Martinez E. In vitro analysis of the microbiological sealing of tapered implants after mechanical cycling. Clin Oral Invest. 2016;20:2437-45.
259. Gross M, Kozak D, Laufer B, Weiss E. Manual closing torque in five implant abutment systems: an in vitro comparative study. J Prosthet Dent. 1999;81:574-8.
260. Kose O, Karataslı B, Demircan S, Kose T, Cene E, Aya S, et al. In vitro evaluation of manual torque values applied to implant-abutment complex by different clinicians and abutment screw loosening. BioMed Res Int. 2017;2017:7376261.
261. Passos S, May L, Faria R, Ozcan M, Bottino M. Implant-abutment gap versus microbial colonization: clinical significance based on a literature review. J Biomed Mater Res Part B. 2013;101:1321-8.
262. Sahin C, Ayyildiz S. Correlation between microleakage and screw loosening at implant-abutment connection. J Adv Prosthodon. 2014;6:35-8.
263. Vélez J, Peláez J, López-Suárez C, Agustín-Panadero R, Tobar C, Suárez M. Influence of implant connection, abutment design and screw insertion torque on implant-abutment misfit. J Clin Med. 2020;9:2365.
264. Lucena G, Molon R, Moretti A, Shibli J, Rêgo D. Evaluation of microbial contamination in the inner surface of titanium implants before healing abutment connection: a prospective clinical trial. Int J Oral Maxillofac Implants. 2018;33:853-62.
265. Vergara-Llanos D, Koning T, Pavicic M, Bello-Toledo H, Díaz-Gómez A, Jaramillo A, et al. Antibacterial and cytotoxic evaluation of copper and zinc oxide nanoparticles as a potential disinfectant material of connections in implant provisional abutments: An in-vitro study. Arch Oral Biol. 2021;122:105031.
266. Jain S, Schramm S, Siddiqui D, Huo W, Palmer K, Wilson T, et al. Effects of multiple implantations of titanium healing abutments: surface characteristics and microbial colonization. Dental Materials. 2020;36:e279-e91.
267. Canullo L, Rossetti P, Penarrocha D. Identification of Enterococcus Faecalis and Pseudomonas Aeruginosa on and in implants in Individuals with peri-implant disease: a cross-sectional study. Int J Oral Maxillofac Implants. 2015;30:583-7.
268. Flanagan D. Enterococcus faecalis and dental implants. J Oral Implantol. 2017;43:8-11.
269. Canullo L, Penarrocha-Oltra D, Covani U, Rossetti P. Microbiologic and clinical findings of implants in healthy condition and with peri-implantitis. Int J Oral Maxillofac Implants. 2015;30:834-42.
270. Marchese A, Orhan I, Daglia M, Barbieri R, Di Lorenzo A, Nabavi S, et al. Antibacterial and antifungal activities of thymol: a brief review of the literature. Food Chem 2016;210:402-14.

271. Nayak A, Fernandes A, Kulkarni R, Ajantha G, Lekha K, Nadiger R. Efficacy of antibacterial sealing gel and O-ring to prevent microleakage at the implant abutment interface: an in vitro study. J Oral Implantol. 2014;40:11-4.
272. Zekiy AO, Makurdumyan DA, Matveeva EA, Bogatov EA, Kaliiants TV. Antiseptic sealant and a nanocoated implant-abutment interface improve the results of dental implantation. Clin Implant Dent Relat Res. 2019;21(5):938-45.
273. ICCVAM. Test Method Protocol: Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) Test Method. ICCVAM Test Method Evaluation Report: Current Validation Status of In Vitro Test Methods Proposed for Identifying Eye Injury Hazard
Potential of Chemicals and Products. NIH Publication No. 10-75532010.
274. Bender C, Partecke L-I, Kindel E, Döring F, Lademann J, Heidecke C-D, et al. The modified HET-CAM as a model for the assessment of the inflammatory response to tissue tolerable plasma. Toxicol in Vitro. 2011;25:530-7.
275. Schneider-Stock R, Ribatti D. The CAM assay as an alternative in vivo model for drug testing. In: Schäfer-Korting M, Stuchi Maria-Engler S, Landsiedel R, editors. Organotypic Models in Drug Development Handbook of Experimental Pharmacology: Springer; 2020.
276. Schrage A, Gamer A, van Ravenzwaay B, Landsiedel R. Experience with the HET-CAM method in the routine testing of a broad variety of chemicals and formulations. Altern Lab Anim. 2010;38:39-52.
277. Payant L, Williams JE, Zwemer JD. Survey of dental implant practice. J Oral Implantol. 1994;20(1):50-8.
278. Vigolo P, Givani A. Platform-switched restorations on wide-diameter implants: a 5-year clinical prospective study. Int J Oral Maxillofac Implants. 2009;24(1):103-9.
279. Suarez-Lopez Del Amo F, Lin GH, Monje A, Galindo-Moreno P, Wang HL. Influence of Soft Tissue Thickness on Peri-Implant Marginal Bone Loss: A Systematic Review and Meta-Analysis. J Periodontol. 2016;87(6):690-9.
280. Galindo-Moreno P, Leon-Cano A, Ortega-Oller I, Monje A, F OV, Catena A. Marginal bone loss as success criterion in implant dentistry: beyond 2 mm. Clin Oral Implants Res. 2015;26(4):e28-e34.
281. Andreasi Bassi M, Lopez MA, Confalone L, Gaudio RM, Lombardo L, Lauritano D. Clinical outcome of a two-piece implant system with an internal hexagonal connection: a prospective study. J Biol Regul Homeost Agents. 2016;30(2 Suppl 1):7-12.
282. Mishra SK, Chowdhary R, Kumari S. Microleakage at the Different Implant Abutment Interface: A Systematic Review. J Clin Diagn Res. 2017;11(6):ZE10-ZE5.
283. Nascimento C, Pita MS, Santos Ede S, Monesi N, Pedrazzi V, Albuquerque Junior RF, et al. Microbiome of titanium and zirconia dental implants abutments. Dent Mater. 2016;32(1):93-101.
284. Park SD, Lee Y, Kim YL, Yu SH, Bae JM, Cho HW. Microleakage of different sealing materials in access holes of internal connection implant systems. J Prosthet Dent. 2012;108(3):173-80.
285. Karl M, Taylor TD. Parameters determining micromotion at the implant-abutment interface. Int J Oral Maxillofac Implants. 2014;29(6):1338-47.
286. Strietzel FP, Neumann K, Hertel M. Impact of platform switching on marginal peri-implant bone-level changes. A systematic review and meta-analysis. Clin Oral Implants Res. 2015;26(3):342-58.

287. Santiago JF, Jr., Batista VE, Verri FR, Honorio HM, de Mello CC, Almeida DA, et al. Platform-switching implants and bone preservation: a systematic review and meta-analysis. Int J Oral Maxillofac Surg. 2016;45(3):332-45.
288. 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(5).
289. Dental S, Candotto V, Gabrione F, Oberti L, Lento D, Severino M. The role of implant-abutment connection in preventing bacterial leakage: a review. J Biol Regul Homeost Agents. 2019;33(3 Suppl. 1):129-34.
290. Besimo CE, Guindy JS, Lewetag D, Meyer J. Prevention of bacterial leakage into and from prefabricated screw-retained crowns on implants in vitro. Int J Oral Maxillofac Implants. 1999;14(5):654-60.
291. Nayak AG, Fernandes A, Kulkarni R, Ajantha GS, Lekha K, Nadiger R. Efficacy of antibacterial sealing gel and O-ring to prevent microleakage at the implant abutment interface: an in vitro study. J Oral Implantol. 2014;40(1):11-4.
292. Lorenzoni FC, Coelho PG, Bonfante G, Carvalho RM, Silva NR, Suzuki M, et al. Sealing Capability and SEM Observation of the Implant-Abutment Interface. Int J Dent. 2011;2011:864183.
293. Seloto CB, Strazzi-Sahyon HB, Dos Santos PH, Assuncao WG. Effectiveness of Sealing Gel on Vertical Misfit at the Implant-Abutment Interface and Preload Maintenance of Screw-Retained Implant-Supported Prostheses. Int J Oral Maxillofac Implants. 2020;35(3):479-84.
294. Annibali S, Bignozzi I, Cristalli MP, Graziani F, La Monaca G, Polimeni A. Peri-implant marginal bone level: a systematic review and meta-analysis of studies comparing platform switching versus conventionally restored implants. J Clin Periodontol. 2012;39(11):1097-113.
295. Gherlone EF, Cappare P, Pasciuta R, Grusovin MG, Mancini N, Burioni R. Evaluation of resistance against bacterial microleakage of a new conical implant-abutment connection versus conventional connections: an in vitro study. New Microbiol. 2016;39(1):49-56.
296. Toyoshima T, Tanaka H, Ayukawa Y, Howashi M, Masuzaki T, Kiyosue T, et al. Primary Stability of a Hybrid Implant Compared with Tapered and Cylindrical Implants in an Ex Vivo Model. Clin Implant Dent Relat Res. 2015;17(5):950-6.
297. Sammour SR, Maamoun El-Sheikh M, Aly El-Gendy A. Effect of implant abutment connection designs, and implant diameters on screw loosening before and after cyclic loading: In-vitro study. Dent Mater. 2019;35(11):e265-e71.
298. 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(3):561-7.
299. Zipprich H, Rathe F, Pinz S, Schlotmann L, Lauer HC, Ratka C. Effects of Screw Configuration on the Preload Force of Implant-Abutment Screws. Int J Oral Maxillofac Implants. 2018;33(2):e25-e32.
300. Flanagan D. Enterococcus faecalis and Dental Implants. J Oral Implantol. 2017;43(1):8-11.

301. Najafi K, Ganbarov K, Gholizadeh P, Tanomand A, Rezaee M, Mahmood S, et al. Oral cavity infection by Enterococcus faecalis: virulence factors and pathogenesis. Rev Med Microbiol. 2020;31:51-60.
302. de Sousa CA, Taborda MBB, Momesso GAC, Rocha EP, Dos Santos PH, Santiago-Junior JF, et al. Materials Sealing Preventing Biofilm Formation in Implant/Abutment Joints: Which Is the Most Effective? A Systematic Review and Meta-Analysis. J Oral Implantol. 2020;46(2):163-71.
303. Najafloo R, Behyari M, Imani R, Nour S. A mini-review of Thymol incorporated materials: Applications in antibacterial wound dressing. J Drug Deliv Sci Technol. 2020:101904.
304. Colas A, Curtis J. Silicones. In: Modjarrad K, Ebnesajjad S, editors. Handbook of Polymer Applications in Medicine and Medical Devices William Andrew Publishing; 2013. p. 131-43.
305. Costa MF, Durco AO, Rabelo TK, Barreto RSS, Guimaraes AG. Effects of Carvacrol, Thymol and essential oils containing such monoterpenes on wound healing: a systematic review. J Pharm Pharmacol. 2019;71(2):141-55.
306. da Silva-Neto J, Nóbilo M, Penatti M, Simamoto PJ, das Neves F. Influence of methodologic aspects on the results of implant-abutment interface microleakage tests: a critical review of in vitro studies. Int J Oral Maxillofac Implants. 2012;27(4):793-800.

