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**IN VITRO COMPARISON OF THE MICROBIAL LEAKAGE IN
TWO DIFFERENT IMPLANT-ABUTMENT SYSTEMS**

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MASTER THESIS

DEPARTMENT OF PROSTHODONTICS

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I. STATEMENT

Hereby I declare that this thesis study is my own study, I had no unethical behavior in all stages from planning of the thesis until writing thereof, I obtained all the information in this thesis in academic and ethical rules, I provided reference to all of the information and comments which could not be obtained by this thesis study and took these references into the reference list and had no behavior of breaching patent rights and copyright infringement during the study and writing of this thesis.

Basmah A. M. Sheikh



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IV. ABBREVIATIONS and SYMBOLS

3D: Three-Dimensional.

ANOVA: Analysis of variance.

CAD: Computer-aided design.

CAM: Computer aided manufacturing.

CEREC: Chairside economical restoration of esthetic ceramics or ceramic reconstruction.

FDPs: Fixed dental prostheses.

LSD : Least significant difference.

P: Probability value.

SD: Standard deviation.

Sig: Significance.

SPSS: Statistical Package for the Social Sciences.

µm: Micron.

µL: Microliter.

SEM: Scanning electron microscopy

M-CT: Microcomputed tomography

IH: Internal hex

EH: External hex

MT: Morse taper

IAI: Implant abutment interface

FAI: Fixture abutment interface

TiBase: Titanium base

CFU: Colony for unit

E. coli: *Escherichia coli*

S. aureus: *Staphyococcus aureus*

GEPT: Gas-enhanced Permeation Test

PEEK: Polyetheretherketone

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1. ÖZET

İki Farklı İmplant-Dayanak Sisteminin Mikrobiyal Sızıntısının In Vitro Olarak Değerlendirilmesi

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Anabilim Dalı: Protetik Diş Tedavisi Anabilim Dalı

Amaç: İmplant-dayanak arayüzündeki mikro boşluk, kaçınılmaz olmasına rağmen, uygun dayanak seçilerek en aza indirilebilir. Çalışmanın amacı, statik durumda ve dinamik yükleme sonrasında iki implant-dayanak sisteminin mikrobiyal sızıntısını araştırmak ve karşılaştırmaktır.

Gereç ve yöntem: Çalışmada AstraTech Osseospeed implant ve iki farklı abutment kullanıldı; acuris konometrik abutment ve ti-base abutment (Dentsply Implants, İsveç). lityum disilikat (IPS e.max CAD, Ivoclar Vivadent, Lihtenştayn) kronlar ile restore edildi. İmplantın iç kısmına bakteri süspansiyonu ile aşılandı. Numuneler statik testi için, 7 gün triptik soya suyuna yerleştirildi. Dinamik yükleme altında için çiğneme simülatörüne yükleni ve her iki durumda bakteri örnekleri alındı.

Bulgular: Bu incelemede her iki grupta da mikrosızıntı olmasına rağmen farklı seviyelerdeydi. Statik koşullar altında ti-base abutment herhangi bir sızıntı göstermedi ($0,0 \pm 0,0$), ancak Acuris konometrik abutment 6 örnekten 3'ü minimum sızıntı gösterdi ($500,0 \pm 547,7$). her iki sistem de yükleme sonrası sızıntı bulguları üretti, Grup A ($388333,3 \pm 487048,9$) ve Grup B ($20000,0 \pm 15491,9$). Her iki durumda gruplar arasında ve gruplar içi anlamlı fark yoktu ($p>0.50$).

Sonuç: bu in vitro çalışmanın sınırlaması dahilinde, her iki sistemin de yüklemeden önce ve sonra benzer bakteri sızıntısı gösterdiği sonucuna varılabilir.

Anahtar kelimeler: Mikrobiyal sızıntı, implant, konometrik konsept, dinamik yükleme

2. SUMMARY

In-Vitro Comparison of the Microbial Leakage in Two Different Implant-Abutment Systems.

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Objective: The microgap in the implant-abutment interface although unavoidable can be minimized by choosing the suitable abutment. The aim of this study is to compare the microbial leakage of two implant-abutment systems before and after loading.

Materials and Methods: AstraTech Osseospeed EV implant and two different abutments were used; prefabricated ti-base abutment (Group A) and Acuris conometric abutment (Group B) (Dentsply Implants, Sweden). Lithium disilicate (IPS e.max CAD, Ivoclar Vivadent, Liechtenstein) crowns were fabricated. Bacterial suspension was inculcated into the implant. The samples were immersed in tryptic soy broth for static condition, and then been loaded into a chewing simulator. Bacterial samples were obtained and cultivated after both static and dynamic loading.

Results: Although there was microleakage in both groups in this investigation it was at different levels. Under static conditions ti-base abutment did not show any leakage ($0,0 \pm 0,0$) however 3 out of 6 Acuris conometric abutment showed minimal leakage ($500,0 \pm 547,7$). Both systems produced leakage findings in 4 out of 6 after loading for Group A ($388333,3 \pm 487048,9$) and Group B ($20000,0 \pm 15491,9$). There was no significant difference between and within the groups in both conditions ($p>0.50$).

Conclusion: within the limitation of this in-vitro study it can be concluded that both systems showed similar bacterial leakage before and after loading.

Key words: Microbial leakage, dental implants, conometric concept, dynamic loading

3. INTRODUCTION and AIM

3.1. Introduction

Titanium implants were first introduced by Dr. P. Branemark, which have revolutionized the oral treatment of partially and fully edentulous patient (Branemark et al., 1969). Although dental implants have a high success rate, complications can lead to implant failure. A balance of biological and mechanical factors is required for a successful implant therapy. A lack of stability or a misfit at the implant/abutment (I/A) interface has been related to failures in these procedures (Bressan et al., 2014).

Peri-implantitis is with no doubt one of the most common biological complications seen in clinical practice, with the potential to cause severe bone loss and implant failure (Berglundh et al., 2018). Several studies have indicated that bacterial colonization of implant can occur as soon as the implant is placed surgically. microorganisms related to periodontitis have been found to cause early bacterial colonization of the implant interface and that it is one of the most significant risk factors in the development of inflammatory infiltration of peri-implant tissues (Rimondini et al., 2001). In two-piece implant systems, preventing microbial invasion of the implant's internal components via the implant-abutment interface (IAI) microgap is challenging. Loss of bone and lately osseointegration breakdown can occur (Mombelli et al., 1987; Winkelhoff et al., 2000; Koutousiz et al., 2016).

Microgaps and misfits in the implant abutment interface have been found to encourage bacterial penetration and possibly lead to the development and progression of peri-implantitis in in vitro studies; the design of the interface has been found to affect its bacterial sealing capacity (Do Nasimento et al., 2012; Broggini et al., 2003). Internal abutment-implant connections, in which a piece of the abutment is put into the implant's body, and external abutment-implant connections, in which the abutment is positioned above the implant, are the most prevalent. Internal Morse taper connection implants had less microleakage than external connection implants, according to studies (Tripodi et al., 2015). Internal Morse taper connection implants had less microleakage than external connection implants, according to studies. It must also be able to avoid or reduce microleakage into the implant's surrounding tissue, which may lead to

infections (Gherlone et al., 2016). In principle, the fixation of an implant restorations can be achieved by either screw-retaining or cement-retaining. Both systems have some advantages and disadvantages. In order to overcome the complications associated with these systems, the option of a conical coupling retention to support single crowns have newly been introduced. A cone-in-cone morse tapered connection between the abutment/crown is used for fixation in this conometric concept. A conical titanium nitride-coated (TiN) cap is cemented extraorally into the crown, then fixed by friction on a conometric abutment without screws or cement (Acuris Cap/Acuris Abutment, all Dentsply Sirona implants, Mölndal, Sweden) (Degidi et al., 2016; Gherlone et al., 2021).

3.2. Aim of the Study

The aim of this in vitro study was to compare the microbial leakage before and after dynamic loading in two different implant-abutment systems; Astra Tech OsseoSpeed EV implants connected to Acuris conometric abutment and conometrically seated Acuris single crown, compared to implants connected to TiBase abutment with internal hex connection restored with screw-retained single crown.

3.3. Research Hypothesis

The null hypothesis of this in-vitro study suggests:

1. There is no significant difference in the microbial seal between the two implant-abutment systems; Acuris conometric abutment and conometric crown, and ti-base abutment with a screw-retained crown (Dentsply Sirona implants, Mölndal, Sweden).

4. GENERAL INFORMATION

4.1. Dental Implants

There are many methods to replace a missing tooth but dental implant restoration is widely accepted treatment to restore and fulfill chewing function. Due to the American Academy of Periodontology, a dental implant is an artificial root which is placed surgically in the bone to support a restoration. Dental implants can be an alternative for people who have lost a tooth or teeth because of periodontal disease, harm, or any other reason (Quirynen et al., 2006).

Although the osseointegrated implant treatment has a very high success rate, some microbiological and mechanical factors might cause the failure of the implants (Piattelli et al., 2001). Some of the main factors that are mainly responsible of the complications seen in implants in long-term are; occlusal overload and the diversity of microorganisms associated with periodontal disease located in the oral cavity (Covani et al., 2006). Peri-implantitis is undoubtedly one of the most prevalent biological complications seen in clinical practice, it has the potential to cause irreversible bone loss and result in loss of the implant (Berglundh et al., 2018).

Titanium implants, initially used by Dr. P. Branemark, have caused a revolution in the treatment plan of partially and for patient with no teeth (Brånemark et al., 1977). In the past, dental implants were produced of pure titanium in a screw shaped form with polished surface between the grooves. The implant abutment connection was provided by a platform on which the abutment could be connected on the implant defined as "external hex". There are more than 30 years of studies on implants of this connection type (Albrektsson et al., 1991).

Some unavoidable problems have been stated in long-term studies on external connection type dental implants, and it has been accepted that bone loss is seen in these implants over time. Bone loss is a major problem, especially in anterior restorations. As the bone loss occurs, recession in the gingival/gum tissue covering the bone will occur causing undesirable aesthetic complications. screw loosening and/ or screw fracture are some other complications seen in these implant system (Albrektsson et al., 1991; Schwarz et al., 2000).

Several changes to the design of dental implants have been developed throughout the years to address the shortcomings of the previous design. These modifications include the enhancement of the internal hex instead of the external hex along with the roughening of the implant surface. The most important advantages of the rough surfaces of the implants are; more implant-bone interface, higher blood clot adhesion, high bone healing simulation and providing superior primary stability. The internal connection has been developed to reduce prosthetic complications seen in the external connection (Feighan et al., 1995).

4.1.1. Dental implant body

There are 3 parts in a Dental Implant: Implant fixture, implant abutment, and implant crown. The implant body is the component that goes into the bone and is normally a little higher than the bone level. It consists of the crest module, body and apex sections (Figure 1). The body of implant can be shaped like a screw, the firm screw shape is the most common design, because it presents high primer stability and offers more bone-implant contact surface (Sitbon, 2009).

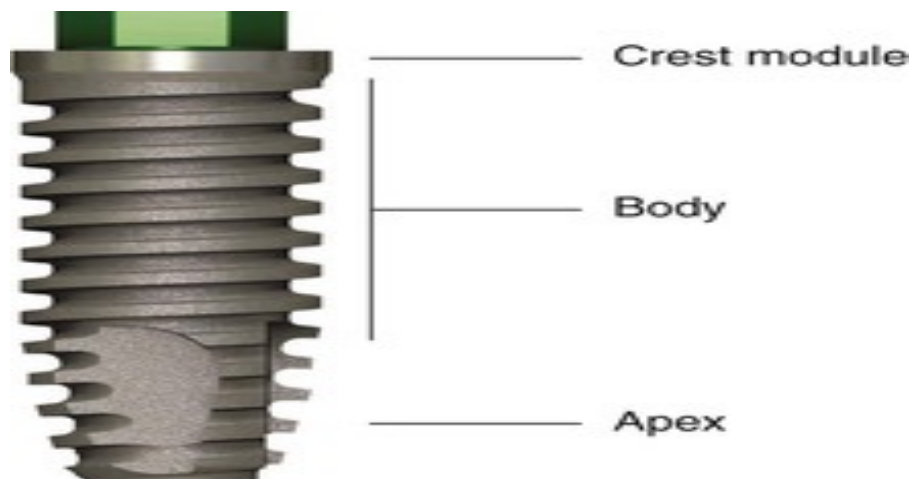


Figure 1. Implant body (Sitbon , 2009).

The crest module has taken this name because it is located on the bone crest and is the part where the abutment is connected. The crest module is usually polished unlike the body to reduce plaque attachment in case of bone loss (Misch et al., 2008).

4.1.2. One-piece and two-piece implant systems

There are a number of implant options from which to choose. The options are based on properties such as type of implant design, material and type of connection. There are one-piece and two-piece implant systems. In one-piece system is that the abutment and implant fixture is one piece during the fabrication which eliminate the microgap. When comparing one-piece versus two-piece designs, Varying implant designs have different effects on tissue response, according to Hermann et al. It has been proposed for example that one-piece implants with no microgap demonstrate little bone resorption, either because there is less bacterial colonization or because there is no micromovement between the components. Some of the drawback of the one-piece system is that there are less options for the abutment selection and the crown in most restorations will have to be cemented. Abutments in one-piece implants are not precise as they don't have a finish margin and there is no CAD/CAM software can be detected for a single piece implants (Hermann et al., 2001).

Implants where the prosthodontic platform is provided by a separate abutment component are called two-piece implants and this is the most common system. Surgically, two techniques can be performed in this system; two-stage (bone-level) implant surgery or single-stage (tissue-level) implant surgery. Many manufacturers recommend that the minimally polished neck of the implant be embedded in the alveolar bone, especially in aesthetic areas. Thus, in both techniques, the microgap will be at or below the alveolar crest level (Broggini et al., 2003).

