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Mechanical tests for evaluation of the integrity of the implant abutment connection

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Trabalho apresentada à Universidade Fernando Pessoa, como parte dos requisitos para a obtenção do grau de Mestre em Medicina Dentária.

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Resumo

Nos últimos trinta anos, a reabilitação oral por sistemas de implantes tornou-se uma das técnicas mais bem-sucedidas.

Um sistema de implante tem habitualmente 2 corpos, um endosteal e um dispositivo de suporte para a prótese conectados por um parafuso.

Ao longo desta interface, as microgaps podem se desenvolver.

Estes espaços constituem um abrigo para bactérias, o que pode aumentar o risco de peri-implantites, associadas a uma maior infiltração de células inflamatórias e perda de osso.

Para reduzir esse risco e, portanto, o tamanho e número de microgaps, são realizados testes mecânicos para adquirir um maior conhecimentos sobre o desempenho de cada sistema de implantes.

O comportamento dos dispositivos fora testados recorrendo a : análise microbiológica, extração de DNA, análise por estereomicroscópio, microscópio eletrônico de varredura, modelagem de elementos finitos, análise radiográfica e análise espectrofotométrica.

Materiais e métodos : A pesquisa foi realizada entre novembro de 2016 e abril de 2017. Estudos experimentais, artigos de revisão e livros escritos em inglês com texto de versão de acesso completo, data de edição da publicação entre 2005 e 2017, e condições do teste mais realistas, como as condições na cavidade oral são critérios de inclusão.

Os resultados foram posteriormente analisados, com o objetivo de avaliar a integridade da conexão de implante-pilar.

Palavra-chave: « testes mecânicos e implantes », « integridade do implante », « implante-pilar microgap »

Abstract

Over the last thirty years, the oral rehabilitation by implants systems has become one of the most successful techniques.

An implant system has two main parts, an endosteal fixture and a prosthesis-supporting abutment connected to fixture with a screw.

Along this interface, microgaps can develop; they are defined as a microscopic space.

This localisation play a role of safe house for bacteria, which can increase the risk of peri-implantitis, they are associated with a higher inflammatory cell infiltration and bone loss.

To reduce this risk and therefore the size and number of microgaps, mechanical tests are carried out in order to develop knowledge about the capacities of each implant system.

As tests, it was found: microbiological analysis, DNA extraction, analysis by stereomicroscope, scanning electron microscope, finite element modeling, radiographic analysis and spectrophotometric analysis.

Material and methods: the research was done between November 2016 and April 2017. Experimental studies, review articles and books written in the English with a full access version text, the edition date of the publication between 2005 and 2017, and conditions of the test more realistic, like the conditions in oral cavity are inclusions criteria.

Their results are subsequently analysed, for the aim of an evaluation of the integrity of the implant-abutment connection.

Keys words: « implant mechanical tests », « implant integrity », « implant-abutment microgap »

Dedication

À mes quatre parents pour m'avoir soutenue dans toutes les épreuves, d'avoir été fiers de moi même quand il n'y avait pas de raison, de m'avoir appris à être forte et combattante, de m'avoir montré que même lorsque le chemin n'est pas conventionnel, l'arrivée est encore plus extraordinaire.
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To Porto for the warm welcome, for making this great city our second home.

To Professor Jorge for the trust he placed in me in the realization of this project.

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To Fernando Pessoa University, for welcoming us, and helping us realize our dreams and our plans for the future.

General index

I. Introduction 1
1) Materials and Methods 2
II. Development 3
1) Microbiologics analysis 3
2) DNA extraction 5
3) Stereomicroscope 6
4) Scanning electron microscope 7
5) Finite element modelling 9
6) Radiographic analysis 10
7) Spectrophotometric analysis 12
III. Results 13
IV. Discussion 14
V. Conclusion 15
VI. References bibliographic 16
VII. Annexes 19

Table of contents

Table 1: Results of all protocols. Do Nascimento C. et alii. 2008; Koutouzis T. et alii. 2014 ; Khorshidi H. Et alii. 2016 ; El Haddad E. et alii. 2016 ; Smith Nicole A et alii. 2014 ; M. Prado A. et alii. 2016 ; Pereira J. et alii 2015 ; Kano S.-C. et alii. 2007; Macedo J.P. et alii. 2017; Scarano A. et alii. 2016 ; Rack A. et alii. 2010; Berberi A. et alii. 2014. 19

Index of abbreviations

ATCC = American Type Culture Collection

BHI medium = Brain heart infusion medium.