APPENDICES

APPENDIX 1

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RESEARCH AND EDUCATION

Microgap and bacterial microleakage during the osseointegration period: An in vitro assessment of the cover screw and healing abutment in a platform-switched implant system

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ABSTRACT

Statement of problem. Microgap and bacterial microleakage at the implant-prosthetic abutment interface are recognized concerns for implant-supported restorations, leading to inflammation of the peri-implant tissues, with deleterious consequences for crestal bone levels. However, little is known regarding the interface established between the implant and the healing abutment or cover screw placed for the osseointegration phase.

Purpose. The purpose of this in vitro study was to characterize the implant-cover screw and implant-healing abutment interfaces of a platform-switched implant system to determine the microgap and bacterial microleakage of the system and evaluate the biological response and functionality of an interface sealing agent.

Material and methods. The interfacial microgaps of the implant-healing abutment and implant-cover screw interfaces were characterized by scanning electron microscopy (n=10), and bacterial microleakage was evaluated after colonization with *Enterococcus faecalis* in a 30-day follow-up (n=10). The sealing efficacy and irritation potential of a silicone-based sealer were determined by using the hen's egg test on chorioallantoic membrane assay. The 2-sample *t* test was performed to compare means between groups, and data presented with the Kaplan-Meier method were compared statistically by using the log-rank test (α=.05).

Results. The interfacial microgap was less than 2.5 μm for both systems. Bacterial microleakage was noted in approximately 50% of the specimens, particularly at early time points, at both the healing abutment and cover screw interfaces. The silicone-based sealer prevented bacterial leakage in the experimental setting.

Conclusions. The implant-healing abutment and implant-cover screw interfaces of the tested system, despite the low microgap, allowed for bacterial microleakage after internal colonization. The use of a nonirritating silicone-based sealing agent effectively sealed the system. (J Prosthet Dent 2021;■:■-■)

Dental implants are reliable therapeutic options for the oral rehabilitation of partially and completely edentulous patients, with documented long-term predictability, effectiveness, and high success rates.[1] The long-term survival of dental implants has been reported to be closely related to the structural and biological functionality of the peri-implant crestal bone, with resorption leading to implant failure, before or after osseointegration.[2] Crestal bone loss has a multifactorial etiology and has been considered an indicator of bone loss progression and the initiation of peri-implantitis.[3] Factors associated with crestal bone loss include bone and soft-tissue features, implant

Supported in part by PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the project UIDB/50006/2020.

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Clinical Implications

The microgap between implants and healing abutments and/or cover screws allows for bacterial microleakage, possibly fostering a pathogenic biofilm during the early healing stages that may impair the sealing of epithelial tissues at the implant surface and promote peri-implant inflammation and then early- and late-stage complications. Preventing bacterial access to the microgap with a sealant should improve the outcome of implant-supported restorations.

placement location and formation of the biological width, occlusal and surgical trauma, implant structure and design, and the characteristics of the implant-abutment interface.[3,4]

Implant-supported restorations rely on either a 2-stage or 1-stage surgical procedure, both with high success rates.[5] In the 2-stage approach, the implant is initially inserted and submerged into the bone and soft tissues and sealed with a cover screw. The implant is load-free and protected from the oral environment during osseointegration. Subsequently, and after the bone healing process is completed, a healing abutment is placed for soft-tissue stabilization, and a transmucosal prosthetic abutment is then attached to support the restoration. In the 1-stage approach, the implant and a transmucosal healing abutment are placed in a single procedure, with the implant remaining exposed to the oral environment throughout the osseointegration period and before the connection of the prosthetic abutment.[5]

In either approach, the connection of the prosthetic abutment with the implant involves an interface—the implant-abutment interface (IAI)—characterized by a microgap between components.[6] This microgap may initiate bacterial leakage, with the accumulation of microorganisms at the interface,[7] eventually resulting in direct and/or indirect inflammation of the peri-implant tissues with deleterious consequences for the bone level.[8-10] The connection design and its influence on the microgap and bacterial microleakage outcomes have been evaluated,[11-14] and it is generally concluded that internal and combination connection systems outperform external connection designs, with the most positive outcomes being associated with platform-switching approaches. Other factors have been further addressed, especially related to the screw-tightening torque.[15-19]

However, research on the interface between the implant and the covering screws and abutments used during the osseointegration phase is sparse. This interface is of clinical significance given the recent evidence of early contamination of the implant with periodontal pathogens that may occur before the placement of the healing abutment.[20,21] This contamination suggests that a pathogenic biofilm may develop which hinders the sealing of epithelial tissues at the implant surface, triggering and sustaining peri-implant inflammation, promoting corrosion and structural damage, and leading to complications at both the early and late healing stages.[22]

Therefore, the aim of this study was to characterize the in vitro microgap and bacterial microleakage of *Enterococcus faecalis*[23-25] to the implant-cover screw and implant-healing abutment interfaces in a platform-switched implant system and to determine the efficacy of an antibacterial silicone-based sealer (GapSeal; Hager & Werken)[26-28] for preventing microleakage within these interfaces. The null hypothesis was that no significant difference would be found in the microleakage of the implant-healing abutment and implant-cover screw interfaces in the presence or absence of the silicone sealer.

MATERIAL AND METHODS

Implants with a combination of two internal connection systems and a platform switched design (4.0×12-mm–Vega; Klockner), titanium healing abutments (height of 5 mm, Vega; Klockner), and cover screws (Sealing cap; Klockner) were supplied by the manufacturer (Fig. 1). To determine the microgap between the implant and the healing abutments or cover screws, 20 assemblies were prepared with healing abutments (n=10) or cover screws (n=10). These were tightened at a 10-Ncm torque. The sample size calculation assumed a mean difference of 0.5 μm between groups, a power of 90%, and a significance level of 5%, indicating a sample size of 9 in each comparison group. The assemblies were embedded in an autopolymerizing acrylic resin (Vertex Orthoplast; Vertex-dental) and then longitudinally sectioned with a diamond saw (Struers Accutom-5; Struers Inc) at a feed speed of 0.150 mm/s. The sections were polished, washed, and dried and then observed by scanning electron microscopy (SEM) (Quanta 400 FEG ESEM/EDAX Genesis X4M; FEI Co) for microgap determination. The assessment was performed in 6 positions for each assembly, 3 on each side, corresponding to the highest values spaced at least by 2.5 μm. Measurements were determined and averaged. Data analysis was conducted independently by 2 researchers (L.G., P.S.G.) and repeated 3 times.

E. faecalis (ATCC 29212) was used for the assessment of bacterial microleakage. A standard bacterial suspension of *E. faecalis* was prepared in a brain heart infusion (BHI; Liofilchem) broth at a density of 10^8 colony forming units (CFU)/mL. Under sterile conditions, the implants were inoculated with 0.2 μL of bacterial suspension, previously determined as the ideal

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Figure 1. Macroscopic image and respective radiographs of implant and healing abutment and cover screw fixtures (Klockner). Scale bar corresponds to 2 mm.

volume for inoculation in both assembled systems. After bacterial inoculation, healing abutments (n=10) and cover screws (n=10) were installed and tightened to 10 Ncm. To prevent external contamination, the assemblies were immersed for 30 seconds in tubes containing sterile BHI broth, which were incubated at

Figure 2. Application of sealing agent inside implant, followed by installation of healing abutment.

37 °C for 48 hours. If bacterial growth was identified by cloudiness, the assembly was excluded.

The assemblies were incubated for 30 days in tubes with sterile BHI broth at 37 °C and 100 rpm. As a positive control, untightened assemblies were used (n=5), and as a negative control, assemblies not subjected to bacterial inoculation were tested (n=5) and maintained under the defined experimental conditions. Every 24 hours, bacterial microleakage was evaluated and recorded by the presence of turbidity in the broth or deposits at the bottom of the tubes—a visual macroscopic indication of bacterial leakage. After 30 days, if no bacterial leakage had been observed, the assemblies were carefully unscrewed, and the bacterial viability was assessed by sampling with sterile paper points. Any specimens without viable bacteria were excluded from the assay. At defined periods, randomly selected assemblies (Research Randomizer; https://www.randomizer.org/) were characterized by SEM imaging. Briefly, the specimens were washed with phosphate-buffered saline, fixed for 30 minutes with 1.5% glutaraldehyde, and dehydrated in an ascending series of alcohols. Assemblies were critical point dried (CPD 7501; Polaron Range), sputter-coated with a thin film of gold and palladium, and imaged by SEM (Quanta 400 FEG ESEM/EDAX Genesis X4M; FEI Co).

The microleakage of the assemblies was further assessed after applying a commercially available sealer (GapSeal; Hager & Werken). Twenty implants were inoculated with *E. faecalis* suspension as previously described, filled with the sealer according to the manufacturer's instructions, and immediately connected to the healing abutments (n=10) or the cover screws (n=10), which were tightened to 10 Ncm (Fig. 2).

The assemblies were incubated up to 30 days as previously described, and bacterial microleakage was assessed as previously described. To address the possible influence of the sealer in the removal torque of the abutments or cover screws, another set of assemblies were tightened according to the manufacturers' recommendations, with or without the sealer. After 48 hours, the assemblies were unscrewed, and the removal torque was recorded, which was not significantly different.