An average of 2 mm crestal bone loss has been reported in implants placed with two-piece and two-stage surgery, and this finding depends on the location of the microgap relative to the alveolar bone. In monolithic systems, if the rough/polished border is at the level of the crestal bone, minimal or no bone loss occurs after a similar healing process (Albrektsson et al., 1986; Hermann et al., 2001)

4.1.3. Dental implants materials

Titanium and zirconia are the materials of the implants. Titanium has been desired as an implant material for more than 35 years. It has been proven to be the ideal material

due to its long-term success and biocompatibility. In the last few years, monolithic zirconium-oxide has been advertised as an alternative material to titanium to avoid the use of metal. Although it looks promising like all new materials, its long-term success and stability must be tested in order to be an alternative to titanium implants (Adell et al., 1981).

4.2. Dental Abutments

The abutment is the part or fixation device that connects between the implant and the crown. Usually a screw is used to attach the implant to the abutment. This screw access channel is blocked with an occluding material (i.e. cotton pellets, foam or silicone) to protect the screw inside the channel. Most of long-term studies on implant-abutment external connection have reported some inevitable problems and it has been accepted that bone loss is seen in the implants with this kind of connection over time (Albrektsson et al., 1991).

Standard or custom-made implant abutments are available. When the implant is placed surgically in an ideal position prefabricated abutment is indicated. The benefits of normal abutments are shortening in the overall treatment period and hence the time of the technical manufacturing. misaligned implants that support multi-unit prosthesis It's possible to fix it with angled abutments. the ideal design of a standard abutment should follow the gingival margin contour and be similar to a tooth preparation (Giglio et al., 1999).

Although the basic form of the endosseous dental implant has remained relatively unchanged, the material composition, surface modifications, and abutment connection designs have since changed dramatically. In spite of the type of a dental implant is a transmucosal piece is necessary to support the prosthetic restoration. Abutments have a large range of forms, designs, and concepts. Most of dental abutments are purely made me titanium. However, titanium abutments might cause aesthetic problems in some special cases with reflecting a dark color under the gingiva, such as when the gingiva is thin. In addition, light transmission in the anterior region can also impair the aesthetic appearance (Vigolo et al., 2005). Ceramic abutments with a color closer to the tooth color were produced to the market to solve this kind of problems. There

are still some shortcomings of ceramic abutments. Ceramics are fragile in nature and have low tensile strength. If cracks are present, even if there are minimum load on the implant it can spread easily during chewing and these cracks can cause failure of the implant and restoration (Yildirim et al., 2003).

There is different mechanism of retention of the abutment and the crown, the abutment retention to the implant body can be achieved with screw or friction. The crown's retention to the abutment can occur by using screw, cement or friction. One of the most important problems that have been reported about using cement for retention is that cements lead to peri-implantitis and this should be taken in consideration during selecting a retention design. the more completely seated, or the more precisely the system fits together, the less micromovements will occur, which may prevent microleakage and mechanical failures (Giglio, 1999).

4.2.1. Classification of dental abutments

Implant abutments classification depends on two main categories; 1) method of retention to the prosthesis 2) the way it is fixed to the implant body. An abutment that requires a screw for retention of the prosthesis, an abutment that requires dental cement to retain the prosthesis, an abutment that retains the prosthesis with friction (Conometric Acuris), and an abutment that requires an attachment device such as a magnet O-ring to retain a removable prosthesis are all classified as part of the first category. The implant abutments in the second category are classified as cement, screw in, and friction fit. Straight or angled abutments are the further subclassification of each kind of the abutments. In addition to that, abutments can be classified into either prefabricated or costume abutments (Sadaqah et al., 2010)

4.2.2. Dental abutments materials

Titanium, zirconia, titanium base with zirconia superstructure, titanium with cast gold (UCLA abutment, telescopic crowns), e.max (Ivoclar Vivadent), resin, PEEK (Polyetherketone) and alumina are the current choices for definitive abutments. With the release of zirconia and e.max abutments to the market, alumina abutments are not the first choice in the market today. Titanium has a rich record of being highly

biocompatible and have high mechanical properties; however, although they are very stable from a biomechanical point of view, titanium abutments have limitations in esthetic cases. Anodizing (gold or pink hue) has been tried as a solution, however it has had a low success rate. Attempts to address the esthetic flaws by placement abutment margins subgingivally should be avoided; a growing number of cement-induced cases of peri-implantitis have been recorded (Linkevicius et al., 2013).

4.2.1.1. Titanium abutments

Titanium is the only element in nature that have many unique properties combined all together like high strength and durability, light weight, high biocompatibility and being extremely durable (Shafie and White, 2014).

4.2.1.1.1. Commercially pure titanium

Due to its corrosion resistance, high strength, and biocompatibility, commercially pure (CP) titanium is commonly used in medical treatment. The mechanical features of pure titanium are affected by the addition of low amounts of oxygen and iron. It is the most formable grade of pure titanium material with the least amount of oxygen and iron added; However, increasing the amount of oxygen increases the durability of the material. Titanium abutments are produced with a gold or silver coating (Figure 2). Titanium nitride is the gold-colored coating on the abutment's surface. The titanium nitride (TiN; also known as "Tinite," "TiNite," "TiN") coating procedure is used which combines titanium and nitrogen ions with TiN, which is later chemically bonded to the titanium material of the abutment (Shafie and White, 2014).



Figure 2. Gold titanium nitride abutment (left), Silver titanium abutment (right) (Shafie and White, 2014).

Titanium nitride is usually coated over titanium components to enhance the surface properties of the substrate as well as to obtain a warm, esthetic color beneath the gingiva due to its goldish color. Except for the contact region between the abutment/implant and the screw/abutment, the TiN coating covers the entire abutment. This kind of titanium abutment is suitable for esthetic cases with narrow soft tissue or when a zirconium crown is being used. In most applications the TiN coating thickness is mostly less than 5 micrometers. This coating is significant in cases of CAD/CAM milled abutments that are not modified. Prefabricated abutments are modified, and any strength gained by the nitrates is usually lost as a result of the abutment modification (Shafie and White, 2014).

4.2.1.1.2. Titanium alloy (Ti-6Al-4V)

Titanium alloy includes 6% aluminum, 4% vanadium, 0.25% (maximum) iron, 0.2% (maximum) oxygen, and the rest titanium. The Ti-6Al-4V alloy is remarkably more durable and has higher strength than commercially pure titanium, with higher fracture resistance and tensile strength. Titanium abutments are the first option for posterior implants due to their distinctive physical properties. Prefabricated abutments and CAD/CAM made customized abutments are available (Shafie and White, 2014).

4.2.1.2. Zirconia abutments

Zirconia resembles titanium with its many physico-chemical properties such as corrosion resistance, durability and biocompatibility. Pure zirconium; it is in the monoclinic phase at room temperature. While the structure is in tetragonal form which is stable in range of 1170 and 2370 ° C, it changes to cubic phase after 2370 ° C.

4.3. Implant Success Criteria

There is significant controversy about the criteria of success of the implants. Many short-term and long-term studies have stated and accepted that the main criteria for success implants are immobility, no infection, absence of peri-implant radiolucency

and sufficient width of attached gingiva. Factors related to the patient are also important in evaluating the success of an implant such as existing medical conditions and smoking (Karthik et al., 2013).

Albrektsson (1986) reported a possible criteria for implant success:

- 1) No mobility in implant.
- 2) There should be no radiolucent areas on the radiograph that may indicate the presence of a peri-implant.
- 3) Mean vertical bone loss less than 0.2 mm annually after the implant's first year of function.
- 4) There is no ongoing pain or infection.
- 5) According to these criteria, a success rate of 85% at the end of a 5-year observation period and 80% at the end of a 10-year observation period are minimum success levels (Albrektsson et al., 1986).

Smith and Zarb (1989) introduced the creteria for implant success:

- 1) Clinically immobile implant.
- 2) No evidence of peri-implant radiolucency is shown radiographically.
- 3) Mean vertical bone loss is less than 0.2 mm annually after the implant's first year of function.
- 4) no continuous pain, discomfort of infection in implant.
- 5) Implant design does not preclude placement of prosthesis with the appearance that is satisfactory to the patient and dentist.
- 6) There is an 85 an 85% success persentage at the end of a 5 year post restorative period, with an 80% success rate at the end of 10 years of function (Smith and Zarb, 1989).

Health scale for dental implant table (Table 1) were introduced in the International Congress of Oral Implantologists, Pisa, Italy, Consensus Conference, 2007 as following (Misch et al., 2008).

Table 1. Dental implants health scale (Misch et al., 2008)

Implant Quality Scale Group	Clinical Condition
I. Success (optimum health)	a) No pain during function b) immobile c) less than 2 mm radiographic bone loss d) No exudates history
II. Satisfactory survival	a) No pain on function b) immobile c) 2–4 mm radiographic bone loss d) No exudates history
III. Compromised survival	a) May have sensitivity on function b) No mobility c) Radiographic bone loss less 4 mm d) Probing depth less 7 mm e) May have exudates history
IV. Failure (clinical or absolute failure)	Any of following: a) Pain on function b) Mobility c) Radiographic bone loss >1/2 length of implant d) Uncontrolled exudate e) No longer in mouth

The outcome of implant treatment is influenced by ongoing marginal bone loss. If minor bone loss around an implant persists for many years, the implant's success may be jeopardized. The implant may be recovered if the bone loss is identified and treated. This topic has been investigated in terms of implant position, size, and geometry, resulting in a number of variables that could influence bone loss surrounding implants (Table 2). Bacterial colonization of the fixture-abutment interface microgap has been identified as one of the elements influencing the marginal bone location (Roos-Jansaker, 2007).

Table 2. Criterion for implant success proposals (Roos-Jansaker, 2007)

Author	Crestal Bone Loss	Radiography
Schnitman & Shulman (1979)	Bone loss less than a third of the vertical height of the implant.	No suggested criteria
Albrektsson et al. (1986)	Vertical bone loss <0.2 mm annually following the implant's first year in service	No of peri- implant radiolucency
Smith & Zarb (1989)	Mean vertical bone loss <0.2 mm annually after the first year in service	No of peri- implant radiolucency as assessed on an undistorted radiograph
Albrektsson & Isidor (1993)	Average bone loss <1.5 mm the first year in service, and thereafter <0.2 mm annually	No evidence of peri-implant radiolucency
Wennstrom & Palmer (1999)	Maximum bone loss of 2 mm between prosthesis installation and the 5th year, with the majority of the loss occurring during the first year	No suggested criteria
Ostman et al. (2007)	Success grade 1 <2 mm bone loss the first year in service	No radiographic signs of pathology No radiographic signs of pathology

4.4. Implant-Abutment Connection

I-A connection (IAC) is the point of turning from surgical to prosthetic phase and is initially determinant of strength and stability of the implant supported prosthesis. The types of implant-abutment connection play an important role in the prosthetic prognosis (Singla et al., 2018).

It was reported that loosening and fracture of the abutment screw are the most important and most common complications in implant restorations (Goodacre et al., 1999). Metal fatigue due to screw loosening is the main reason of screw fracture. To avoid complications such as screw loosening and screw fracture, various implant-abutment connections designs were produced by manufacturers. Screw-retained implant-abutment connections are preferred nowadays. Screwless morse-taper connection is another option in addition to the screw-retained connection. In the screwless abutment system, friction and compression forces that occurs between the connection surfaces are the forces that hold the implant and abutment together (Bozkaya and Müftü, 2003).

Internal and external connections are another design variation of the implant-abutment connection (Figure 3). Implants with exterior hexagon connections were previously used, which caused issues such as loosening of screw and rotational mismatch, as well as microbiological leakage, which resulted in revisions to the external hexagon and internal connection (Kitagawa et al., 2005).

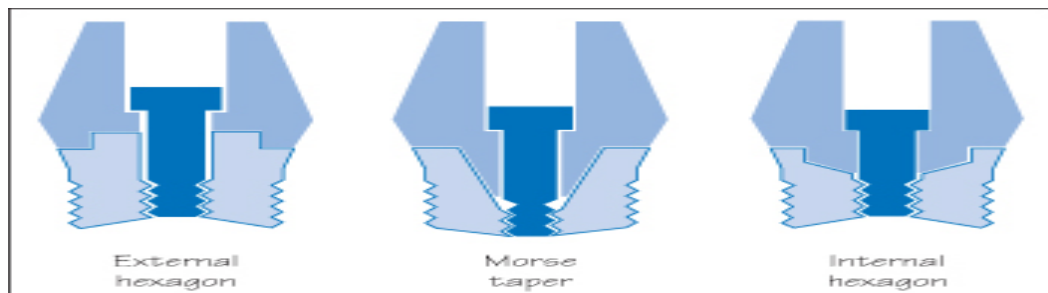


Figure 3. Implant-abutment connection (Kitagawa et al., 2005).

The presence or absence of an appendage projecting out of the implant body distinguishes external and internal connections. External connection involves a geometric projection extending above implant body, while in internal connection

mating components are recessed into the implant body. The most frequent designs are hexagonal, octagonal, morse taper, and hex morse taper (Goodacre et al., 1999).

There is anti-rotational component in both systems, these can come in many shapes like hexagonal and octagonal. It is required to serve the purpose of antirotation, prosthetic indexing and also resist bacterial penetration. Manufacturer companies try to provide the most ideal design with all components in order to create an implant-abutment connection with the best mechanical and biological features (Singla et al., 2018).

4.4.1. External connection

Branemark invented the external connection, which has been in use since the dawn of modern implantology. External connections have some disadvantages such as abutment micromovements which leads to mechanical and biological complications. this system is contraindicated in situations of overloads (Figure 4).