The irritation potential of the sealant, as supplied, was tested with the chorioallantoic membrane (CAM) assay according to the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) guidelines.[29-32] In short, fertilized chicken eggs were incubated with hourly rotation at 37 °C in a 60% humidified atmosphere in an incubator (Octagon Advance; Brinsea Products, Inc). At day 9 after fertilization, an eggshell window was created to access the CAM. Subsequently, 300 μL of the sealer was distributed into the CAM silicone O-rings used to retain the sealer and delimitate the area of analysis. For a contact period of up to 5 minutes, the CAM was observed and imaged by using a stereomicroscope (Stemi 305; Zeiss) and an attached imaging system (Axiocam 208 color; Zeiss), and the time taken for injury to occur was recorded. Specimens were tested in quintuplicates. The irritation index (II) was scored according to the severity and speed of damage regarding hemorrhage, vascular lysis, and coagulation from the following formula:

$$II = \frac{(301-H)}{300} \times 5 + \frac{(301-L)}{300} \times 7 + \frac{(301-C)}{300} \times 9,$$

where H, L, and C correspond to the time in seconds required to initiate hemorrhage, lysis, and coagulation, respectively. The Irritation Index is classified in accordance with the following score: 0–0.9, no irritation; 1–4.9 weak irritation; 5–8.9, moderate irritation; and 9–21, severe irritation.

For quantitative assays, data were presented as the mean ±standard deviation of replicates. Normality was evaluated by the Shapiro-Wilk test, and as variables were normally distributed, a 2-sample t test was performed to compare means between the 2 groups under study. Regarding microleakage, data were presented by using the Kaplan-Meier method and compared statistically by using the log-rank test (α=.05). A statistical analysis was performed with a statistical software program (IBM SPSS Statistics, v26.0; IBM Corp).

RESULTS

The microgap of the healing abutment-implant interface and cover screw-implant interface were determined by SEM evaluation of the sectioned assemblies (Fig. 3). The quantitative assessment revealed a mean ±standard deviation value of 1.65 ±0.34 μm at the healing abutment-implant interface and a significantly greater value of 2.18

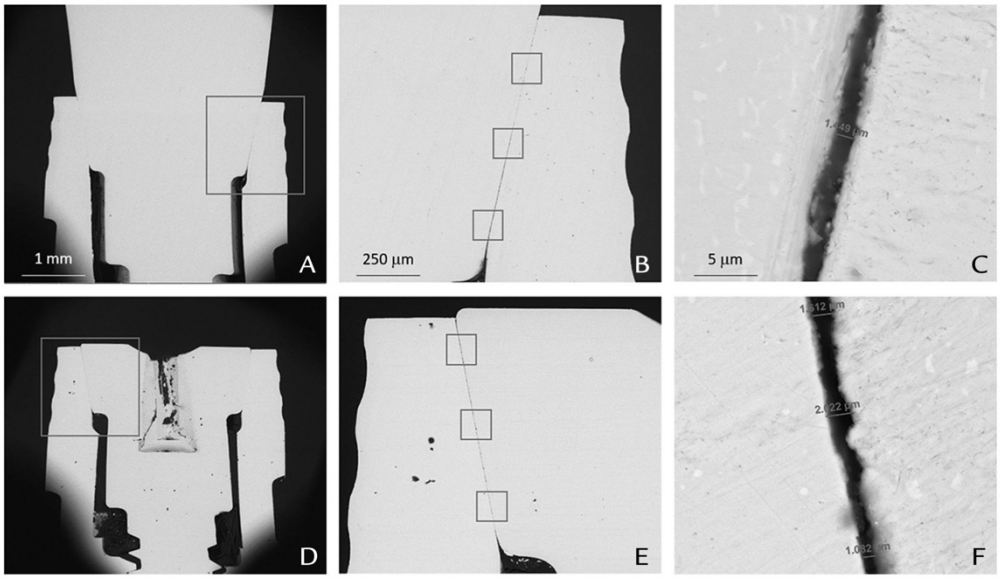

Figure 3. Representative scanning electron micrographs of implant-healing abutment and implant-cover screw interfaces and assessment of interfacial microgap. A-C, Healing abutment. D-F, Cover screw. Original magnification: A, D, ×50; B, E, ×200; C, F, ×10 000.

±0.29 µm at the cover screw-implant interface (P=.002) (Fig. 4).

Both assemblies exhibited bacterial leakage in the first days, broadly up to day 9 of the incubation period (Fig. 5). For the remaining assemblies, 44% of the healing abutments and 56% of the cover screws showed no bacterial leakage up to 30 days despite the confirmed viability of the bacteria inside the implants. No significant differences were found between conditions (P=.142). SEM imaging of selected assemblies, collected 24 hours after bacterial leakage, revealed the infiltration of *E. faecalis* through the microgap for both the healing abutment-implant and cover screw-implant systems, with a progressive organization of biofilm (Fig. 6).

As shown in Figure 7, the application of the sealer homogenously filled the interface and spread to the neighboring surfaces. The sealer completely prevented bacteria leakage throughout the assayed period of 30 days, for both interfaces (Fig. 8).

From the HET-CAM irritation assay, the direct application of the sealer on the CAM induced no significant adverse vascular alterations, hemorrhage, coagulation, or vascular lysis at the 5-minute observation period, similar to the negative control. The irritation index for the sealer and the negative control were both 0.07, corresponding to a no irritation score. The positive control induced an irritation index of 12.80, corresponding to severe irritation. Representative macrographs of the CAM assay are presented in Figure 9.

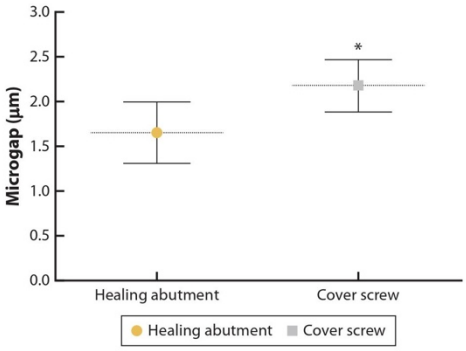

Figure 4. Mean of highest microgap values of system interfaces tightened to 10 Ncm. *Significantly different from healing abutment (t test=3.713, P=.002).

DISCUSSION

This in vitro study was designed to address the microgap and bacterial microleakage of the implant-cover screw and implant-healing abutment interfaces of a platform-switched implant system, further characterizing the

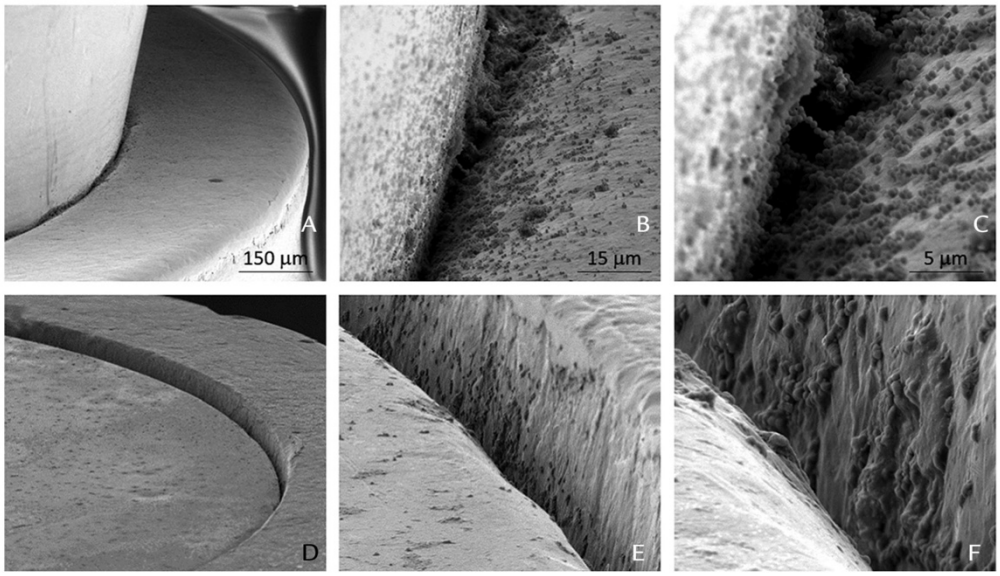

Figure 5. Representative scanning electron micrographs of healing abutment/implant and cover screw/implant interfaces *E. faecalis* leakage. A-C, Healing abutment. D-F, Cover screw. Original magnification: A, D, ×400; B, E, ×4000; C, F, ×10 000.

functionality of an interface sealing agent. Based on the results, the null hypothesis that no significant difference would be found in the bacterial microleakage of the assayed system in the presence or absence of the silicone sealer was rejected.

Despite the high success rate of implant-supported restorations,[1] early and late failures of the osseointegration process have been reported and correlated with the colonization of pathogenic microorganisms.[7] During the bone remodeling phase, the microgap at the implant-prosthetic abutment interface allows the leakage of bacteria and bacteria-derived products. These may trigger and sustain local immunoinflammatory activation, possibly culminating in periimplant bone loss.[8,9] Nevertheless, information on the role of the interface between the implant and the cover screw or healing abutment, in terms of bacterial microleakage, is sparse.

The interfacial microgap was accessed by SEM, and maximal values were found to be within the 1.5 to 2.0 µm range for both interfaces. These values were lower than reported values for the microgap of prosthetic abutment-implant interfaces (0.1 to 10 µm, before loading).[6] The formation of space at the implant-fixture interface seems to be inevitable because of the technical precision limit on the components' fabrication[14] and may further increase with the cyclic loading during oral function. Space at the interface may allow the movement of bacteria and related metabolic products, promoting the colonization and biofilm development within the internal implant surfaces.

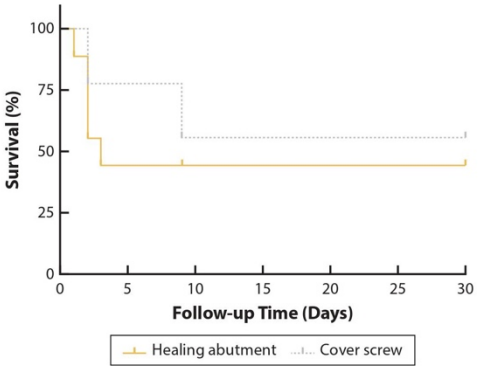

Figure 6. Bacterial microleakage assessment of implant-healing abutment and implant-cover screw interfaces upon *E. faecalis* inoculation and incubation for up to 30 days.

Bacterial microleakage was assayed with *E. faecalis*, which has been used in in vitro bacterial microleakage studies addressing implant fixture interfaces.[12] *E. faecalis*

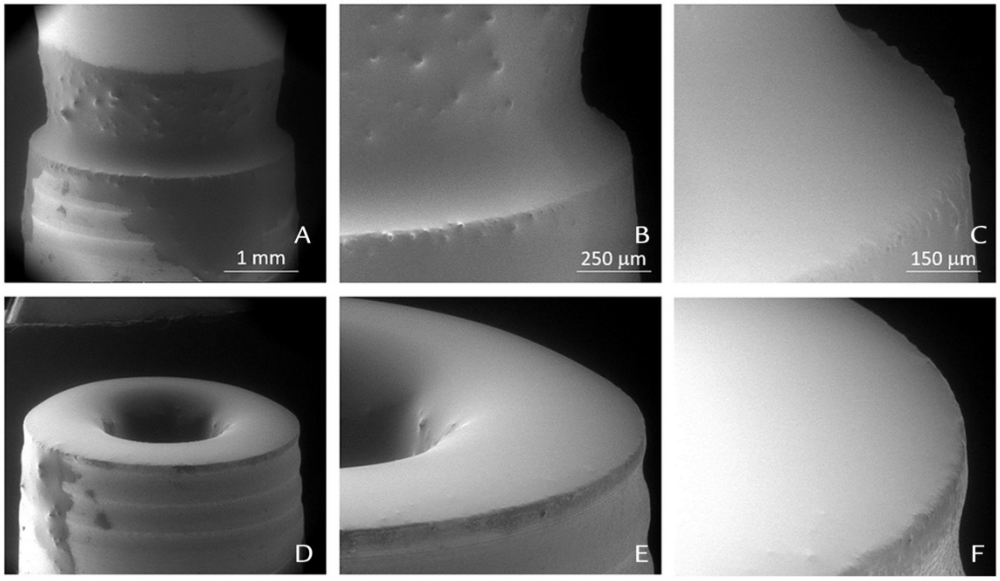

Figure 7. Representative scanning electron micrographs of implant-healing abutment and implant-cover screw interfaces with silicone sealant. A-C, Healing abutment. D-F, Cover screw. Original magnification: A, D, ×60; B, E, ×150; C, F, ×400.

are Gram-positive cocci involved in the pathogenesis of oral infections, associated with periodontitis,[25] and are frequent colonizers of oral implant surfaces.[23] The role of *E. faecalis* in the pathogenesis of peri-implantitis has been reported.[24] In the present experimental setting, bacterial microleakage was ≈50% of the conditions, with most of the leakage occurring early for both interfaces. The similar microleakage results for both fixtures might be related to the attained microgap levels—found to be similar for both systems—given the correlation between microgap and interfacial microleakage.[6]

Another factor that may influence microgap and microleakage is the tightening torque of the system's components.[19] In the present experiment, and despite the manufacturers' recommendations to conduct manual tightening without a specific torque value, the insertion torque was adjusted to 10 Ncm for reproducibility. This value was based on previous studies reporting a typical manual tightening torque within a 7 to 14 Ncm range[15] and an average of approximately 10 Ncm (range 7.2 to 10.9 Ncm).[16] A reduction in the interfacial gap with increasing torque values has been reported,[13] resulting from the increased clamping force between the implant and the interfacial fixture, increasing the surface contact area and, thus, decreasing the interfacial gap.[6] Additionally, higher tightening torques seem to minimize biomechanically related complications, reducing screw loosening and micromotion. Micromotion may interfere with the stability of the soft-tissue seal that protects the underlying bone tissue and the bone-implant interface[17] and further contribute to a micropumping effect at the microgap, intensifying the leakage of bacteria and related by-products, as well as increasing the movement of saliva, blood, and crevicular fluid.[18]

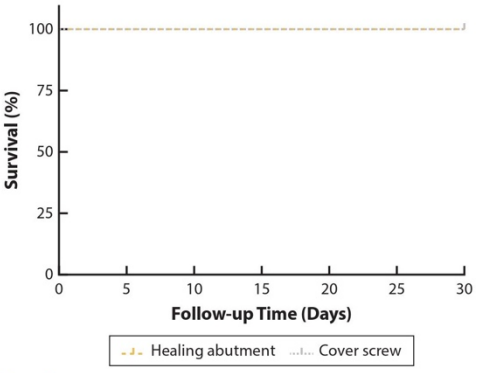

Figure 8. Bacterial microleakage assessment of implant-healing abutment and implant-cover screw interfaces upon *E. faecalis* inoculation and application of sealing agent up to 30 days.

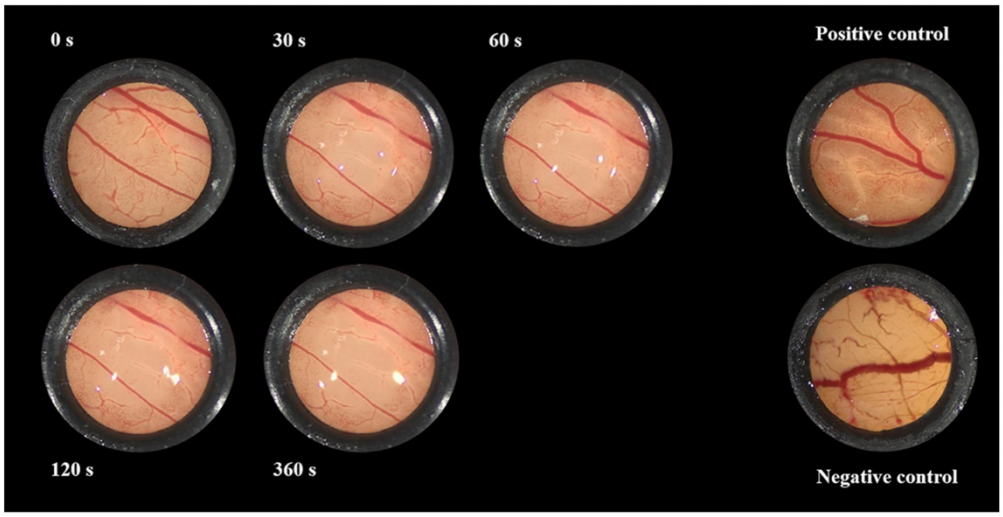

Figure 9. Representative images of HET-CAM assay of sealant at different time points indicating no significant vascular alterations.

A silicone sealing agent was assessed for functionality and biological response. This agent is composed of a viscous silicone matrix and thymol, a monoterpene phenol with established antifungal and antibacterial activity.[26] The product is bioactive and provides a physical and chemical barrier against pathogen colonization of the interfacial microgap. Given its antibacterial activity and ability to fill in the gap spaces, the sealing agent has been used for implant-prosthetic abutment interfaces, with documented in vitro[27] and clinical[28] effectiveness. However, the authors are unaware of studies that evaluated its efficacy on healing abutments and implant cover screws. In the present study, the application of the sealing agent was found to prevent bacterial leakage throughout the assessed 30-day period of follow-up. The biological response to the sealant was further addressed in the HET-CAM irritation assay. This characterization system allows the assessment of irritation and inflammation of implanted substances[30-32] and has been reported to be a reliable alternative to in vivo assessment given its suitable reproducibility and sensitivity, which is based on the alteration of vascular endpoints.[31] No significant vascular alterations were detected, with the silicone sealer inducing a response similar to that of the negative control, thus supporting its nonirritation potential.

Limitations of the present study included that the microleakage was evaluated in the absence of mechanical loading. Future studies should evaluate microleakage upon the combined application of dynamic mechanical testing and hydrothermal cycling, modeling for short- and long-term clinical use. Future studies may also include an extended biological characterization of the sealing agent for further endpoints such as cytotoxicity, genotoxicity, and hemocompatibility.

CONCLUSIONS

Based on the findings of this in vitro study, the following conclusions were drawn:

1. Despite the low microgap values for both interfaces, bacterial microleakage was found in around 50% of the assayed specimens in a 30-day follow-up period.
2. The silicone sealer tested had no irritation potential according to the HET-CAM assay and provided effective sealing for the systems tested.

REFERENCES

1. Howe M, Keys W, Richards D. Long-term (10-year) dental implant survival: a systematic review and sensitivity meta-analysis. J Dent 2019;84:9-21.
2. Albrektsson T, Buser D, Sennerby L. Crestal bone loss and oral implants. Clin Implant Dent Relat Res 2012;14:783-91.
3. Caricasulo R, Malchiodi L, Ghensi P, Fantozzi G, Cucchi A. The influence of implant-abutment connection to peri-implant bone loss: a systematic review and meta-analysis. Clin Implant Dent Relat Res 2018;20:653-64.
4. de Medeiros R, Pellizzer E, Vechiato Filho A, Dos Santos D, da Silva E, Goiato M. Evaluation of marginal bone loss of dental implants with internal or external connections and its association with other variables: a systematic review. J Prosthet Dent 2016;116:501-6.
5. Esposito M, Grusovin M, Chew Y, Coulthard P, Worthington H. One-stage versus two-stage implant placement. A Cochrane systematic review of randomised controlled clinical trials. Eur J Oral Implantol 2009;2:91-9.