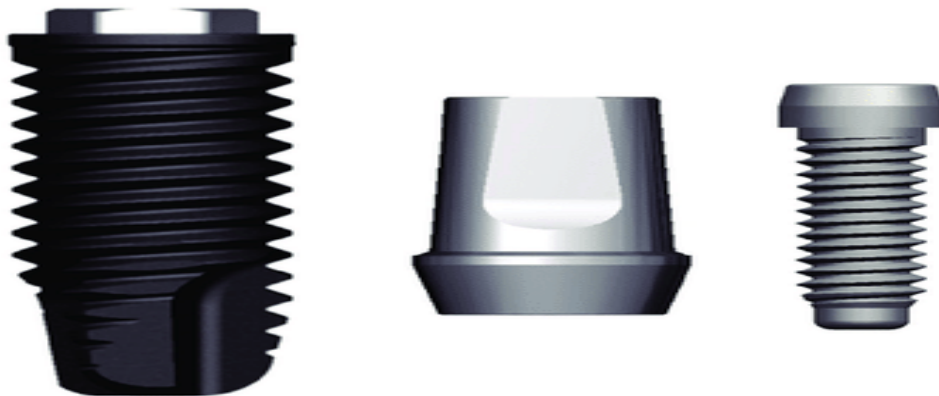


Figure 4. External connection

The external hex system has its advantages:

- 1) Appropriate for two stage surgical procedure.
- 2) Retrievability and an anti-rotation mechanism
- 3) Compatibility among different systems.

The following are some to the potential drawbacks of using an external hex:

- 1) Micro-movements because of the size of the hex.
- 2) Low resistance to rotation and lateral movements due to the fact that the center of

rotation is located higher

- 3) Bone resorption might occur due to the micro-gap in the IAC (Maeda et al., 2006; Lemos et al., 2018).

4.4.2. Internal connection

The internal connection is an evolution of the external connection, it offers some advantages like less fracture and screw loosening and ideal stress absorption. Its design provides for consistent load distribution around the implants, reducing crestal bone stress (Figure 5).

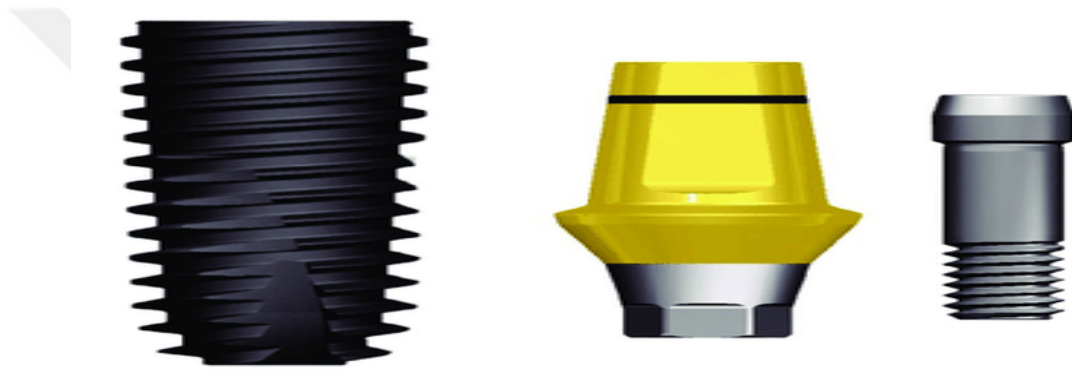


Figure 5. Internal connection

It may be explained by the taper shape of the hexagon socket in the prosthesis and the greater depth of the internal hexagon within the implant, which decrease the lever arm and change the prosthesis-implant fulcrum to the middle third of the implant. This reduces the likelihood of screw breakage and restoration failure by distributing stress more evenly on the bone and increasing the stability of the prosthesis retaining screw. Internal hexagon implants showed higher detorque values and less screw loosening under cyclic loading, due to better abutment screw protection. (Pita et al., 2011).

The internal hex system has a number of advantages, including:

- 1) Simplicity in abutment connection.
- 2) Suitable for one stage implant installation.
- 3) Due to the wider area of connection, it has better stability and anti-rotation properties, better prosthesis retention and it's ideal for single-tooth restoration.
- 4) Since the center of rotation is lower, the resistance to lateral loads is higher.

5) Homogeneous stress distribution.

While its disadvantages are:

1) Thinner lateral fixture wall at the connecting part.

2) Difficulty in adjusting deviations in angles between implants (Maeda et al., 2006). implants with an external connection have high rate of screw loosening complications up to 40%. (Jemt et al., 1991; Becker et al., 1995). In the other hand, Levin et al. introduced a low percentage of abutment loosening (3.6%-5.3%) with the internal connection abutments (Levine et al., 1999). Considering these differences in the literature, researchers continue to compare different internal and external connection systems. It was reported that internal connection had no failure after dynamic loading. on the other hand, screw loosening and fracture was seen in the specimens with external connections in various stages of loading (Khraisat et al., 2002).

4.4.2.1. Morse taper connection

The “Morse taper” was introduced by Sutter et al., who proposed an 8-degree internal taper connection between the fixture and the abutment. Astra Tech offers a similar cone screw connection with an 11-degree taper. Also, Nentwig and Moser have produced a 4-degree cone screw connection for the Ankylos implant system. However, a true Morse taper without any threaded component has been developed by the Bicon system. The abutment has a 1-2-degree tapered post that fits into the implant's smooth mirror-image shaft (Figure 6). Such a connection depends solely on frictional fit to provide optimal stability (Shadid et al., 2013).

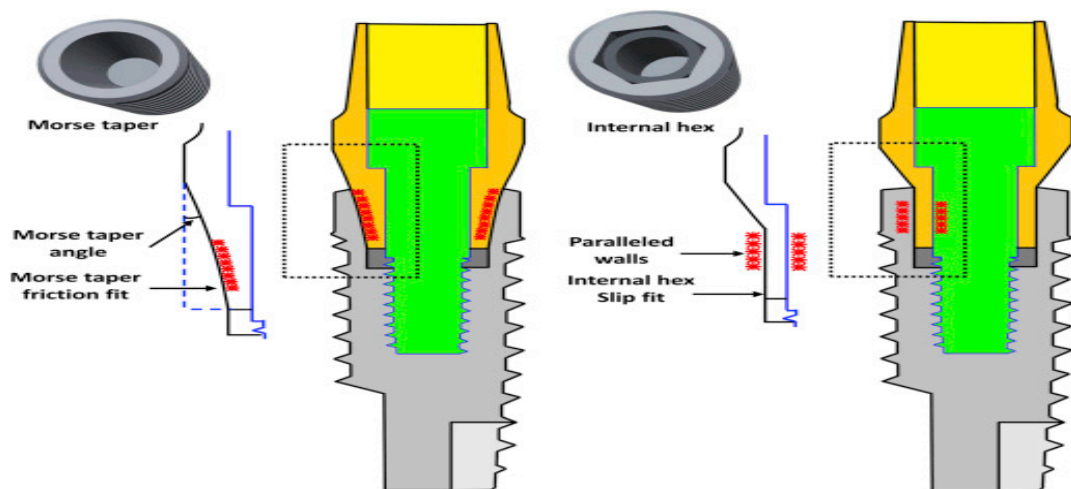


Figure 6. Morse taper connection (Hung et al., 2019).

Internal hexagonal and the Morse Taper connection are two of the most common internal abutment-internal connection designs used nowadays. The Morse taper implant abutment connection has a unique internal joint design between two conical components. In implant dentistry, a conical “male” abutment is tightened into a “female” conical implant design. The high propensity of parallelism between the two structures within the joint space causes significant friction due to this internally tapered shape. (Souza et al., 2016). Morse taper connection when compared with external hex offers:

- 1) Better biological and mechanical stability.
- 2) Better stress distribution.
- 3) Higher sealing capability.
- 4) Sufficient soft tissue and bone crest stability.
- 5) Increased prosthetic versatility.
- 6) High esthetic. (Shadid et al., 2013)

4.4.2.2. Screwless morse taper connection

A choice to traditional external or internal screw-retained (ISR) implant-abutment connections are the screwless morse taper (SMT) implant-abutment connection. In this connection, locking is provided through the contact of two conical surfaces (Figure 7). Since the opposite surfaces of the abutment and the implant are locked together with friction, the screw, which is the weakest link in the system, is not used in the screwless Morse taper connection (Ugurel et al., 2015).

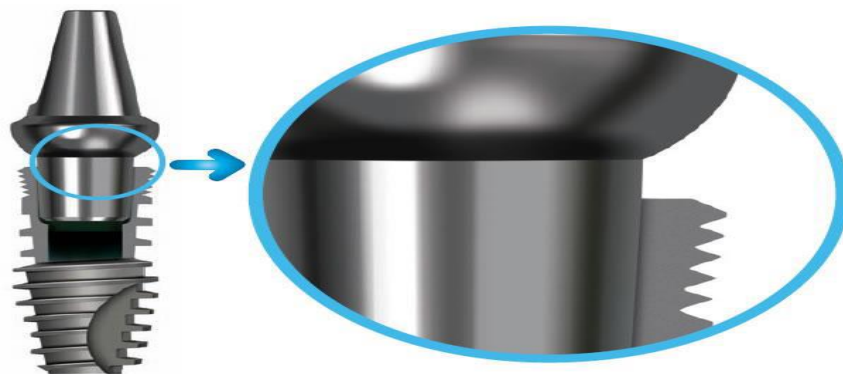


Figure 7. Screwless Morse taper connection (Ugurel et al., 2015).

The high friction between the surfaces of two conic components of identical size perfectly links them. It is characterized as "cold welding" because surfaces that are not completely smooth at the level undergo a sort of interpenetration and fusion between their asperities as a result of contact pressure. The formation of micro-welding points between the surfaces allows the implant and abutment to behave as a single body. In comparison to conventional screw-retained systems, where the greatest tensions are located in the connecting screw itself, in this system, stress distribution can be considered homogeneous across the entire system. The biggest advantage of this system is its mechanical stability and absence of micromovements and therefore less complications occur (Sannino and Barlattani, 2013).

It was reported that the screwless Morse Taper implant system has a lower mechanical resistance than ISR implant systems, which may lead to more clinical complications (Ugurel et al., 2015).

4.4.3. Platform switching concept

Platform switching is a technique for preserving alveolar bone levels surrounding dental implants. The use of an abutment with a diameter smaller than the implant's diameter is the unique characteristic of this technique. As a result, marginal bone loss at the end of the first year is reduced because stress is distributed more homogeneously around the implant (Figure 8). The protection of the bone against bacterial leakage in platform switching method is provided by biological sealing of the implant-abutment interface. Thus, the surrounding tissue preserve the crestal bone and more aesthetic results are obtained in the interdental papilla (Lazzara et al., 2006).



Figure 8. Dental implants without (left) and with (right) platform switching

4.5. Implant-Supported Fixed Restorations

For a long time, the choice between screw-retained and cement-retained implant prostheses has been a topic of contention, but the ideal type of implant prosthesis remains a controversial among dentists. The following factors are determined by various methods of prosthesis retention to the implants: All factors to consider include access, occlusion, retention, incidence of loss of retention, retrievability, passivity of fit, and restriction of implant position, as well as the effect on peri-implant tissue health, previsualization, direct loading, impression procedures, porcelain fracture, and clinical performance. (Shadid and Sadaqa, 2013).

4.5.1. Cement-retained implant-supported fixed restorations

Cement-retained implant supported restoration has some advantages as:

- 1) Cement-retained restorations are more esthetic. specially in cases where implants are not placed ideally, they provide better aesthetics.
- 2) Easy Fabrication and clinical procedure.
- 3) Lower in cost.
- 4) It's easier to get to the back of the mouth., especially for patients with limited jaw opening.
- 5) Inherently more passive due to the cement space buffer.

Disadvantages and concerns:

- 1) Residual extra cement is one of the most important drawbacks of cement-retained implants, which makes a huge risk factor developing peri-implant disease.
- 2) When a condition emerges that necessitates crown removal, cement-retained restorations are frequently severely damaged beyond repair during the removal process.
- 3) In cases with insufficient interocclusal space or malaligned implants, cement-retained implant restorations are contraindicated (Shadid and Sadaqa, 2013).

4.5.2. Screw-retained implant-supported fixed restorations

Screw retention was validated by the Branemark system. Screw is used to attach prosthesis to and abutment to implant. Different size, design, properties and materials are available for the screw. Screw hole is restored with composite.

Advantages:

- 1) It is the choice in cases with limited interocclusal space.
- 2) A screw-retained prosthesis simplifies the management process of when a complication arises requiring the removal of the crown. The screw is removed without damaging the restoration.
- 3) Fewer biologic complications compared to cement-retained restorations.
- 4) For immediate loading, screw-retained restoration is the preferred option.

Disadvantages:

- 1) Due to the visible screw access hole it is considered less esthetic
- 2) Extra components needed which makes it more expensive.
- 3) Difficulty of access to the posterior of the mouth. In addition to the difficulty of reach, using screw-retained prosthesis in the posterior may result in the screw or screwdriver being swallowed or aspirated.
- 4) Technical difficulties such as retaining screw loosening and screw fracture (Shadid and Sadaqa, 2013; Jivraj, 2018).

4.5.3. Conometric prosthetic connection:

Screw-retaining and cement-retaining are two options for the fixation of an implant supported restorations. Aside from the practitioner's personal preference and the advantages and drawbacks in the clinical application of the respective fixation method, the retention type can have an effect on the prosthetic aftercare of the implant patients. The use of a conical coupling retention to support single crowns has newly been produced to solve the complications associated with cement-retained and screw-retained fixed implant-supported restorations (Figure 9).



Figure 9. Schematic figure of the Acuris conometric concept (Gehrke et al., 2021).

The Morse taper conometric approach can also be used to provide a fixed connection between the implant and the prosthesis. In the conometric system, a tapered coping is affixed to the prosthesis and put into a tapered abutment. The concept includes a conical titanium nitride-coated (TiN) cap that is cemented extraorally into an all-ceramic crown and then fixed by friction on a conical abutment using the fixation tool (Acuris Cap/Acuris Abutment, both Dentsply Sirona implants, Mölndal, Sweden). Without the use of screws or cement, the final restoration is connected to the abutment. Wedge effects gently deform the cervical margin of the coping when an insertion force is applied, creating elastic stress fields within both the coping and the abutment when the system is engaged. Despite the fact that the insertion force is no longer there, certain stresses will persist in some manner (Bressan et al., 2013). Since the cementation process between the coping and the crown is done extraorally by a dental technician, the Acuris restorative concept differs from previously published conometric approaches. This particularity is possible because of the existence of a newly added antirotation feature. There are very few data on the microbial sealing of the conometric Acuris connection (Gehrke et al., 2021).

Some of the reported advantages are:

- 1) Ease of maintenance
- 2) Improved esthetics, due to no visible screw access holes
- 3) Less risk of biological complications due to the absence of intra-oral cementation.

4) The formation of an anatomical soft tissue profile

Hybrid-acrylic prostheses and lithium disilicate or monolithic zirconia fixed partial dentures (FPDs) and single crowns (SCs) retained by conical friction showed minimal problems and healthy peri-implant soft-tissues (Degidi et al., 2016).

Microbial sealing ability along the prosthetic connection of conometrically seated Acuris single crowns was found to be preventing bacterial translocation under non-dynamic loading conditions (Gehrke et al., 2021).

4.6. Peri-implantitis Pathogenesis and the Role of Microgap

When plaque forms on teeth and dental implants, the tissue reaction is nearly identical, according to clinical and experimental studies. Peri-implant disease is divided into two categories, according to the definition of periodontal disease at teeth: peri-implant mucositis, which is similar to gingivitis, and peri-implantitis, which is similar to periodontitis. Irreversible Inflammatory reactions in the mucosa associated with the loss of supporting bone around a functioning implant is referred to as peri-implantitis (Zitzmann and Berglundh, 2018). the microbiota associated with the progression of periimplantitis and periodontitis occurring concurrently in partially edentulous mouths are similar (Mombelli et al., 1987).

In human biopsies, the histological characteristic of peri-implant mucositis and peri-implantitis lesions were studied. The inflammatory cell lesion in peri-implant mucositis sites was found to be dominated by T cells with an apical extension restricted to the barrier epithelium. The lesion in peri-implantitis extended apically to the pocket epithelium and included large numbers of plasma cells and lymphocytes, as well as PMN cells and macrophages (Zitzmann and Berglundh, 2018).

4.6.1. The role of microgap in periimplantitis

Implant-abutment connection must have maximum and continuous resistance against chewing forces and bacterial penetration. Microgap formation between implant and abutment cannot be prevented in two-piece implant systems. Even in 2-piece implants with good marginal fit of implant components, gaps and cavities have been recorded,

and these small spaces can be a raising ground for bacteria that can cause inflammation in the peri-implant soft tissues (Piattelli et al., 2003).

Inadequate fit between the implant and the abutment, like incorrectly suited dental restorations, may be a risk factor, leading to clinical and microbiological alterations in the peri-implant tissues. Furthermore, because a misfit between the abutment and the implant allows for micromovements, a misfit between the two poses a biomechanical risk, as it allows the set to be subjected to unfavorable loads, which could result in the prosthetic screw loosening or fracturing, or the implant body fracture (Passos et al., 2013). Jansen et al. researching the size of the IAI interface, found mean values between 1 mm and 10 mm; Dellow et al. between 0 and 7.15 mm; and Callen et al. between 30 mm and 13 mm. Due to the existence of the interface, it is clear that fluid and microorganism transfers are evident (Passos et al., 2013). The existence of these gaps at the IAI near the bone crest is unavoidable, and implant systems are not able to control and prevent microbial colonization of this interface. Several authors have looked into measuring the IAI gap in order to see if there is a relation between the increase in space and the potential of further microbial leakage. Jansen et al. studied and compared the size of the gaps and measure of contamination. The amount of spaces was measured with scanning electron microscopy, and it was reported that there was no statistically significant relationship between the size of these spaces (means) and the proportion of the leakage, by means of the microbiological test. Although some implants were accordance with these results, others that had low maladaptation means obtained a high contamination index, when the tapered prosthetic connection was used (Jansen et al., 1997; Passos et al., 2013). Several materials have been described over the years to seal the implant-abutment interface: adhesive, a silicone O-ring washer, stick wax, a silicone hermetic washer, chlorhexidine-thymol varnish, a seal washer, and 2% chlorhexidine solution. The sealing capacity of numerous materials was investigated in vitro at distinct implant-abutment surfaces as part of a study on bacterial contamination in implants. Bacterial leakage was seen after 14 and 35 days in the control and experimental groups, respectively, and majority of the materials examined failed to prevent contamination after 63 days. Dental implants exhibited bacterial infiltration regardless of their exterior or interior hexagonal layouts, despite the fact that the materials investigated had equivalent sealing capacities (Duarte et al.,

2006). Many implant companies are working on and producing designs that will prevent microleakage in order to prevent peri-implant complications. Many manufacturers use the screwless morse-taper abutment design because it decreases microleakage. The effectiveness of the sealing provided by the locking taper used in the implant-abutment connection was studied in vitro and it was reported that the seal created by the locking taper design has been shown to be tight in terms of bacterial invasion (Dibart et al., 2005). In Morse-taper systems, microbial leakage and bacterial growth on the implant's interior surface are unavoidable. The frequency of bacterial leakage along the implant-abutment interface between two systems of morse taper dental implants was investigated in another in vitro study. Different methods of activating the taper abutments were tried, including tapped-in and screwed-in, and both techniques demonstrated bacterial leakage along the implant–abutment contact (Aloise et al., 2010).

Both in-vitro and in-vivo studies have shown that within minutes after implant placement, contamination of the internal parts of the implant and bacterial colonization of the implant surface and peri-implant tissues can occur. It has been reported in the literature that the microgap at the implant-abutment interface can be seen in all implant systems. Tightening the screw at the torque values recommended by the manufacturer is very important because tightening the screw at these values is very effective in reducing the amount of microgap. Repeated clinical closing and opening of abutment screws during impression and prosthesis fabrication can cause component wear and reduce frictional fit of the mating parts, resulting in progressive torque loss, altered resistance to opening, and the risk of preload loss in function (Weiss et al., 2000). According to current research, the position of the microgap influences the bone crest level alterations in all 2-part implants. In cosmetic instances when crestal bone loss is to be reduced, it is recommended that the implants be positioned 1 to 2 mm above the alveolar crest, with the microgap 1 to 2 mm above the alveolar crest. It indicates that when the microgap is positioned coronally to the crest, less bone loss happens, and when the microgap is located apically to the crest, more bone loss occurs (Piattelli et al., 2003).

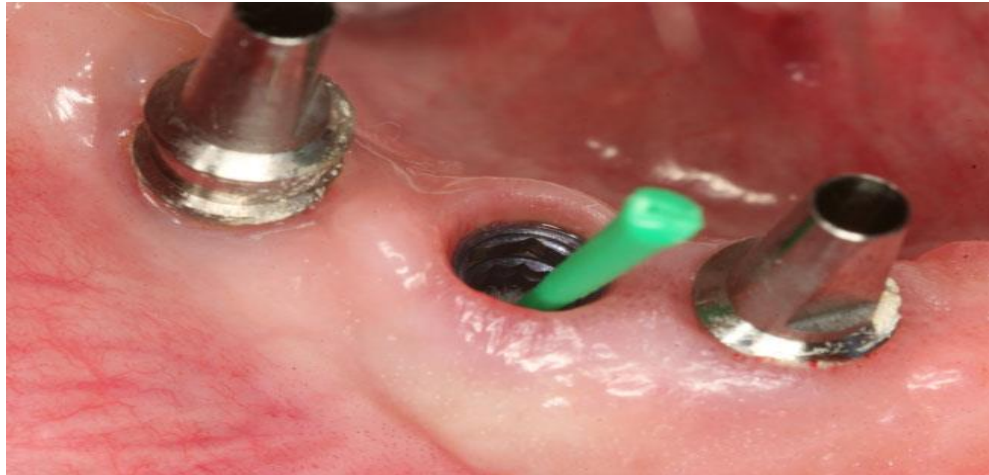
4.7. Implant-Abutment Microbial Leakage Control Methods

Leakage testing has a long history, the first microleakage test was done in 1961 (Swartz and Phillips, 1961). Leakage in Implants have been tested using the same leakage test techniques that have been used in other dental fields with some modification for implants. Radiographic evaluation of the microgap in the interface is possible. SEM, optical microscopy and laser scanning microscopy can be used for direct observation in vitro. However, these methods are not completely reliable as they do not provide information about the continuity and depth of the gap. Microbial leakage or bacterial seal testing (Assenza et al. 2012), endotoxin or other molecular microleakage tests (Harder et al. 2010), spectrophotometric dye penetration determination (Park et al. 2012), and the gas permeability test are all acceptable testing methods. The later method not only determines whether or not the implant leaks, but also the quantity of leakage over time (speed of leaking), allowing for quantitative comparison of different implant systems (Al-Jadaa et al., 2015).

4.7.1. In vivo microleakage detection methods

Mawhinney et al. compared internal bacterial colonization in two implant systems; external hexagon connection and Morse taper internal connection. The implant restoration was removed, and a sterile glass syringe was used to inject 10 mL of sterile saline into the implant well. The saline solution was redrawn and sent to the lab for microbiological testing. The number of aerobic and anaerobic colony forming units per millilitre was calculated, and the dominating bacterium in each sample was identified using 16s rRNA gene amplicon sequencing. It was reported that bacterial contamination was seen in both systems (Mawhinney et al., 2015).

Romanos et al. studied and evaluated the bacterial composition of implant-abutment connections of immediately loaded implants. Samples were taken after 2 years of function (Picture 1). Bacterial samples were taken from two sites; the abutment and the inner part of the implant (Romanos et al., 2016).



Picture 1. Taking bacterial samples from the internal part of the implant using a mini brush

4.7.2. In vitro microleakage detection methods

In order to evaluate the possibility of bacterial infiltrate at the implant-abutment interface, in vitro studies used two main methods; Outgrowth and ingrowth testing methods. Inoculating the interior of the implant with a microbial agent, assembling the implant-abutment complex, and evaluating the bacteria's release into the external environment are all part of the outgrowth approach. The ingrowth method is the alternative approach, it involves assembling the implant abutment complex in a sterile environment, submerging the complex in a bacterial solution, and analyzing if bacteria have penetrated the implant's internal surface (Koutouzis et al., 2000).

There are different methods of to evaluate the results, some of the reported methods are DNA checkerboard, culture methods, real time quantitative PCR, colony count, gas enhanced permeation test, turbidimetry test, and the histological method (Mishra et al., 2017).

In vitro studies that examine microbial penetration in the internal parts of the implant evaluate different implant-abutment interface geometries in loading and non-loading conditions. The majority of in vitro studies testing the possibility of bacterial infiltration into and out of the implant-abutment interface were done in non-loading conditions. very few studies were incorporated with loading conditions (Koutouzis et al., 2000).

Gherrlone et al. used the outgrowth method to evaluate the bacterial penetration from

the inside to the outside the implant-abutment interface in a new design of internal conical connection compared to eight different internal connections. Bacterial suspension was inoculated to the inner part of the implant. After assembling the components samples were immersed in nutrient solution that covered the implant-abutment interface and turbidity was checked daily (Picture 2) (Gherlone et al., 2016).



Picture 2. Samples in nutrient solution. Left: turbidity of the broth as a sign of bacterial leakage. Right: no contamination (Gherlone et al., 2016).

The chewing simulator is the most commonly used device in dynamic loading environments with the ability of simulating human chewing parameters by performing cyclic loading with uniaxial or multi-axis movements and bite forces. To simulate 5 years of clinical service, it is suggested that 1,200,000 chewing cycles be performed and 240,000 cycles simulates 1 year of service (Steiner et al., 2009).

Steinebrunner et al. conducted the first research to use loading conditions to determine the sealing capability of various implant abutment interface designs. The internal parts of implants were inoculated with a bacterial suspension and attached to the superstructure with the recommended torque. In the chewing simulator, the specimens were immersed in a nutrient solution and loaded with 1,200,000 cycles of 120 N (Steinebrunner et al., 2005).

Do Nascimento C et al. evaluated bacterial leakage to the internal part of the implant along the implant-abutment interface from human saliva under loaded and unloaded

conditions using DNA Checkerboard. The assemblies were immersed in human saliva and the experimental group were loaded with 500,000 cycles at 120 N and the unloaded group were incubated in static conditions for 7 days at 35°C. after 7 days samples were taken from the internal part of the implants using micro brush (do Nasimento et al., 2012).



5. MATERIALS and METHOD

5.1. Materials Used in the Study

Astra Tech™ OsseoSpeed™ EV implants (Dentsply Sirona Implants, Mölndal, Sweden) were chosen and divided to two groups according to the abutment and restoration type as shown in the following table (Table 3).

Table 3. Materials used in the study

	Group A	Group B
Implant	Astra Tech™ OsseoSpeed™ EV/ 4.2 S - 11 mm	Astra Tech™ OsseoSpeed™ EV/ 4.2 S - 11 mm
Abutment	TiBase 4.2GH1 L	Conometric Abutment EV 4.2/3.0/0°/4.5/I
Screw	Uncoated Screw	Uncoated Screw
Crown	Screw-Retained	Conometric Acuris

5.1.1. Implants used in the study

A total of 12 Astra Tech OsseoSpeed EV straight implants were used with these properties; 4.2 diameter, bone level and 11 mm length (Dentsply Sirona Implants, Mölndal, Sweden). The OsseoSpeed EV implant with an internal hex has a unique interface for one-position-only placement, it also allows for the flexibility of six-position indexing of prefabricated abutments six-position and it includes an option for index-free placement (Figure 10).



Figure 10. OsseoSpeed EV, the indexing portion has seven slots: six symmetrically placed and one additional slot.

5.1.2. Abutments used in the study

Two types of abutments were used in this study; Prefabricated TiBase Abutment and Acuris conometric abutment EV (Dentsply Sirona Implants, Mölndal, Sweden). Conometric abutment EV (Figure 11) was with the following properties; straight 4.2 in diameter, 4.5 in diameter at the abutment's equator, 3 mm high and with the option to be seated in 6 positions. Straight tibase abutment 4,2 in diameter was used (TitaniumBase EV, Dentsply Implants Manufacturing, Sweden).