6. Liu Y, Wang J. Influences of microgap and micromotion of implant–abutment interface on marginal bone loss around implant neck. Arch Oral Biol 2017;83:153-60.
7. Dias E, Sperandio M, Napimoga M. Association between implant-abutment microgap and implant circularity to bacterial leakage: an in vitro study using tapered connection implants. Int J Oral Maxillofac Implants 2018;33:505-11.
8. Tallarico M, Canullo L, Caneva M, Özcan M. Microbial colonization at the implant-abutment interface and its possible influence on periimplantitis: a systematic review and meta-analysis. J Prosthet Res 2017;61:233-41.
9. Koutouzis T. Implant-abutment connection as contributing factor to peri-implant diseases. Periodontol 2000 2019;81:152-66.
10. Sasada Y, Cochran D. Implant-abutment connections: a review of biologic consequences and peri-implantitis implications. Int J Oral Maxillofac Implants 2017;32:1296-307.
11. 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 2020;13:1131.
12. Mishra S, Chowdhary R, Kumari S. Microleakage at the different implant abutment interface: a systematic review. J Clin Diagn Res 2017;11:10-5.
13. Verdugo C, Núñez G, Avila A, San Martín C. Microleakage of the prosthetic abutment/implant interface with internal and external connection: in vitro study. Clin Oral Implants Res 2014;9:1078-83.
14. Alves D, de Carvalho P, Elias C, Vedovatto E, Martinez E. In vitro analysis of the microbiological sealing of tapered implants after mechanical cycling. Clin Oral Invest 2016;20:2437-45.
15. Gross M, Kozak D, Laufer B, Weiss E. Manual closing torque in five implant abutment systems: an in vitro comparative study. J Prosthet Dent 1999;81:574-8.
16. Kose O, Karatash B, Demircan S, Kose T, Cene E, Aya S, et al. In vitro evaluation of manual torque values applied to implant-abutment complex by different clinicians and abutment screw loosening. BioMed Res Int 2017;2017:7376261.
17. Passos S, May L, Faria R, Ozcan M, Bottino M. Implant-abutment gap versus microbial colonization: clinical significance based on a literature review. J Biomed Mater Res Part B 2013;101:1321-8.
18. Sahin C, Ayyildiz S. Correlation between microleakage and screw loosening at implant-abutment connection. J Adv Prosthodont 2014;6:35-8.
19. Vélez J, Peláez J, López-Suárez C, Agustín-Panadero R, Tobar C, Suárez M. Influence of implant connection, abutment design and screw insertion torque on implant-abutment misfit. J Clin Med 2020;9:2365.
20. Lucena G, Molon R, Moretti A, Shibli J, Rêgo D. Evaluation of microbial contamination in the inner surface of titanium implants before healing abutment connection: a prospective clinical trial. Int J Oral Maxillofac Implants 2018;33:853-62.
21. Vergara-Llanos D, Koning T, Pavicic M, Bello-Toledo H, Díaz-Gómez A, Jaramillo A, et al. Antibacterial and cytotoxic evaluation of copper and zinc oxide nanoparticles as a potential disinfectant material of connections in implant provisional abutments: an in-vitro study. Arch Oral Biol 2021;122:105031.
22. Jain S, Schramm S, Siddiqui D, Huo W, Palmer K, Wilson T, et al. Effects of multiple implantations of titanium healing abutments: surface characteristics and microbial colonization. Dent Mater 2020;36:e279-91.
23. Canullo L, Rossetti P, Penarrocha D. Identification of Enterococcus faecalis and Pseudomonas aeruginosa on and in implants in individuals with peri-implant disease: a cross-sectional study. Int J Oral Maxillofac Implants 2015;30:583-7.
24. Flanagan D. Enterococcus faecalis and dental implants. J Oral Implantol 2017;43:8-11.
25. Canullo L, Penarrocha-Oltra D, Covani U, Rossetti P. Microbiologic and clinical findings of implants in healthy condition and with peri-implantitis. Int J Oral Maxillofac Implants 2015;30:834-42.
26. Marchese A, Orhan I, Daglia M, Barbieri R, Di Lorenzo A, Nabavi S, et al. Antibacterial and antifungal activities of thymol: a brief review of the literature. Food Chem 2016;210:402-14.
27. Nayak A, Fernandes A, Kulkarni R, Ajantha G, Lekha K, Nadiger R. Efficacy of antibacterial sealing gel and O-ring to prevent microleakage at the implant abutment interface: an in vitro study. J Oral Implantol 2014;40:11-4.
28. Zekiy AO, Makurdumyan DA, Matveeva EA, Bogatov EA, Kaliiants TV. Antiseptic sealant and a nanocoated implant-abutment interface improve the results of dental implantation. Clin Implant Dent Relat Res 2019;21:938-45.
29. ICCVAM. Test Method Protocol: Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) Test Method. ICCVAM test method evaluation report: current validation status of in vitro test methods proposed for identifying eye injury hazard potential of chemicals and products. NIH Publication No. 10-75532010. 2010.
30. Bender C, Partecke L-I, Kindel E, Döring F, Lademann J, Heidecke C-D, et al. The modified HET-CAM as a model for the assessment of the inflammatory response to tissue tolerable plasma. Toxicol in Vitro 2011;25:530-7.
31. Schneider-Stock R, Ribatti D. The CAM assay as an alternative in vivo model for drug testing. In: Schäfer-Korting M, Stuchi Maria-Engler S, Landsiedel R, editors. Organotypic Models in Drug Development Handbook of Experimental Pharmacology. 1st ed. New York: Springer; 2021. p. 303-23.
32. Schrage A, Gamer A, van Ravenzwaay B, Landsiedel R. Experience with the HET-CAM method in the routine testing of a broad variety of chemicals and formulations. Altern Lab Anim 2010;38:39-52.

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Acknowledgments
The authors thank Klockner for providing the materials used in this study.

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https://doi.org/10.1016/j.prosdent.2021.07.030

APPENDIX 2

Odontology
https://doi.org/10.1007/s10266-021-00652-x

ORIGINAL ARTICLE

Microgap and microleakage of a hybrid connection platform-switched implant system in the absence or presence of a silicone-based sealing agent

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Received: 15 July 2021 / Accepted: 21 August 2021
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Abstract
The present study aims to characterize, for the first time, the microgap and bacterial microleakage of a platform-switched implant system with hybrid connection, screwed at distinct torque values (manufacturer recommended torque—25 N cm—and a reduced torque—5 N cm—mimicking the long-term functional use), in the absence or presence of a silicon-based sealing agent. Microgap was determined through scanning electron microscopy and bacterial microleakage was evaluated in vitro, upon *Enterococcus faecalis* colonization of the system. The sealing efficacy was evaluated in the absence or presence of a commercially available silicon-based sealer. The cytotoxicity of the sealer was further addressed in vitro, with a fibroblastic cell line, in accordance with reference standards. A low microgap of the implant system was verified, regardless of the applied torque load—maximal values ranged around 0.25 and 1.25 µm, for 25 and 5 N cm torques, respectively. No bacterial microleakage was reported at 25 N cm, while at 5 N cm, leakage was verified on 38% of the samples. The application of a silicon-based sealer—with an adequate cytocompatible profile—was effective on preventing the bacterial microleakage on the assayed experimental setting. The assayed platform-switched implant system with hybrid connection presented a low interfacial misfit and an effective sealing capability at manufacturer recommended torque. Despite the increased microleakage at low torque conditions, the application of a cytocompatible silicon-based sealing agent restored the sealing effectiveness of the system. The use of a silicon-based sealing agent can assist on the maintenance of the sealing effectiveness even at low torque conditions.

Keywords Microgap · Microleakage · Implant · Abutment · Hybrid connection · Platform-switching

Abbreviations

IAI	Implant–abutment interface
SEM	Scanning electron microscopy
BHI	Brain heart infusion
CFU	Colony-forming units
DMSO	Dimethyl sulfoxide
PI	Propidium iodide

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Published online: 27 August 2021

Introduction

In recent years, there has been a great evolution in the field of implantology—both in terms of materials and techniques—and what started as an empirical act of teeth replacement became a well-differentiated therapeutic approach with high success rates [1].

Osteointegration—the direct apposition of bone within a mechanically stable implant surface—became a fundamental requirement for the predictable outcome of implant-based treatments, in spite of different factors that seem to hinder the predictability and success of oral rehabilitations [2]. Marginal bone loss seems to be one of the most common complications throughout the distinct bone healing phases, potentially compromising the long-term stability and success of oral rehabilitations. Diverse factors, including peri-implantitis, occlusal overload, surgical trauma, implant–abutment design and physic-chemical properties,

as well as microleakage, have been associated with the loss of marginal bone [3–5].

Currently, the vast majority of implant-based rehabilitations rely on the use of two-piece components—the implant and the abutment, given the system capability to protect the implant from undesirable forces during loading, as well as the ability to orientate the prosthetic angle of the rehabilitation [6]. Regardless of the connection type, the interface between both pieces cannot be precisely matched due to the technical precision limits of production, which originates a microgap at the implant–abutment interface (IAI) [7]. In the vast majority of the systems, the microgap ranges from 1 to 50 µm upon connection and prior to loading, whether it may increase with cyclic loading under function [8, 9].

Oral bacteria are within the range of 0.2–1.5 µm (width) and 2–10 µm (length), and though can easily penetrate the microgap and access the internal cavity of the implant, promoting a product exchange between the implant interior and the oral environment [10]. The movement of bacteria, as well as of bacteria-derived metabolic products and other small molecules and fluids, into and out the internal cavity of the implants, is defined as the IAI microleakage [11]. The accumulation and perpetuation of pathobionts within the gap and cavity of the IAI, neighboring the peri-implant tissues, seems to induce bone resorption, either directly—via the release of cytotoxic byproducts, or indirectly—via the activation of the immune-inflammatory response [12–15]. Microleakage is further increased by micromovements that elapse from the microabrasion, microshift and/or microrotation that occur between the implant and abutment components during function [16].

In order to lessen microleakage-related complications, the design of the implant systems has been improved. Platform-switching—relying on a connection platform smaller than the diameter of the implant collar, aim an inward reposition of the IAI, thus distancing microleakage and micromotion events away from the alveolar ridge [17, 18]. Different meta-analytical reviews and clinical trials have reported favorable outcomes in the use of platform-switching, as compared to platform matching, regarding marginal bone level and bacterial load at the peri-implant tissues, within conical, internal and external polygonal connections [19, 20]. Notwithstanding, no data have been reported on the most recent platform-switched systems with hybrid connections—conjoining a conical interface with an internal polygonal connection, despite the fact that literature supports that all implant–abutment connections present an amount of microgap and associated microleakage, that needs to be addressed [21, 22].

Accordingly, different strategies have been developed to minimize/prevent bacterial leakage, ranging from the use of disinfectants and antibacterial drugs (e.g., chlorhexidine, doxycycline—aiming to reduce bacterial colonization) to sealing agents (e.g., silicones, o-rings—aiming to physically close the microgap, preventing microleakage) [23, 24]. Whether some data have been published for platform matching systems [25–27], to the best of the authors' knowledge, the efficacy of sealing agents has not been assayed in platform-switched interfaces.

Hence, the present study aims to, for the first time: (1) evaluate, in vitro, the microgap and microleakage of a platform-switched implant system with hybrid connection, screwed at distinct torque values; and (2) address the efficacy and biological response of a commercial sealing agent, applied within the referred platform-switched implant system with hybrid connection.

Materials and methods

Experimental groups

Hybrid connection implants with platform-switching (4 mm in diameter and 11 mm in length—KLOCKNER Vega®, Barcelona, Spain) and titanium straight abutments (total length of 8 mm—KLOCKNER®, Barcelona, Spain), directly supplied by the manufacturer, were selected and assigned to two equal groups according to the torque applied to the assemblies, i.e., 25 and 5 N cm (Fig. 1). To minimize the effect of sedimentation on the screws, which may lead to

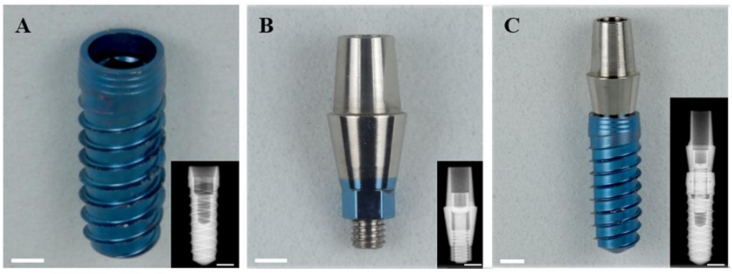

Fig. 1 **A** Implant Klockner Vega RV 4×11 mm; **B** Klockner Vega RV 8 mm straight abutment; and **C** assembled implant and straight abutment. Inset images: respective X-ray micrography. Scale bar 2 mm

preload reduction, components were retightened after 10 min, with the initial torque value. Before every adjustment, the torquemeter was checked for correct calibration.