Figure 11. Acuris conometric abutment

5.1.3. Microbiological materials used in the study

- 1) Tryptic soy broth.
- 2) Tryptic soy agar.
- 3) MacConkey agar.
- 4) Bile esculin agar.
- 5) *E. coli* and *E. Faecales* bacterial suspension.
- 6) Sterile disposable inoculation loop

5.2. Sample Preparation

A special reservoir was prepared in the laboratory to be used in the study (Analitik Medikal, Gaziantep, Turkey). A second piece was prepared to be added to the original chamber to increase the depth of the chamber and provide sufficient space for solution.

The implants were mounted individually in auto-polymerizing acrylic resin (Meliodent, Heraeus Kulzer, Berkshire, UK) blocks. Every implant was attached to a dental survey (Kavo EWL, Typ 990, Kavo Elektrotechnisches Werk GmbH, Leutkirch im Allgau, Germany) using red laboratory wax while inserting it to the block to ensure that the long axis of the implant was perpendicular to the top surface of acrylic resin block. The implant-abutment connection was 1-2 mm above the acrylic block.

5.3. Fabrication of the Crown

Scanbody for each abutment type were used. IPS E-Max CAD (Ivoclar Vivadent, Schaan, Liechtenstein) were the material of use to fabricate the crowns. Samples were divided into two groups according to the type of abutment:

Group A: LT A2/A6 were used to fabricate the screw-retained prothesis(n=6).

Group B: LT A2/C16 blocks were used to fabricate the conometric Acuris crown(n=6).

Each crown was designed with CEREC MC XL software, the crowns were designed according to each material manufacturer instructions (1,5 occlusal thickness and 1mm axial thickness) (Picture 3).



Picture 3. Crowns design

Then and the digital information was sent to the accompanying milling unit. The crowns were milled using CAD\CAM milling machine (InLab MC X5; Sirona Dental Systems) (Picture 4). New milling burs were used for the milling process.



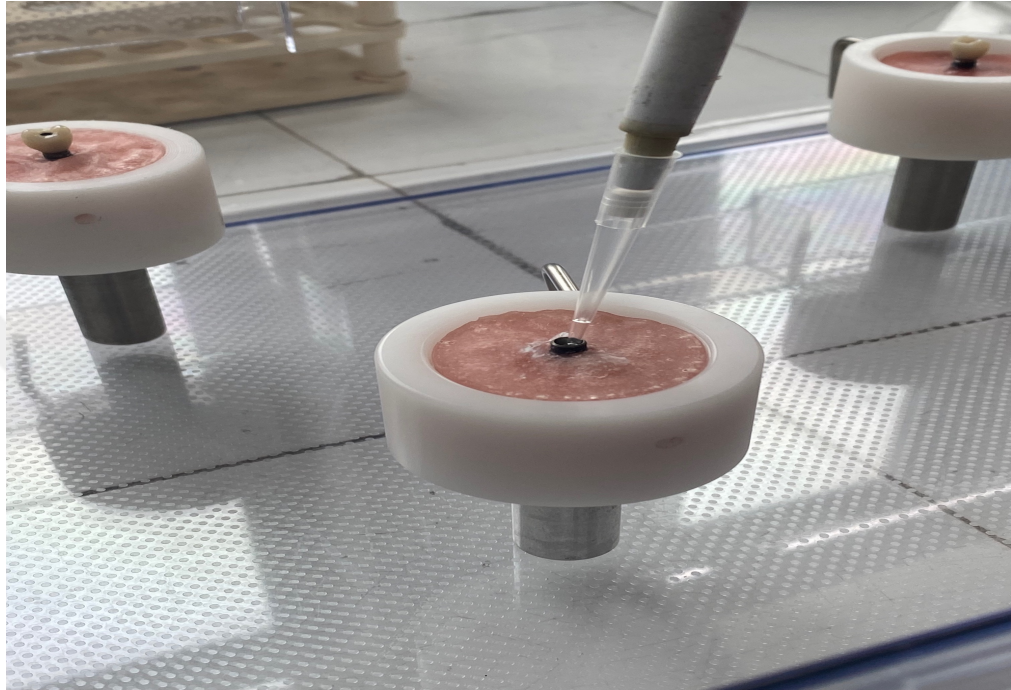
Picture 4. InLab MC X5 milling machine

Finally, all the crowns were glazed and polished according to the manufacturer's instructions. Cementation of the crowns were done very carefully in order not to face any problems like decementation during the dynamic loading that would affect the values of microleakage test. DTK Kleber adhesive were used to cement the screw retained crowns to the tibase abutment. G-CEM LinkForce (GC Inc, Tokyo, Japan) translucent dual cure adhesive resin cement was used to cement the acuris crown to the final cap following the manufacturer's instructions. Bisco's Porcelain etchant (9.5% HF) was applied to the inner surface of the crown then rinsed and dried after 20 seconds. After that primer (GC Inc, Tokyo, Japan) were applied and dried with an air syringe. After cementation of the crowns, 24 hours were waited to ensure that the polymerization of the resin cement was complete.

5.4. Inoculation of the Bacteria

After several trials, 3 μL was determined to be the ideal quantity of bacterial suspension for inoculation. A suspension of *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 was prepared from overnight culture on tryptic

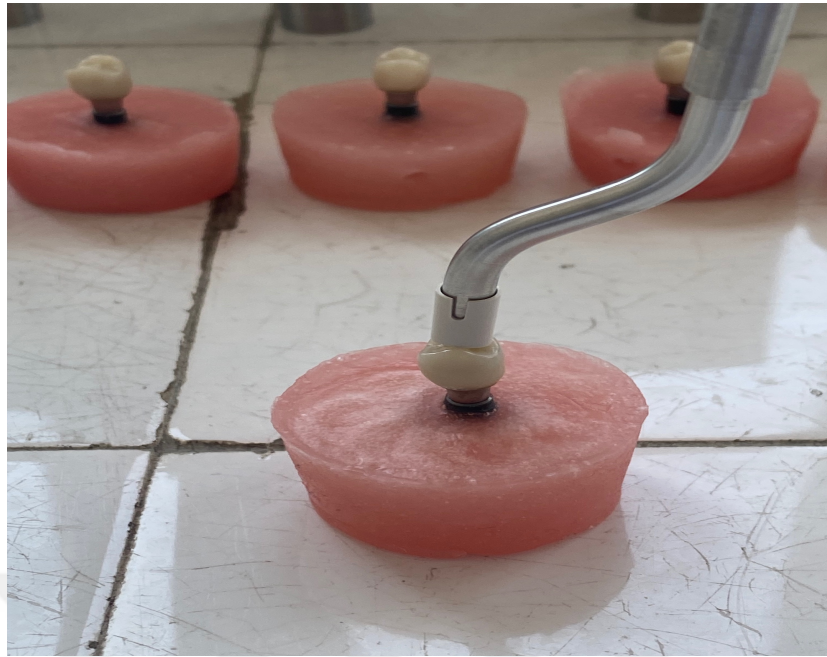
soy agar medium in a 0.5 McFarland turbid tube in tryptic soy broth medium. With maximum caution not to contact the implants' exterior or inner surfaces about 3 μL (3×10^6 cfu) of this suspension mix were carefully pipetted with micropipette to the deepest point of the internal lumen of each implant (Picture 5).



Picture 5. Inoculation of bacteria

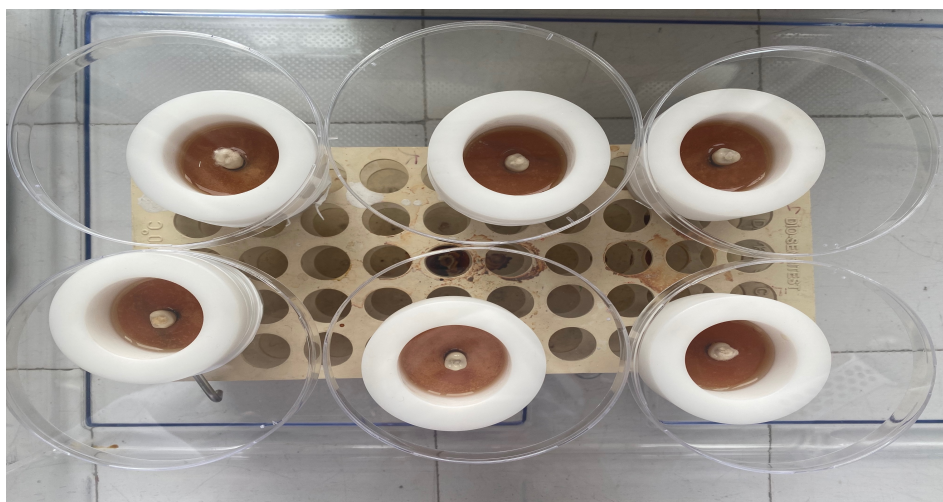
5.5. Fixation of the Crowns and the Unloaded Test

After the inculcation of the bacterial suspension, the abutments were then connected using a manual torque following the manufacturer's instructions to 25Ncm with extreme caution and carefulness not to touch the outer or inner surfaces of the implants. In group A the screw-retained crowns were torqued to 25Ncm following the manufacturer's instructions and the screw hole of the screw-retained crowns was blocked with cotton and composite, and in Group B the crowns were fixed by an axially directed force from a calibrated striker (Conometric fixation tool, Dentsply Sirona Implants, Mölndal, Sweden) (Picture 6).



Picture 6. Fixation of the Acuris crown using the conometric fixation tool

All assembled specimens were then disinfected with 70% aqueous ethanol (EtOH), then the chambers were filled with tryptic soy broth solution and the specimens were partially immersed in the solution that came halfway up to the crown. This insured bacterial penetration along the implant-abutment interface but not along the screw access hole. Implants were incubated at room temperature for seven days and were checked daily (Picture 7). Samples were taken daily of the tryptic broth.



Picture 7. Samples incubated for 7 days in room temperature covered with plastic petri.

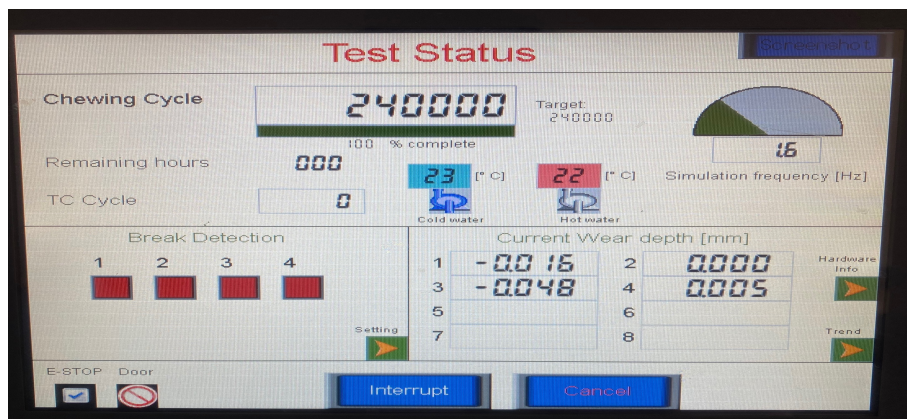
5.6. In Vitro Dynamic Loading

Dynamic loading was performed using a dual-axis chew simulator (CS-4, SD Mechatronic GmbH, Feldkirchen-Westerham, Germany) (Picture 8).



Picture 8. Dual-axis chewing simulator

In the chewing simulator, there are 4 force arms to apply load to all 4 samples simultaneously. The axis extending in the horizontal direction carries all the arms, causing the force to be applied simultaneously. The chewing load that is planned to be applied is provided by the weights placed on the force arms in the chewing simulator. These weights are transmitted to the antagonists (crowns) using tapered tips and create contact pressure during the abrasive action. Bits are subject to wear due to this movement. The machine is controlled by the touch screen connected to the simulator (Picture 9).

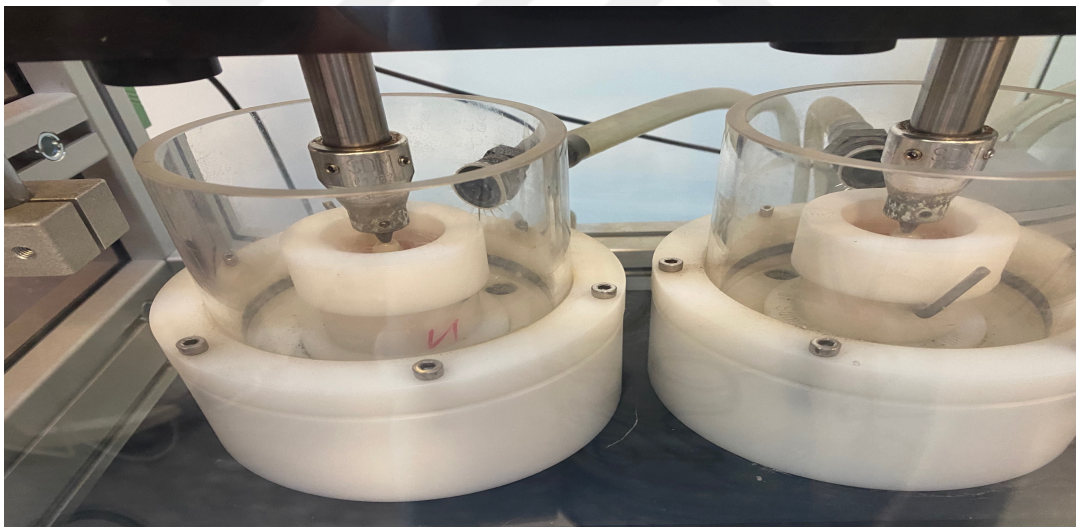


Picture 9. Touchscreen connected to the chewing simulator

The dynamic loading conditions are as follows:

- Dynamic Load Amount: 50 N
- Chewing Frequency: 1.6 Hz
- Number of Dynamic Cycles: 240,000 Cycle/ 1 year.

A load of 50 N was applied to a 30° inclined surface 3 mm from the center of the crown. After the dynamic load was applied on the inclined surface, a sliding movement of 3 mm occurred in the lateral direction and the friction movement continued until the load of 50 N reached the midpoint of the crown. After the crown came to the middle point, the contact of the tip that applied the force arised, and the simulator returned to its initial state and continued to be exactly the same as this cycle. After the samples were placed in the chewing simulator, the test chamber was filled with the tryptic broth (Picture 10).