Microgap determination

To determine the width of the gap between the implant and abutment, 20 abutments were connected to the implants and tightened at 25 N cm ($n = 10$) and 5 N cm ($n = 10$). The assemblies were following included in a self-polymerizable acrylic resin (Vertex Orthoplast, Vertex-dental, Netherlands) and cut along their longitudinal axis with a diamond wheel mounted on a precision cut-off and grinding machine, with a feed speed of 0.150 mm/s (Struers Accutom-5, Struers Inc, United States). Sections were following polished, washed and dried. Images of the IAI were obtained under scanning electron microscopy (SEM, Quanta 400 FEG ESEM/EDAX Genesis X4M). Data collection was performed in six positions, 3 on each side of the IAI (Fig. 2A, red squares on center micrographs). Three measures, corresponding to the highest values spaced at least 2.5 µm, were determined and averaged. Data analysis was conducted independently by two researchers and repeated three times.

Microleakage assessment

To evaluate the bacterial microleakage, 20 implants were removed from their packaging under sterile conditions and inoculated with 0.2 µL of *Enterococcus faecalis* (ATCC 29212) suspension in BHI (Brain–Heart Infusion, Liofilchem) broth, at 10^8 colony-forming units (CFU)/mL. After inoculation, the abutments were carefully screwed to the implants at 25 N cm ($n = 10$) and 5 N cm ($n = 10$), under sterile conditions. To avoid false positives and discard potential external contaminations, all assemblies were pre-incubated in tubes containing sterile BHI broth for 30 s, that were further incubated at 37 °C for 48 h. Tubes with a cloudy broth (evidencing bacterial growth) led to the exclusion of the respective assembly from further observations. The assemblies, transferred to new tubes with sterile BHI broth, were incubated at 37 °C and 100 rpm up to 30 days. For negative control, abutments were laid on but not tightened to implants free of bacterial inoculation. For positive control, the abutments were laid on but not tightened to implants inoculated with bacterial suspension, as the experimental groups. Visual inspection of the tubes was performed daily. A turbid broth indicated bacterial leakage. If no turbidity was detected after 30 days of incubation, the abutments were

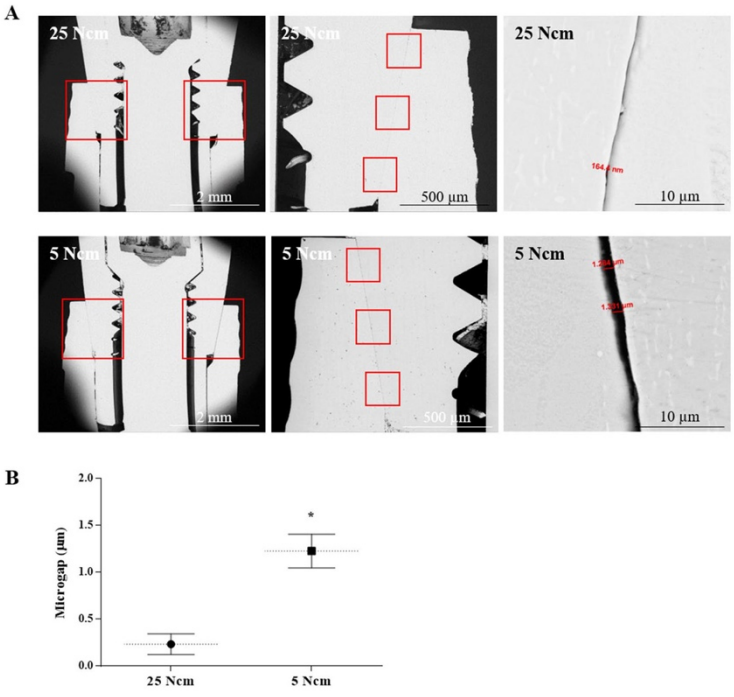

Fig. 2 **A** SEM micrographs of the implant–abutment interface at different magnifications, and **B** mean of the highest microgap values in the implant–abutment assemblies, tightened at 25 and 5 N cm. *Significantly different from 25 N cm conditions, $p < 0.05$

carefully unscrewed, and the inner surface of the implants were sampled using sterile paper points to assess the viability of the bacteria. Additionally, bacterial microleakage was qualitatively evaluated by SEM. At selected time-points of incubation, implant–abutment assemblies were washed twice with warm phosphate buffered saline (PBS), fixed for 30 min with 1.5% glutaraldehyde in sodium cacodylate (0.14 M) and dehydrated gradually with a series of ethanol solutions. Assemblies were critically point dried (CPD 7501, Polaron Range), sputter-coated with gold/palladium (Au/Pd) thin film and visualized by SEM (FEI Quanta 400FEG ESEM/EDAX Genesis X4M).

Microleakage assessment after implant–abutment sealing

For the efficacy assessment of the sealing strategy, implant–abutment assemblies were inoculated with *E. faecalis*, as aforementioned, loaded with Gapseal® (Hager Werken, Germany) according to the manufacturer's instructions and carefully screwed at 5 N cm ($n = 10$). Gapseal® is a silicone matrix sealer with thymol antiseptic. The sealed assemblies were characterized by SEM (FEI Quanta 400FEG ESEM/EDAX Genesis X4M). Bacterial microleakage was assessed, as previously detailed, up to 30 days.

To evaluate whether the use of sealer could change the removal torque of the abutments, another set of implant–abutment assemblies were sealed with Gapseal® and screwed as previously described. After 48 h, all abutments were unscrewed, and the torque of removal recorded. No significant differences were attained regarding torque removal, in the presence or absence of Gapseal® application.

Biological characterization of the sealing agent: in vitro cytotoxicity assessment

The biological response of Gapseal® was addressed in vitro, with a fibroblastic cell line, in accordance with ISO 10993-5 standards—Biological evaluation of medical devices—Part 5: Tests for in vitro cytotoxicity.

Sealer' extracts were prepared on 24-well plates, upon incubation in complete cell culture medium (α-minimum essential medium (α-MEM) supplemented with 10% (v/v) foetal bovine serum, 100 IU/mL penicillin and 100 µg/mL streptomycin (all from Gibco®)), for 24 h, at 37 °C and a 5% CO_2 humidified atmosphere.

L929 mouse fibroblast cells (NCTC clone 929, ATCC) were grown in complete cell culture medium (supplemented α-MEM, as described above). Cells were seeded at a density of 10^5 cells/mL into 96-well plates and incubated for 24 h, at 37 °C, under a 5% CO_2 humidified atmosphere. After incubation, the cells were exposed to undiluted and diluted (30, 10, 3, 1 and 0.1%) extracts, in cell culture medium. As negative control, cells were incubated with fresh cell culture medium and as positive control, cells were incubated with dimethyl sulfoxide (DMSO, Panreac®). The plates were incubated for an additional period of 24 h. Cell viability and cell metabolic activity were evaluated by live/dead staining and MTT assay, respectively. For live/dead staining, the extracts were removed, and cells were incubated with a Calcein AM (1 µM, BioLegend®) and propidium iodide (PI, 50 µL/mL, BD Biosciences®) solution for 15 min at 37 °C, protected from light. Fluorescent cells were visualized and recorded using a Celena S digital imaging system (Logos Biosystems®), equipped with green (488 nm) and red (594 nm) filters. Concerning MTT assay, at the end of the culture period, cells were incubated with 10% MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium, Sigma-Aldrich), for 3 h, at 37 °C. The formazan precipitates formed were solubilized in DMSO and the absorbance was measured at 550 nm on a microplate reader (Synergy HT, BioTek®). The results were expressed as percentage of control culture (negative control).

Statistical analysis

Quantitative data are presented as mean ± standard deviation of replicates. Normality was evaluated by the Shapiro–Wilk test and as variables were normally distributed, a two-sample *T* test was performed to compare means between the two groups under study. Regarding microleakage, data were presented using the Kaplan–Meier method and compared statistically using the log rank test. The significance level was set at 5% (p values ≤ 0.05 were considered significant). Statistical analysis was performed in the IBM SPSS statistics 26.0 software.

Results

The microgap at the implant–abutment assemblies was determined upon SEM imaging, on 25 and 5 N cm torque groups. Representative SEM micrographs and defined areas of evaluation are depicted in Fig. 2A. Briefly, a reduced microgap length was identified on the 25 N cm group (within the 0.25 µm range), as compared to the 5 N cm group (within the 1.25 µm range), as shown on representative SEM images (Fig. 2A) and calculated averaged maximal values (Fig. 2B).

Bacterial microleakage was assessed for 30 days, following implant inoculation with *E. faecalis* and abutment assembly, at either 25 N cm or 5 N cm torques. Assemblies of the 25 N cm group showed no leakage during the experimental period. On the other hand, assemblies tightened at 5 N cm allowed bacteria microleakage. Until day 3, 38% of the assemblies presented turbidity in the culture medium, as consequence of microbial leakage, while the remaining

implants showed no signs of leakage over the 30 days of follow-up (Fig. 3A). SEM imaging revealed no bacterial leakage for 25 N cm condition and the gradual infiltration of *E. faecalis* trough the IAI, for assemblies tightened at 5 N cm (Fig. 3B). Higher magnifications images showed the presence of *E. faecalis* organized in biofilm and extensively colonizing implant and abutment's surfaces.

Given the absence of bacterial microleakage at the 25 N cm group, the assessment of the efficacy of the sealing agent (Gapseal®) was conducted exclusively at 5 N cm torque. SEM imaging revealed a complete filling/covering of the IAI (Fig. 4A) and a complete efficacy on preventing the microleakage of bacterial suspension throughout the experimental period of 30 days (Fig. 4B).