Picture 10. Application of forces on the crown during dynamic loading

5.7. Taking Samples

Daily observations were made of the samples. Samples were taken in both situations; unloaded and after dynamic loading. In the unloaded implants samples of the tryptic broth were taken daily for seven days using disposable sterile plastic inoculation loop (Picture 11).



Picture 11. Taking sample of tryptic broth using inoculation loop

However, in the dynamic loading situation, the samples were taken twice; half way the cycles and when the cycles were done. To take the samples, the machine was paused to take 1 cc from each chamber and then continued again. After the cycles are done the samples were removed from the chewing simulator and 10 μ l of the solution was drawn with sterile 1-time-use syringes. Streaking were made using disposable steril plastic inoculation loop in Bile Esculin agar and McConkey agar medium, then incubated at 37°C for 24 hours to confirm the purity of the microorganism inoculated in the inner part of the implant and to determine the presence of bacteria. The number of grown colonies were counted. The development of *E. coli* and *E. Faecalis* that resulted demonstrated that bacteria had indeed escaped from the inner section of the implant into the surrounding solution via the tested interface. The unloaded implants underwent the same procedure, but without the loading.

In the case of no growth, the expression "no bacterial growth" was used. After the evaluation, the numbers (cfu/mL) were made according to the following category (Table 4).

Table 4. Classification of bacterial count results

cfu/mL	Reproduction Level
0	No bacterial growth
10-1000	Low bacterial growth
1000-10.000	Medium bacterial growth
10.000-100.000	High bacterial growth

5.8. Statistical Analysis

Data were analyzed with IBM SPSS V23 (Version 23, SPSS Inc., Chicago, Illinois, United States). Conformity to the normal distribution was evaluated using the Shapiro-Wilk test. Mann-Whitney U test was used to compare the data that were not normally distributed according to the groups. Wilcoxon test was used to compare the data within the group that was not normally distributed according to the pairwise time, and the Friedman test was used to compare the data that were not normally distributed according to three or more times. Analysis results were presented as mean $\pm$ SD for quantitative data, deviation and median (minimum – maximum). Significance level was taken as $p < 0.050$.

6. RESULTS

6.1. Bacterial Count Results

For preparation of the bacterial suspension, the test organism *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 was first plated onto Tryptic soy agar and then incubated for 24 hours at 37°C. Suspension was made from the culture by diluting a few colonies in Tryptic soy broth to a density of 0.5 McFarland Standard. Approximately 3 µL (3×10^6 cfu) of this suspension mix was pippered into the inner part of the implants. After assembling the components Tryptic soy broth was added to the sample's chamber covering up half of the crown to make sure there is no leakage from the occlusal screw that might give a false positive result. The prepared samples were covered with sterile Petri dish and kept at room temperature for about 7 days. Everyday 10 µL of tryptic soy broth were taken of each sample's chamber and streaking were done on Bile Esuclin agar and McConkey agar medium. The cultured cultures were incubated at 37 °C and 24 hours. The number of growing colonies was counted (cfu) in both conditions as seen in the tables bellow for Group A (Tables 5 and 6) and for Group B (Tables 7 and 8).

Table 5. Preloading Bacterial count results of Group A.

	No.1 (cfu)	No.2 (cfu)	No.3 (cfu)	No.4 (cfu)	No.5 (cfu)	No.6 (cfu)
Day 1	0	0	0	0	0	0
Day 2	0	0	0	0	0	0
Day 3	0	0	0	0	0	0
Day 4	0	0	0	0	0	0
Day 5	0	0	0	0	0	0
Day 6	0	0	0	0	0	0
Day 7	0	0	0	0	0	0

Table 6. Bacterial count results of Group A after dynamic loading

	No.1 (cfu)	No.2 (cfu)	No.3 (cfu)	No.4 (cfu)	No.5 (cfu)	No.6 (cfu)
120 Cycle	0	0	3×10^3	3×10^3	10^6	10^6
240 Cycle	0	0	3×10^6	3×10^4	10^6	10^6

Table 7. Preloading Bacterial count results of Group B

	No.1 (cfu)	No.2 (cfu)	No.3 (cfu)	No.4 (cfu)	No.5 (cfu)	No.6 (cfu)
Day 1	0	0	0	0	0	0
Day 2	0	0	0	0	0	0
Day 3	10^3	10^3	10^3	0	0	0
Day 4	10^3	10^3	10^3	0	0	0
Day 5	10^3	10^3	10^3	0	0	0
Day 6	10^3	10^3	10^3	0	0	0
Day 7	10^3	10^3	10^3	0	0	0

Table 8. Bacterial count results of Group B after dynamic loading

	No.1 (cfu)	No.2 (cfu)	No.3 (cfu)	No.4 (cfu)	No.5 (cfu)	No.6 (cfu)
120 Cycle	3×10^{-4}	3×10^{-4}	3×10^{-4}	3×10^{-4}	0	0
240 Cycle	3×10^{-4}	3×10^{-4}	3×10^{-4}	3×10^{-4}	0	0

6.2. Preloading Statistical Results

There was no statistically significant difference between the groups in terms of the presence of bacterial microleakage in both conditions of unloaded and dynamic loading ($p > 0.50$).

In Group A; there is no statistically significant difference in the value of leakage between the samples in the same group before and after loading ($p > 0.50$).

In Group B; there is no statistically significant difference in the value of leakage between the samples in the same group before and after loading ($p > 0.50$) as shown in the following table (Table 9).

Table 9. Comparison of preloading values between and within groups

Day		Group A	Group B	p
D1	Mean $\pm$ s. deviance	0,0 $\pm$ 0,0	0,0 $\pm$ 0,0	1,000
	Median (min. - max.)	0,0 (0,0 - 0,0)	0,0 (0,0 - 0,0)	
D2	Mean $\pm$ SD	0,0 $\pm$ 0,0	0,0 $\pm$ 0,0	1,000
	Median (min. - max.)	0,0 (0,0 - 0,0)	0,0 (0,0 - 0,0)	
D3	Mean $\pm$ SD	0,0 $\pm$ 0,0	500,0 $\pm$ 547,7	0,180
	Median (min. - max.)	0,0 (0,0 - 0,0)	500,0 (0,0 - 1000,0)	
D4	Mean $\pm$ SD	0,0 $\pm$ 0,0	500,0 $\pm$ 547,7	0,180
	Median (min. - max.)	0,0 (0,0 - 0,0)	500,0 (0,0 - 1000,0)	
D5	Mean $\pm$ SD	0,0 $\pm$ 0,0	500,0 $\pm$ 547,7	0,180
	Median (min. - max.)	0,0 (0,0 - 0,0)	500,0 (0,0 - 1000,0)	
D6	Mean $\pm$ SD	0,0 $\pm$ 0,0	500,0 $\pm$ 547,7	0,180
	Median (min. - max.)	0,0 (0,0 - 0,0)	500,0 (0,0 - 1000,0)	
D7	Mean $\pm$ SD	0,0 $\pm$ 0,0	500,0 $\pm$ 547,7	0,180
	Median (min. - max.)	0,0 (0,0 - 0,0)	500,0 (0,0 - 1000,0)	
Test statistic		$\chi^2=0,000$	$\chi^2=18,000$	
p		1,000	0,051	

χ^2 : Friedman test statistic, U: Mann-Whitney U test statistic

There was no statistically significant difference between the median values of preloading at different times according to the groups ($p>0.050$). When the in-group comparison results were examined, no statistically significant difference was found between the median values of preloading according to time in groups A and B ($p>0.050$).

6.3. After Dynamic Loading Statistical Results

The numbers of grown colonies were counted, analyzed statistically and compared between and within the groups after dynamic loading (Table 10).

Table 10. Comparison of after dynamic loading values between and within groups

		Group A	Group B	p
120 cycle	Mean $\pm$ SD	334333,3 $\pm$ 515624,9	20000,0 $\pm$ 15491,9	1,000
	Median (min. - max.)	3000,0 (0,0 - 1000000,0)	30000,0 (0,0 - 30000,0)	
240 cycle	Mean $\pm$ SD	388333,3 $\pm$ 487048,9	20000,0 $\pm$ 15491,9	0,394
	Median (min. - max.)	165000,0 (0,0 - 1000000,0)	30000,0 (0,0 - 30000,0)	
Test statistic		Z=-1,342	Z=0,0	
p		0,180	1,000	

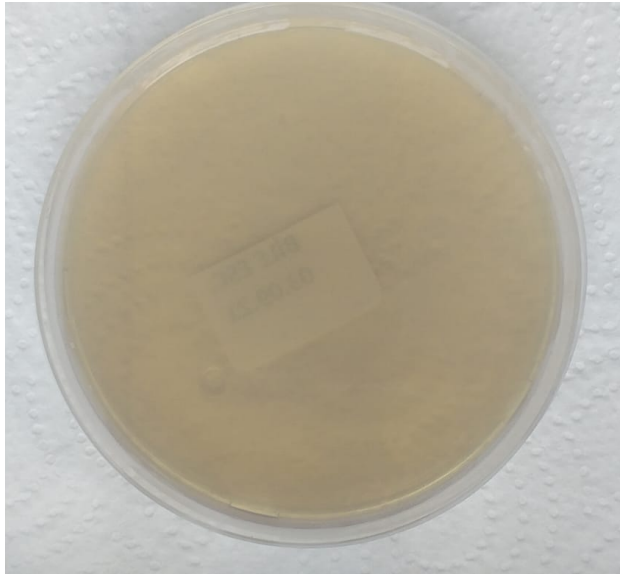
U: Mann-Whitney U test, Z: Wilcoxon test

There was no statistically significant difference between the median values of after dynamic loading at different times according to the groups ($p > 0.050$). When the in-group comparison results were examined, there was no statistically significant difference between the median values of after dynamic loading according to 120 and 240 cycles in groups A and B ($p > 0.050$). No differences in bacterial leakage at the Implant-abutment interface was observed between the used 2 different bacteria.

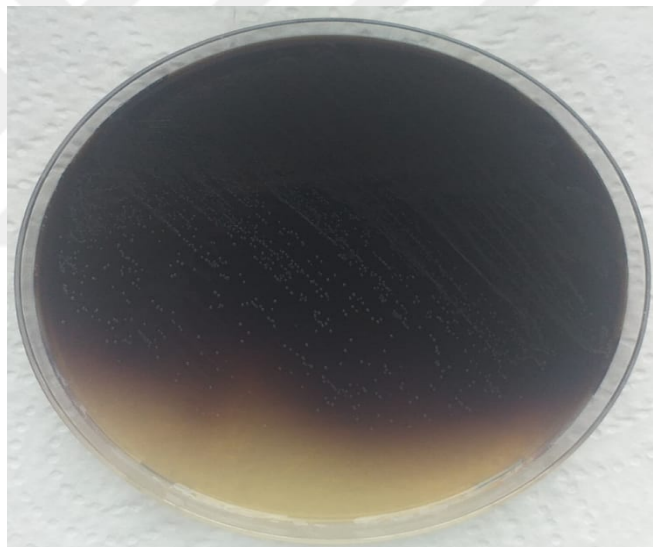
6.4. Bacteria Count Classification

The following classification was chosen to translate the number of colony forming units into clinical outcomes for bacterial enumeration.

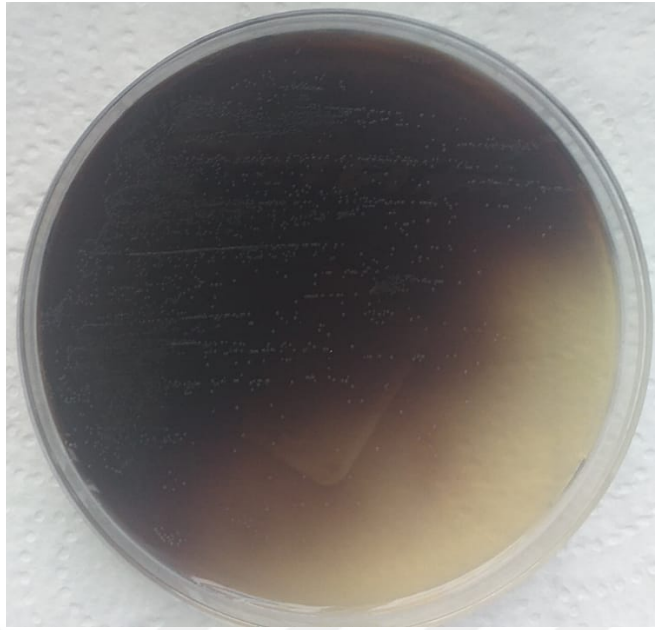
1. 0, No bacterial growth in Bile esculin agar (Picture 12).
2. 10-1000, low bacterial growth in Bile esculin agar (Picture 13).
3. 1000-10,000, moderate bacterial growth in Bile esculin agar (Picture 14).
4. 10,000-100,000, high bacterial growth in Bile esculin agar medium (Picture 15).



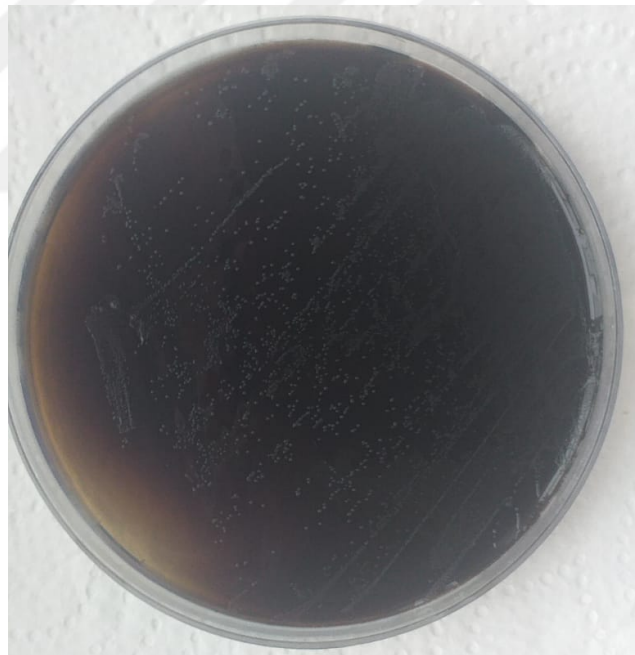
Picture 12. 0, No bacterial growth in Bile esculin agar



Picture 13. 10-1000, low bacterial growth in Bile esculin agar.