The biological characterization of the sealer was further assessed in vitro, with fibroblastic cells grown in the presence of sealer' extract at distinct concentrations (ranging from the undiluted extract—100%, to a 0.3% dilution), in accordance with ISO 10993-5 standards. Undiluted sealer' extract significantly decrease the metabolic activity of the cultures, as compared to control. On the other hand, diluted Gapseal® extracts, within the 0.3–30% range, presented no significant differences to control (Fig. 5A). For these dilutions and through live/dead fluorescent imaging, cultures revealed a high viability evidenced by the predominant green fluorescence, with cells presenting an elongated morphology and significant cytoplasmic spreading, similar to negative control (Fig. 5B).

Discussion

This study aimed to characterize, for the first time, the microgap and microleakage of a platform-switched implant system with a hybrid connection, with distinct torques at the implant–abutment assembly (i.e., 25 and 5 N cm), further addressing the efficacy and biological response

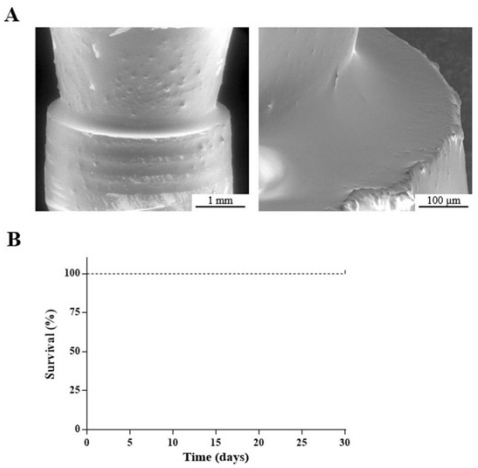

Fig. 4 **A** SEM micrographs of the implant–abutment interface following the application of the sealing agent, at two different magnifications. **B** Bacterial microleakage assessment for implant–abutments assemblies tightened at 5 N cm, after *E. faecalis* inoculation and application of the sealing agent, up to 30 days of follow-up

of a commercially available sealing agent applied at the implant–abutment interface (IAI).

Platform-switched systems, based on the inward shifting of the abutment contour over the implants, reduce the stress concentration at the crestal bone level and improve soft tissue stabilization, allowing an increased preservation of the peri-implant bone level [18] 28. Biologically, platform-switching allows more surface area for the establishment of the biological width and, by distancing leakage—and IAI motion-related events away from the crestal ridge, further prevents bacterial contamination and inflammatory cell infiltration that were associated with the marginal bone loss

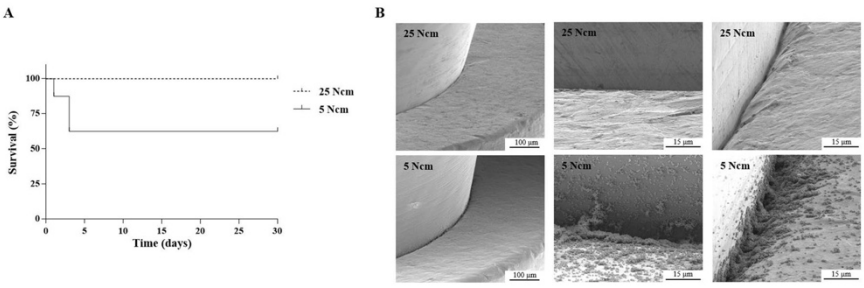

Fig. 3 **A** Bacterial microleakage assessment for implant–abutments assemblies tightened at 25 and 5 N cm, after *E. faecalis* inoculation, up to 30 days of follow-up. **B** SEM imaging of the IAI of 25 and 5 N cm torque conditions

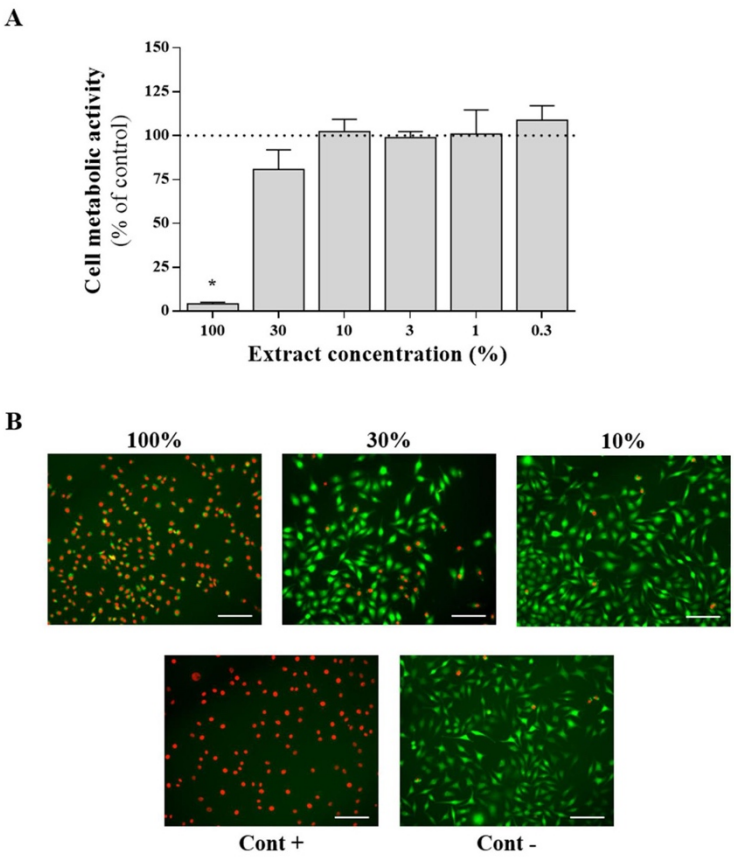

Fig. 5 In vitro biological evaluation of the sealing agent extracts following culture with L929 cells, for 24 h, as determined by **A** metabolic activity of the cell cultures and **B** fluorescence live/dead cell staining. Live cells stain green and dead cells stain red. Negative control: cells exposed to culture medium and positive control: cells exposed to DMSO (scale bar 100 µm). *Significantly different from negative control (dotted line), $p < 0.05$

[17]. Additionally, platform-switched systems have been combined with conical connections, further reducing microleakage by the cold-welding effect, thus restricting bacterial penetration and pumping effect inwards and outwards of the implant chamber [8]. Conical connections demonstrated an improved sealing performance and abutment stability, as well as long-term clinical functionality and resistance to bending forces and fatigue, as compared to external and internal polygonal connections [29–31].

Recent modifications on implant systems introduced hybrid connections, as the one evaluated within this study, elapsing from the combination of a conic and an internal polygonal connection, claiming an increased component fit and antirotational characteristics [11]. Whether these connections seem to display improved clinical outcomes given the lower rotation center and improved mechanic stability [32–34], no data is available regarding bacterial penetration and microleakage of these systems, as addressed in this research.

Furthermore, and despite the influence of the implant–abutment connection on the system microleakage [8], the torque of the IAI also seems to be a major determinant [35]. In the present study, distinct torque values were used to mimic the clinical application—the 25 N cm torque reflects the recommendations of the manufacturer, while the 5 N cm torque simulates a decreased stability at the IAI, elapsing from a long-term function under loading. Screw loosening, rotational forces and the settling effect decrease the screw preload and interface tolerance of the implant–abutment assembly [36], with a decreased torque level being selected to mimic this clinical situation.

In this study, microgap was evaluated by SEM imaging revealing very low IAI misfit regardless of the applied torque load—maximal values ranged around 0.25 and 1.25 µm, for

25 and 5 N cm torques, respectively. Hybrid implant–abutment connections are expected to display a reduced misfit, given the improved surface area of contact, load distribution and anti-rotational fit of the components, performing superiorly to external and internal connections [21, 34]. Additionally, a close relationship between the adjustment of the IAI and the torque of the prosthetic abutment screw has been established, as presently demonstrated, with the highest gap values being attained at decreased torque levels [37, 38]. Mechanistically, the reduction of the strengthening torque decreases the stress responsible for the elongation of the screw, thus lessening the compressive force that approximates the implant and abutment surfaces, diminishing the linear area of contact and increasing misfit [39].

The gap at the IAI seems to be directly related to the microleakage of the system, potentially associated with the passage of bacteria, bacteria-derived products, molecules and fluids through the IAI, contributing to the inflammation of peri-implant tissues and subsequent bone loss that may compromise the success of implant-based rehabilitations [11]. In the present work, microleakage assessment was evaluated for 30 days upon colonization of the implant chamber with *E. faecalis* suspension. *E. faecalis* is a Gram-positive coccus, commonly associated with a wide range of infections of the oral cavity, such as primary and secondary endodontic infections, dental caries, periradicular abscesses, periodontitis and peri-implantitis, being considered a keystone pathogen within the peri-implantitis-related bone loss [40, 41]. *E. faecalis* has been routinely used on microleakage studies at the IAI [8]. In the present study, bacterial leakage was not identified on the 25 N cm group, while for the 5 N cm group, leakage was established on 38% of the samples up to 3 days of the experimental period. These data support the high efficacy of the system on precluding microleakage when recommended torque values were used, i.e., 25 N cm. Notwithstanding, low torque conditions allowed microleakage, supporting the need of further therapeutic interventions to prevent bacterial penetration into the IAI.

Whether distinct approaches have been assayed to physically close the IAI, and consequently the microgap, the use of sealing agents seems to be effective on minimizing bacterial leakage, as recently reviewed in a meta-analysis [42]. Sealing gels improved the fastening and long-term stability of the IAI and decreased microleakage in both conical/internal and external connection implant systems [27]. Nonetheless, no data is available regarding the sealing efficacy for hybrid connection platform-switched systems. In this research, even in low torque conditions (5 N cm), the application of the sealing agent was effective on preventing microleakage for the 30-day experimental period. The used sealer is composed of a highly viscous silicone matrix with thymol—a natural phenolic monoterpene with effective antibacterial activity [43], and seems to occupy the spaces within the IAI, creating a physic and chemical barrier for microorganisms' colonization. The biological response of the sealer was further addressed in vitro, within a fibroblastic cell line, in accordance with the ISO 10993-5 standards. An adequate cytocompatible profile was attained, with no significant differences being established regarding cultures grown in the presence of diluted sealer extracts and the control. Silicone-based materials report an adequate cytocompatibility and biocompatibility, being commonly used within medical-related applications [44]. Additionally, thymol, apart from the effective antibacterial activity, seems to induce an adequate biological response, thus modulating the inflammation and enhancing the wound healing environment [43, 45].