Picture 14. 1000-10,000, moderate bacterial growth in Bile esculin agar



Picture 15. 10,000-100,000, high bacterial growth in Bile esculin agar medium

7. DISCUSSION

Microgaps and misfits in the implant abutment interface have been found to encourage bacterial penetration and possibly lead to the development and progression of peri-implantitis. Microgap in the implant-abutment interface although inevitable can be minimized by choosing the suitable abutment (Mishra et al., 2017).

In this study the microbial leakage was tested in two implant-abutment systems; Acuris abutment restored with a conometric crown, and TiBase abutment with screw retained crown under nonloading and dynamic loading conditions. Bacterial leakage was seen in both groups in different levels and there was no significant difference within and between the two systems before and after loading ($p>0.50$).

The null hypothesis of this in-vitro study suggests that there is no significant difference in the microbial seal under unloaded and loaded condition between the two implant-abutment systems; TiBase abutment with a screw-retained (Group A) crown and Acuris conometric abutment with conometrically seated crown (Group B). In the light of the results above our null hypothesis is accepted.

A dental implant system comprises the endosseous portion and connects to a superstructure that ultimately restores function and esthetics. There are two types of implant systems; one-piece implant systems and two-piece implant systems. As the latter systems contains of separate components, the microgap in the interface between the abutment and the implant, and at the interface between the prosthetic and the abutment seems unpreventable. In the two-piece system the abutment is connected to the implant on one side and to the prosthesis on the other side. An implant–abutment interface and an abutment–restoration interfaces are formed as a result. The size of the abutment-restoration interface microgap varies depending on the individual manufacturer and though it appears to be limited to less than 50 μm for present implant systems (Bressan and Lops, 2014).

In two-part implant systems, the mechanism responsible for crestal bone remodeling is unclear. The implant-abutment connection surface is one of the issues prompting concern. If incompatibility occurs on the implant-abutment connection surface, microgaps are formed. Microbial penetration through the microgaps between the

implant-abutment junction is inevitable. Bacterial colonization on the inside of the link is demonstrated in in vitro and in vivo studies.

Broggini et al. reported that perfect compatibility between the implant-abutment at the bone crest level reduces the accumulation of inflammatory peri-implant cells and causes minimal bone loss (Broggini et al., 2003). McCartney et al. and May et al. reported that full compatibility of implant components with each other and with the prosthesis is vital for the preservation of marginal bone and the long-term success of dental implants (McCartney et al., 1991; May et al., 1997).

According to Quirynen et al., crestal bone loss is directly associated with the presence of a microgap in implant-abutment interface (Quirynen et al., 2002).

In this study two different implant-abutment systems were used to compare the microbial leakage before and after dynamic loading. Acuris abutment and ti-base abutment were used. Emax were the materials of choice for the restorations, 6 ti-base abutments were restored with a screw-retained restoration (Group A) and 6 Acuris abutment with a conometrically seated crown (Group B). Before loading Group A did not show any leakage ($p > 0.50$), However Group B showed minimal leakage in 3 samples ($p > 0.50$). Both groups had leakage after dynamic loading ($p > 0.50$). It is found as a result of this study that there is no significant difference in the microbial seal between the groups and within each group ($p > 0.50$). In this study, different levels of microleakage were observed at the implant-abutment interface in both groups, confirming that complete hermetic closure was not possible.

There are few researchers who studied the microleakage, marginal/ internal fit, and fracture resistance in implant-supported restorations with different kind of abutments and restorations (Gherke et al., 2021; Degidi et al., 2015). The removal force and the retention of conometric abutment/cap under dynamic loading were studied too (Tebbel et al., 2018). However, no studies were found evaluating the microbial leakage at the IAI in Astratech Osseospeed implant with the acuris conometric abutment and comparing it with the ti-base-abutment system before and after loading. For that reason, two systems were chosen to test the microbial leakage for this study; Ti-base abutment restored with screw-retained crown and Acuris conometric abutment with acuris crown.

Several in vitro investigations focused onto fluid penetration across various implant system interfaces (Aloise et al., 2010). Microbial leakage was intensely examined at implant-abutment interface of two-piece implant systems (Coelho et al., 2008; Aloise et al., 2010; Smith et al., 2014).

Evidence about the differences in bacterial microleakage according to the implant-abutment microgap with different connection designs are very limited. Morse taper connections, according to several studies, have narrower gaps and generate less bacterial microleakage than internal and external connections (Assenza et al., 2012; D'Ercole et al., 2014; Koutouzis et al., 2011; Tripodi et al., 2012). Regardless of the connection type at the implant abutment interface, bacterial microleakage cannot be completely prevented in all implant systems available in the market (Passos et al., 2013; Schwarz, 2000). Information about the bacterial leakage along the prosthetic-abutment connection level are actually very limited. Bressan et al. investigated microbial leakage at the prosthetic connection interface in two Morse taper conometric systems. Bacterial suspension was inoculated into the abutment screw hole, and the coping was inserted on the abutment and was incubated for 72 hours into anaerobic conditions. It can be concluded that conometric interfaces for prosthetic connection may allow minimum bacterial invasion (Bressan et al., 2017).

Gehrke et al. studied the microbial penetration by the prosthetic connection of Acuris crowns in vitro using qrt-PCR and SEM. A two-fold study set-up was proposed in turn to evaluate the bidirectional penetration of bacteria along conometrically seated single crowns. The conometric abutment screw hole was inoculated with bacterial suspension and then titanium nitride-coated (TiN) caps were fixed by friction. The systems were incubated in anaerobic conditions for seven days. It was concluded that It may be inferred that under non-dynamic loading circumstances, the Acuris conometric interface does prevent bacterial translocation.

In a recent study; Gehrke et al. evaluated bacterial leakage and the existence of microgap along the Acuris conometric prosthetic interface of three implant abutment systems (Ankylos, AstraTechEV, Xive) using qrt-PCR tests and SEM. The abutments screw holes were filled with the bacterial suspension and the system were incubated under anaerobic conditions for seven days. It was concluded that there wasn't any

leakage detected from or into the Acuris abutment in the three implant abutment systems (Gehrke et al., 2021).

Because of the high risk of false negative observations, it is recommended that investigating periods longer than 7 days be avoided. It was stated that any bacterial microleakage usually occurs within the first 3 days of evaluation. This may be a result of the reduction of the liquid and nutrients that are necessary for bacterial survival (Bressan et al., 2017). According to that information it was decided to keep the unloaded test period for seven days in our study.

Although many bacterial microleakage studies have been performed in vitro (Abdelhamed et al., 2015; Al-jada et al., 2015; Aloise et al., 2010; Assenza et al., 2012; Covani et al., 2006; do Nasimento et al., 2012; Koutousiz et al., 2014; Bressan et al., 2017), few have been done in vivo (Mawhinney et al., 2015; Romanos et al., 2016). The reasons of that are how challenging to standardize the patients and how difficult it is to take bacterial samples in vivo conditions which are highly susceptible to contamination. In fact, under in vivo conditions, the presence of bacterial contamination in the internal part of the implant-abutment junction is inevitable (Persson et al., 1996).

To imitate clinically occurring microleakage, many approaches have been used. Radiography, SEM, and other optical methods are easy procedures for detecting the existing misfit or gap in the interface. However, these methods are not completely reliable as they do not provide information about the continuousness and depth of the gap. Other accepted test methods; microbial leak or bacterial leak test, endotoxin or other molecular microleakage test, spectrophotometric determination of dye penetration and gas permeability test (Assenza et al., 2012).

Although the microgap at the implant-abutment interface in this recent study was tested by measuring bacterial microleakage using an accepted method, some studies also used two or more methods to measure this microleakage. Al-Jadaa et al. used three different methods for the evaluation of microleakage at the implant abutment interface. These methods are gas permeability test, microbial leak testing and molecular leak testing (Al-Jadaa, 2015).

Abdelhamed et al. compared the microbial leakage between titanium and zirconia abutments. For this, molecular tests were applied using both micro CT technology and toluidine blue staining method (Abdulhamed et al., 2015).

Based on the cross-sections of the implants, there are two areas of sufficient width and space as the bacterial reservoir in case of microleakage, the first is the implant-abutment interface, and the second is the area under the screw at the deepest point of the implant, for that reason the bacterial suspension was inoculated to the deepest part in the implant in this in vitro study.

In order to assess the risk of bacterial infiltrate at the implant-abutment interface, in vitro studies used two main methods. Outgrowth method is one method that involves inoculating the interior of the implant with a microbial agent, assembling the implant-abutment complex, and assessing the bacteria's release into the surrounding environment which was used in our study. The main disadvantage of the outgrowth model is an error in the inoculation process of the internal aspect of the implant which may lead to a false positive result. Ingrowth method is an alternative technique which is based on assembling the implant-abutment complex in a sterile environment, submerging the complex into a bacterial solution and evaluating the penetration of bacteria to the internal aspect of the implants. The main challenge in this method is to assemble and disassemble the complex under sterile conditions and obtain a microbial sample from the internal part of the implant without cross-contamination with the external surfaces of the implant.

Aljadaa et al. stated that it is ideally to examine the microleakage in implants performed under unloaded and dynamic loading conditions in one set of implants without being re-assembled (Aljadaa et al., 2015). Because it would have required disconnection of the implant-abutment components, 'inward' bacterial leakage was not chosen for this present study. As a result, there would be certain scientific drawbacks, as bacteria from the outer portions could be moved to the interior parts during the opening process and therefor resulting in a false-positive result. For the previous reasons mentioned, it was decided to test for 'outward' leakage, on the assumption that bacteria leakage from the inner parts of the implant outward may also migrate in the opposite direction, into the implant.

In previous studies investigating the microbial leakage many bacterias were used such as; *Actinobacillus actinomycetemcomitans*, *Streptococcus sanguis*, *Escherichia coli*, *Streptococcus oralis*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Staphylococcus aureus* and *Enterococcus faecalis*. These bacterias can be used in both ingrowth and outgrowth methods (Dibart et al., 2005; Jansen et al., 1997; D'Ercole et al., 2015).

In this study a mix of *E. coli* (*Escherichia coli*) and *E. Feacalis* (*Entrococcus faecalis*) was used. *E. Feacalis* is a nonmotile, facultatively anaerobic gram-positive coccus. It is an important opportunistic pathogen that exists in the oral cavity, gastrointestinal tract, urinary tract and is approximately 1.0-1.5 µm in size. *E. faecalis* can survive in severe environmental conditions (acidic or basic pH, high salt concentration, presence of heavy metals, aerobic and anaerobic). They can grow in the range of 10 to 45 °C and survive at temperatures of 60 °C for 30 min. This bacterium has the ability to infiltrate the dentinal tubules for more than 200 microns, can survive without the help of other bacteria, and is resistant to a wide range of antibiotics. This species is involved in the pathogenesis of secondary endodontic apical lesions and can also be found in root-filled teeth with no apical lesions and also in primary endodontic lesions. (Stuart et al., 2006; Tripodi et al., 2015).

E. coli is a motile, facultative anaerobic gram-negative bacterium. Their diameter is approximately between 1.1 to 1.5 µm and 2 to 6 µm in length. It is an opportunistic human pathogen occasionally associated with implant failure (Renvert et al., 2015). already used in microbial leakage dental implant studies (Jansen et al., 1997; Koutouzis et al., 2011). *E. coli* and *E. faecales* was chosen over other known periodontal pathogens for this study because both are easy to culture, widely used test microorganism for in vitro studies as mentioned above especially for sterilization, disinfection and contamination purposes. And because of their reduced size, their permeability through the microgap of the I-A interface and their common residence in the peri-implant area. It is not fastidious and it is easy to handle in the laboratory with a short generation time of 20 minutes. Furthermore, it can be found in the oral cavity of healthy individuals.

Microleakage in the microgap at the implant-abutment interface can be increased by dynamic loading stresses on implants. In an in vitro study of Steinebrunner et al. the

researchers theorized that the stability of the implant-abutment connection decreases along with the dynamic loading which increase the chance of bacterial leakage. The bacterial leakage was evaluated in five different implant systems with different components at the implant-abutment connection under dynamic loading. There was a statistically significant increase in bacterial colonization relative to the number of chewing cycles (Steinebrunner et al., 2005).

Using an in vitro dynamic-loading model to assess the potential risk for invasion of oral microorganisms into the fixture-abutment interface microgap Koutouzis et al. showed that 12 out of 14 implants with internal conical connection were contaminated by *Escherichia coli* after 24 hours (Koutouzis et al., 2011).

D'Ercole et al. evaluated the bacterial leakage for two different microbial kinds along the level of the implant–abutment interface, and the marginal fit and size of microgap at the I-A interface in 2 different implant connections. And it was reported that no leakages through the I-A interface were demonstrated for either type of connection evaluated. The study also demonstrated that a decent marginal fit of implant components appeared to be able to prevent microbial penetration (D'Ercole et al., 2015).

Some of the in vitro studies, bacterial leakage has been reported even in the absence of loading at the interface of many implant-abutment systems (Abdulamed et al., 2015; Aljada et al., 2015; Aloise et al., 2010; Assenza et al., 2012; Covani et al., 2006; Duarte et al., 2006; Jansen et al., 1997; Koutousiz et al., 2015; Passos et al., 2013). In these situations, the amount of microbial penetration in the internal part of the implants depends on the fit and torque forces between the implant and abutment (Gross et al., 1999; da Silva-Neto et al., 2012). The microgap between the implant component might get bigger when faced with the eccentric occlusal forces.

Nascimento et al. examined and evaluated the existence of microbial leakage from human saliva along the implant-abutment interface into the interior of the implant using the DNA Checkerboard before and after dynamic loading. As a result, in all connection types evaluated in both loading and non-loading environments; it was concluded that bacterial species can penetrate from human saliva along the implant-abutment interface. However, higher bacterial penetration was observed after dynamic loading in external and internal-hex implants (Nascimento et al., 2012).

Koutousiz et al. examined the influence of dynamic loading on the colonization of oral microorganisms in the Implant-abutment interface microgap of dental implants with internal Morse-taper connection and it was reported that minimum bacterial infiltration found in the IAI. Bacterial leakage increases with the dynamic loading since there was a possibility of micro movement at the IAI which causes a pumping effect and leads to detrimental effects on the marginal bone stability (Koutousiz et al., 2014).

Tripod et al. investigated whether there was bacterial leakage at the Cone Morse implant-abutment connection, with and without loading. While 10 samples in the experimental group were loaded in the special test setup, 10 samples in the control group were not loaded. In conclusion; They did not find any difference in the percentage of microbial leakage between the groups (Tripodi et al., 2015).

da Silva-Neto et al. examined the microbial leakage in different implant abutment connections under loaded and unloaded conditions. Four groups of specially designed implants with an opening at the apex were grouped according to the I-A and screw device. Samples were loaded with 300,000 cycles/ 1 year and the unloaded implants were kept inside the cycling machine to meet the same conditions of the loaded implants. It was concluded that microleakage increased over time in most of the groups, Morse tapers group showed lower values when loaded (da Silva-Neto et al., 2017).

Although these studies compare bacterial leakage with and without dynamic loading, there are also a few studies that discuss microleakage only under dynamic loading condition (Steinebrunner et al., 2005; Koutouzis et al., 2015) or only without loading (Ghrke et al., 2021; Aljada et al., 2015; Nassar et al., 2015; Passos et al., 2013; Covani et al., 2006; Jansen et al., 1997).

In our study, the number of dynamic loading cycles was determined as 240,000 cycles. The loading cycles number of 240,000/1 year used in our study was also used in similar studies (da Silva-Neto et al., 2017; Podhorsky et al., 2016). The loading apparatus in this study aimed to simulate forces the implants can face during mastication, where 240,000 cycles represent 1 year in function. With the current methodology, microleakage was noticed since the first time point analysis in all implant-abutment connection groups. This is in agreement with previous studies that stated that microleakage happens in short period (Gross et al., 1999; Ceolho et al., 2008) however

toxins leak after 5 minutes and bacteria leak in the first 72 hours (Jansen et al., 1997; Broggini et al., 2006).

There are only a few studies in the literature investigating microleakage under dynamic loading (do Nasimento et al., 2012; Koutousiz et al., 2014; Tripodi et al., 2015; da Silva-Neto et al., 2017). In these studies, a force between 50-120N was generally used. Therefore, in our study, a 50N chewing force was applied with a frequency of 1.6 Hz in dynamic loading. Koutousiz et al and Tripodi et al used similar criteria in their studies (Koutousiz et al., 2014; Tripodi et al., 2015).

In a study on bacterial contamination in implants, different sealing materials (adhesive, silicon O-ring washer, wax, silicone hermetic washer, chlorhexidine-thymol varnish and 2% chlorhexidine solution) were used to examine the effectiveness of these materials on the bacterial penetration in the implant-abutment interface. Most of the sealing materials used failed to prevent bacterial contamination for more than 63 days. Bacterial contamination of the implants was observed in the study, regardless of the type of external or internal hexagonal connection (Duarte et al., 2006).

Nayak et al. in their study on the insulating ability of O-rings (polysiloxane) and GapSeal (antibacterial isolation gel), they reported that although microbial penetration was observed in all groups, it was seen at least in the GapSeal group, followed by the O-ring group (Nayak et al., 2014).

Proff et al. tested the ability of gutta-percha to provide adequate isolation in microcavities against bacterial penetration in their in vitro study and reported that bacterial leakage occurred after 24 hours (Proff et al., 2006).

In the in vitro study of Besimo et al. isolation of cavities with Cervitec®, a varnish containing chlorhexidine, ensured implant isolation and bacterial penetration was prevented for 11 weeks in test samples in the environment without loading (Besmio et al., 1999).

Koutousiz et al. evaluated the effect of chlorhexidine in preventing endotoxin penetration at the implant-abutment interface of implants with Morse taper connection, and as a result showed that 0.2% chlorhexidine solution did not have a significant effect on preventing penetration (Koutousiz et al., 2015).

Podhorsky et al. examined the effectiveness of three different methods (filling of the hollow parts with either a disinfectant agent, a setting sealing compound, or a

nonsetting sealing compound) to reduce the bacterial leakage in two implant systems with internal connection. The inner part of the implant body was pretreated in four ways before the connection of the abutment; application of a 0.2% chlorhexidine gel, a special silicone, or a sealing grease, or no pretreatment. Half of the specimens were subjected to thermocycling. It was concluded that none of the pretreatments prevent the leakage but that the applied products may help reducing the bacterial leakage to a minimum level (Podhorsky et al., 2016).

Considering previous studies and their results, generally the use of isolation methods does not have a significant effect in preventing bacterial penetration. In addition, these methods may increase the risk of incorrect seating of the abutment. For these reasons, it was decided not to use any isolation material in this study.

Most studies evaluating the microgap at the implant-abutment interface results are similar to this study results confirming bacterial microleakage. In these studies, different implant types (Assenza et al., 2012; D'Ercole et al., 2015; Koutouzis et al., 2011; Tripodi et al., 2012), sealing methods (Besimo et al., 1999; Koutouzis et al., 2015; Proff et al., 2005), in vivo/in vitro conditions (do Nascimento et al., 2012; Mawhinney et al., 2015; Steinebrunner et al., 2005; Tripodi et al., 2015) with/without loading (Abdelhamed et al., 2015; Al-jadaa et al., 2015 Aloise et al., 2010; Duarte et al., 2006; Tesmer et al., 2009; Tripodi et al., 2015) or different implant-abutment systems (Gehrke et al., 2021; Deconto et al., 2010) were used as in this study.

Koutousiz et al. examined the bacterial penetration into the Implant-Abutment interface (IAI) in implants with sloped marginal design and compare it with implants with conventional marginal design under dynamic loading. 20 Astra tech Osseospeed implants and titanium abutments were used in this study. *E. Coli* colonized IAI microgaps in ten of the first group and eight of Group 2's twenty implants. Between Group 1 and Group 2 implants, there was no statistically significant difference in the identified mean number of *E. Coli* CFU ($p > .050$) (Koutousiz et al., 2016). In the present study although same Astratech osseospeed implants and tibase abutment were used, it resulted that 4 out of 6 showed leakage which is slightly higher than the recently mentioned study, but no statistically significant difference was found.

Abdelhamed et al. compared zirconia and titanium abutments for microleakage in Astratech Osseospeed implants. It was concluded that there was no statistically

significant difference in bidirectional microleakage between titanium and zirconia abutments at different closing torque values in the unloaded condition (Abdelhamed et al., 2015). In this present study same implants and titanium abutments were used and the results mentioned are similar with our study as no significant difference was found ($p>0,050$).

In a study of Guerra et al. the microbial leakage at the implant-abutment interface were tested and compared in different connections (Internal hex, external hex, solid taper, short taper and taper with internal hex) under static conditions. Internal hex connection showed the lowest percentage of bacterial infiltration and no significant difference in bacterial leakage was found among different implant abutment connections (Guerra et al., 2016). In this present study the used implants and abutments was with internal hex connection and had no leakage under static conditions and there was no significant difference which is similar to the results of the study above.

Berberi et al. examined in vitro the microbial leakage in implant–abutment interface of OsseoSpeed™ implants connected to original and compatible abutments (n=28). TiDesign™, Natea™, Dual™ and Implanet™ abutments were used. Rhodamine B was placed inside the implants and spectrophotometric analysis was performed with a spectrophotometer. leakage volume was significantly lower in TiDesign and there was significant difference between the groups. Osseospeed and titanium abutments were used in this present study and showed no significant difference within and between the groups when compared (Berberi et al., 2014). These differences in fact might be due to that the other abutments in the study above were compatible and do not fit perfectly with the OsseoSpeed implants, and that Rhodamine B dye was used not bacterial suspension and the difference in the detection method.

Previous studies showed varying results regarding bacterial leakage under static conditions which ranged from 20-80% for internal hex and 20-60% for taper lock designs (Aloise et al., 2010; D’Ercole et al., 2014) These considerable variations may be related to the differences in study designs.

Comparison of the present findings can only be made with caution, as the available literature data are limited about the microleakage in Acuris conometric system, and bacterial translocation appears to be dependent on the particular geometry and accuracy of the prefabricated components of each system. There are only couple of

studies about microbial leakage in Acuris conometric concept at the prosthetic level in nonloading condition, but there are none about the leakage at the IAI level with or without loading. Therefore, our results of the dynamic loading can't be compared with any other literature, as there are no any study examining the leakage in IAI before and after loading in this system.

Gehrke et al. evaluated the microbial leakage along the prosthetic connection in the Acuris conometric concept of three different implant abutment systems (Ankylos, AstraTechEV, Xive)(n=60). The leakage was testes both ways, into and out of the system. And it was concluded that the prosthetic Acuris conometric concept prevent the bacterial leakage under unloaded conditions in all tested systems. Statistical analysis demonstrated a significant difference for qrt-PCR readings of positive control and all test specimens ($p < 0.0001$) whereas no difference was found between negative control and test specimens and between the three different implant systems (Gehrke et al., 2021). In our recent study Acuris conometric Group showed leakage in 3 samples but no significant difference was found comparing it with Group A that showed no leakage at all under static condition which is similar to the study above. Although differences in the method was seen but similar results were obtained as no statistically significant difference was found, the materials used were the same but the difference is that in this study mentioned above evaluated the leakage at the prosthetic level and the bacterial inoculation was in the abutment, however in our study the leakage evaluated was along the IAI and the bacterial inoculated was into the implant.

Bressan et al. examined and compared the microbial leakage in two conometric systems with the same design and different material under static conditions. PEEK and gold were used for the coping materials. Both groups had minimal microbial leakage, and there was no statistically significant difference between them. The result of our present study showed that Acuris conometric group results were similar with leakage in 3 samples and there was no significant difference between the groups under static conditions although different conometric system and coping materials and was used.

Al-Jadaa et al. studied the effect of dynamic loading on bacterial leakage in implant-abutment interface using a gas-enhanced permeation test. Astra tech implants were used in the study and compared with two other systems. Four out of eight implants

showed leakage by the second day. It was concluded that implants that showed microleakage under unloaded conditions had higher potential of having leakage under loading conditions. There was no significant difference in GEPT values before loading, but there was significant difference in the values after loading (Al-Jadaa et al., 2015). The results of the study mentioned is similar to this present study as it was found that there is no statistically significant difference for AstraTech/TiBase before and after loading. The difference here is that Aljadaa et al. found significant difference after loading and the fact that it might be related to the difference in the detecting method, torque values (20 Ncm vs 25Ncm) and the number of cycles (1'200'000 cycle vs 240,000).

Zipprich et al. examined microbial penetration at the implant abutment interface of 14 different implant systems with two different internal types of Implant-Abutment connections under conditions of controlled dynamic loading using a developed chewing simulator. OsseoSpeed implants with titanium abutments were used. No leakage was seen in both groups under unloaded conditions. However, contaminations were seen in both groups after loading. It was concluded that implant systems with internal conical IACs showed better bacterial seal than implants with flat IACs during the dynamic loading and there was no difference in both conditions (Zipprich et al., 2016). In this study similar results were recorded; no significant difference was found ($p>0.050$).

Tripodi et al. evaluated microbial leakage in implants with internal connection in both loaded and unloaded conditions. 500,000 cycle and a force of 120 N was applied for the dynamic loading. Two out of ten samples in both conditions showed bacterial contamination by the 13th and 14th days (Tripodi et al., 2015). Although higher cycle number were used in this study but no difference was found before and after loading and with that similar to the results of this present study as it was stated that no significant difference was found between and within the groups before and after loading ($p>0.050$).

Koutouzis et al. found significant difference and that the bacterial penetration was influenced by the loading conditions in implants with Morse-taper connection. 1/ 20 samples were found to be colonized by *Escherichia coli* in the unloaded group, while the number of colonized samples increased to 4/20 in the loaded group (Koutousiz et

al., 2014). Although internal connection but different type (Morse taper) were used but the facts that the leakage increased with loading is similar to the results of this study and that no statistical difference was found. The fact that significant difference was found might be due to the lower sample size, higher dynamic loading cycles (500,000), the used of teflon as an antagonist material, as well as to differences in the internal connection characteristics of the studied implants.

da Silva-Neto et al. evaluated the microbial leakage along different implant-abutment connection (Internal hex, External hex, Morse taper) under loaded and unloaded conditions. Considering the internal connections under load, Internal hex group presented the highest values of microleakage. There was significant difference between groups and before and after loading. Demonstrating that the microgap at this interface may not be stable under loading. However, there is no information about the microgap size at the moment of loading (da Silva-Neto et al., 2017). The significant difference found in the study above might be due to that external and morse taper connections were used however in our study same internal connection was used for both group with different abutments and that different detecting methods were used. A previous published review showed that even studies using similar methodologies, the results of microleakage among the different implant systems, showed high variability (da Silva-Neto et al., 2012).

8. CONCLUSION

Within the limitation of this in vitro study. The following conclusion can be made:

- 1- Under non-dynamic loading conditions, the Tibase abutment interface did not allow for bacterial translocation although Conometric Acuris abutments showed minimal leakage by the third day.
- 2- Both systems had leakage after dynamic loading.
- 3- There was no significant difference between the two systems variations in test values in both unloaded and loaded conditions ($p > 0.050$).
- 4- There was no significant difference between the results within each group before and after loading ($p > 0.050$).
- 5- The microbial microleakage can probably be reduced by obtaining an ideal component fit, a reducing or absence of micromovements, and a minimal prosthetic misfit.
- 6- The reduced bacterial passageway at such interfaces may help maintenance of healthy periimplant tissues status.

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