Conclusions

This research addresses, for the first time, the assessment of the microgap and the bacterial microleakage of a hybrid connection platform-switched implant system, further evaluating the efficacy of a sealing agent at low torque conditions. Overall, the implant system presented a very low misfit at the IAI, even in low-torque conditions. The use of recommended torque values (25 N cm) was able to prevent bacterial microleakage, whether reduced torque levels (5 N cm) allowed for bacterial microleakage in 38% of the cases. Notwithstanding, the use of a sealing agent, reporting an adequate cytocompatibility profile, was found to restore the sealing effectiveness of the system.

Acknowledgements This work received financial support from PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the project UIDB/50006/2020. The authors would like to thank Klockner SA for providing the implants and abutments used in this study.

Funding The work was supported by Fundação para a Ciência e Tecnologia (FCT) through the project UIDB/50006/2020.

Declarations

Conflict of interest Pedro Fernandes declares that he has no conflict of interest. Liliana Grenho declares that she has no conflict of interest. Maria Helena Fernandes declares that she has no conflict of interest. João Carlos Sampaio-Fernandes declares that he has no conflict of interest. Pedro Gomes declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent For this type of study, formal consent is not required.

References

1. Payant L, Williams JE, Zwemer JD. Survey of dental implant practice. J Oral Implantol. 1994;20(1):50–8.
2. Misch CE. Endosteal implants for posterior single tooth replacement: alternatives, indications, contraindications, and limitations. J Oral Implantol. 1999;25(2):80–94.
3. Vigolo P, Givani A. Platform-switched restorations on wide-diameter implants: a 5-year clinical prospective study. Int J Oral Maxillofac Implants. 2009;24(1):103–9.
4. Suarez-Lopez Del Amo F, Lin GH, Monje A, Galindo-Moreno P, Wang HL. Influence of soft tissue thickness on peri-implant marginal bone loss: a systematic review and meta-analysis. J Periodontol. 2016;87(6):690–9.
5. Galindo-Moreno P, Leon-Cano A, Ortega-Oller I, Monje A, O'Valle F, Catena A. Marginal bone loss as success criterion in implant dentistry: beyond 2 mm. Clin Oral Implants Res. 2015;26(4):e28–34.
6. Andreasi Bassi M, Lopez MA, Confalone L, Gaudio RM, Lombardo L, Lauritano D. Clinical outcome of a two-piece implant system with an internal hexagonal connection: a prospective study. J Biol Regul Homeost Agents. 2016;30(2 Suppl 1):7–12.
7. Scarano A, Assenza B, Piattelli M, Iezzi G, Leghissa GC, Quaranta A, et al. A 16-year study of the microgap between 272 human titanium implants and their abutments. J Oral Implantol. 2005;31(6):269–75.
8. Mishra SK, Chowdhary R, Kumari S. Microleakage at the different implant abutment interface: a systematic review. J Clin Diagn Res. 2017;11(6):ZE10–ZE5.
9. Quirynen M, van Steenberghe D. Bacterial colonization of the internal part of two-stage implants. An in vivo study. Clin Oral Implants Res. 1993;4(3):158–61.
10. Nascimento C, Pita MS, Santos Ede S, Monesi N, Pedrazzi V, Albuquerque Junior RF, et al. Microbiome of titanium and zirconia dental implants abutments. Dent Mater. 2016;32(1):93–101.
11. Liu Y, Wang J. Influences of microgap and micromotion of implant–abutment interface on marginal bone loss around implant neck. Arch Oral Biol. 2017;83:153–60.
12. Broggini N, McManus LM, Hermann JS, Medina R, Schenk RK, Buser D, et al. Peri-implant inflammation defined by the implant–abutment interface. J Dent Res. 2006;85(5):473–8.
13. Park SD, Lee Y, Kim YL, Yu SH, Bae JM, Cho HW. Microleakage of different sealing materials in access holes of internal connection implant systems. J Prosthet Dent. 2012;108(3):173–80.
14. Jansen VK, Conrads G, Richter EJ. Microbial leakage and marginal fit of the implant–abutment interface. Int J Oral Maxillofac Implants. 1997;12(4):527–40.
15. 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(4):426–34.
16. Karl M, Taylor TD. Parameters determining micromotion at the implant–abutment interface. Int J Oral Maxillofac Implants. 2014;29(6):1338–47.
17. Lazzara RJ, Porter SS. Platform-switching: a new concept in implant dentistry for controlling postrestorative crestal bone levels. Int J Periodontics Restor Dent. 2006;26(1):9–17.
18. Atieh MA, Ibrahim HM, Atieh AH. Platform-switching for marginal bone preservation around dental implants: a systematic review and meta-analysis. J Periodontol. 2010;81(10):1350–66.
19. Strietzel FP, Neumann K, Hertel M. Impact of platform-switching on marginal peri-implant bone-level changes. A systematic review and meta-analysis. Clin Oral Implants Res. 2015;26(3):342–58.
20. Santiago JF Jr, Batista VE, Verri FR, Honorio HM, de Mello CC, Almeida DA, et al. Platform-switching implants and bone preservation: a systematic review and meta-analysis. Int J Oral Maxillofac Surg. 2016;45(3):332–45.
21. 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(5):1131.
22. Dental S, Candotto V, Gabrione F, Oberti L, Lento D, Severino M. The role of implant–abutment connection in preventing bacterial leakage: a review. J Biol Regul Homeost Agents. 2019;33(3 Suppl. 1):129–34.
23. Besimo CE, Guindy JS, Lewetag D, Meyer J. Prevention of bacterial leakage into and from prefabricated screw-retained crowns on implants in vitro. Int J Oral Maxillofac Implants. 1999;14(5):654–60.
24. Nayak AG, Fernandes A, Kulkarni R, Ajantha GS, Lekha K, Nadiger R. Efficacy of antibacterial sealing gel and O-ring to prevent microleakage at the implant abutment interface: an in vitro study. J Oral Implantol. 2014;40(1):11–4.
25. Coelho PG, Sudack P, Suzuki M, Kurtz KS, Romanos GE, Silva NR. In vitro evaluation of the implant abutment connection sealing capability of different implant systems. J Oral Rehabil. 2008;35(12):917–24.
26. Lorenzoni FC, Coelho PG, Bonfante G, Carvalho RM, Silva NR, Suzuki M, et al. Sealing capability and SEM observation of the implant–abutment interface. Int J Dent. 2011;2011:864183.
27. Seloto CB, Strazzi-Sahyon HB, Dos Santos PH, Assuncao WG. Effectiveness of sealing gel on vertical misfit at the implant–abutment interface and preload maintenance of screw-retained implant-supported prostheses. Int J Oral Maxillofac Implants. 2020;35(3):479–84.
28. Annibali S, Bignozzi I, Cristalli MP, Graziani F, La Monaca G, Polimeni A. Peri-implant marginal bone level: a systematic review and meta-analysis of studies comparing platform-switching versus conventionally restored implants. J Clin Periodontol. 2012;39(11):1097–113.
29. Schmitt CM, Nogueira-Filho G, Tenenbaum HC, Lai JY, Brito C, Doring H, et al. Performance of conical abutment (Morse Taper) connection implants: a systematic review. J Biomed Mater Res A. 2014;102(2):552–74.
30. Tsuruta K, Ayukawa Y, Matsuzaki T, Kihara M, Koyano K. The influence of implant–abutment connection on the screw loosening and microleakage. Int J Implant Dent. 2018;4(1):11.
31. Gherlone EF, Cappare P, Pasciuta R, Grusovin MG, Mancini N, Burioni R. Evaluation of resistance against bacterial microleakage of a new conical implant–abutment connection versus conventional connections: an in vitro study. New Microbiol. 2016;39(1):49–56.
32. Kofron MD, Carstens M, Fu C, Wen HB. In vitro assessment of connection strength and stability of internal implant–abutment connections. Clin Biomech (Bristol, Avon). 2019;65:92–9.
33. Toyoshima T, Tanaka H, Ayukawa Y, Howashi M, Masuzaki T, Kiyosue T, et al. Primary stability of a hybrid implant compared with tapered and cylindrical implants in an ex vivo model. Clin Implant Dent Relat Res. 2015;17(5):950–6.
34. Sammour SR, Maamoun El-Sheikh M, Aly E-G. Effect of implant abutment connection designs, and implant diameters on screw loosening before and after cyclic loading: in-vitro study. Dent Mater. 2019;35(11):e265–71.
35. Velez J, Pelaez J, Lopez-Suarez C, Agustin-Panadero R, Tobar C, Suarez MJ. Influence of implant connection, abutment design and screw insertion torque on implant–abutment misfit. J Clin Med. 2020;9(8):2365.
36. Gehrke SA, Delgado-Ruiz RA, Prados Frutos JC, Prados-Privado M, Dedavid BA, Granero Marin JM, et al. Misfit of three

different implant–abutment connections before and after cyclic load application: an in vitro study. Int J Oral Maxillofac Implants. 2017;32(4):822–9.
37. 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(4):344–50.
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(3):561–7.
39. Zipprich H, Rathe F, Pinz S, Schlotmann L, Lauer HC, Ratka C. Effects of screw configuration on the preload force of implant–abutment screws. Int J Oral Maxillofac Implants. 2018;33(2):e25–32.
40. Flanagan D. *Enterococcus faecalis* and dental implants. J Oral Implantol. 2017;43(1):8–11.
41. Najafi K, Ganbarov K, Gholizadeh P, Tanomand A, Rezaee M, Mahmood S, et al. Oral cavity infection by *Enterococcus faecalis*: virulence factors and pathogenesis. Rev Med Microbiol. 2020;31:51–60.
42. de Sousa CA, Taborda MBB, Momesso GAC, Rocha EP, Dos Santos PH, Santiago-Junior JF, et al. Materials sealing preventing biofilm formation in implant/abutment joints: which is the most effective? A systematic review and meta-analysis. J Oral Implantol. 2020;46(2):163–71.
43. Najafloo R, Behyari M, Imani R, Nour S. A mini-review of Thymol incorporated materials: applications in antibacterial wound dressing. J Drug Deliv Sci Technol. 2020;60:101904.
44. Colas A, Curtis J. Silicones. In: Modjarrad K, Ebnesajjad S, editors. Handbook of polymer applications in medicine and medical devices. New York: William Andrew Publishing; 2013. p. 131–43.
45. Costa MF, Durco AO, Rabelo TK, Barreto RSS, Guimaraes AG. Effects of Carvacrol, Thymol and essential oils containing such monoterpenes on wound healing: a systematic review. J Pharm Pharmacol. 2019;71(2):141–55.

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