CFU = Colony forming units

FAI = Fixture-abutment interface

FEM = Finite element modelling

IAI = Implant-abutment interface

IAC = The implant-abutment connection

PBS = Phosphate solution

RhB = Rhodamine B

SB20 medium = Bacitracin sacarose agar

SD = Standard deviation

SEM = Scanning electro magnetic

THB-HM = Todd-Hewitt broth supplemented with hemin and menadione

TSB = Tryptic soy broth

I. Introduction

According to E. El Haddad in 2016 for the oral & implantology journal, the success rate, which is the fraction or percentage of survival among a number of attempts, in implant dentistry is above 80%. The final goals of implants are the aesthetic, full functional restoration, and prevent any implant component from collapse. In fact, implant failures are defined as: biological, mechanical, and iatrogenic (and functional). The two first reasons play a key role above any other; indeed biological complications peri-implantitis, affecting the soft and hard tissues surrounding dental implants. On the other hand mechanical causes involve implant-prosthetic components such as; fracture of implant or abutment, screw loosening and over-structure fracture are complications mechanical. The implant-abutment connection and the functional loading are two characteristics, which will influence the success of implant rehabilitation. The implant-abutment connection (IAC) is influenced by biological factors too, which are going to influence the osseo integration phenomena and the bone level maintenance by the presence of oral and systemic diseases like oral lichen planus, lesions of gastro esophageal, periodontal disease, which model the immune response and may increase the risk of peri-implantitis and material erosion. The connection between abutment and implant will create an interface, with a micro-gap, that allows microorganisms to penetrate and colonize the inner part of the implant. There is, after that, an accumulation of bacteria, which is going to create a growing biofilm and eventually develop peri-implantitis. Generally, the presence of this micro-gap is associated with a higher inflammatory cell infiltration and bone loss. This biofilm is associated with microorganisms, forming complex microbial communities, composed by polysaccharides, proteins, nucleic acids, and water, which is going to compose the extracellular matrix. The growth is totally dependent of condition in the oral cavity. That's why, it is not possible to consider that the oral cavity habitat is the same for all patients, in deed, all characteristics will depend on the saliva composition, oxygen content, pH, temperature, nutriments, tissues and restorative surfaces and resident microorganisms. The first microorganism to colonize is adhesin, it has glycoproteins receptors. 60 to 80% of colonizers are streptococcus species (Pereira J. et alii, 2015*)*. Nowadays, manufacturers are trying to reduce the mobility of the implant-abutment connection to lowdown bacterial leakage. The degree of this penetration into the

connection will depend on the precision of fit between abutment and the implant, the degree of micromovements, and the value of the torque used to connect the components, it means that this degree is specific to each implants systems.

This systematic review highlights different types of mechanical test for an evaluation of the implant-abutment connection. Rehabilitation with oral implants, is more and more common and will have a big role to play in our future, but it still have some issues to fix. Seven experimental methodologies were studied: microbiological, DNA extraction, stereomicroscope, scanning electron microscope, finite element modeling, radiographic and spectrophotometric analysis. The different protocols and all the results founded were distressed approaching microgaps size and its relation to the implant type system, bacterial contamination and leakage.

(El Haddad E. et alii, 2016; Tripodi D. et alii, 2012; Meleo D. et alii, 2012; Scarano A. et alli , 2016).

1) Materials and Methods

The research was accomplished between November 2016 and April 2017. It was conducted online using scientific databases such as « PubMed / Medline », « Research Gate » and « B-on ».

The aim of this review was to evaluate mechanical tests for an estimation of the integrity of the implant abutment connection. Keys-words were used as: « mechanical tests », « implant integrity », « microgap ».

For the inclusion criteria, only experimental studies, review articles and books written in the English were selected and with a full access version text. The edition date of the publication had to be between 2005 and 2017. And a last criterion is when the conditions for of the test were more realistic from the conditions in oral cavity.

Clinical tries on animals and articles with minor abstract information and low interest to this subject were excluded.

At the end, one book was obtained, 43 articles were found but only 18 were selected for the preparation of this paper.

II. Development

1) Microbiologic analysis

This new process is revelled by Do Nascimento C. et alii. in 2008. 20 Branemark compatible implants with a 3.75 mm diameter external hexagonal platform are used. They are separated in 2 groups randomly: the first one is pre machined cobalt-chromium alloy (Co–Cr) with plastic sleeve, cast in nickel-chromium alloy and the second one is a plastic abutments cast in Ni–Cr alloy. The microbiological evaluation is perform with a pure culture of F. nucleatum in anaerobic conditions, by cultivating the microorganism in TSBy, during 48 h at 35 ° C. The nutrient broth is diluting at the time of the density is about to be 0,5 McFarton Standard. The inner portion of each 10 implants was inoculated into a suspension of 3.0 mL F. nucleatum. The abutments and implants are connected with a torque of 32 Ncm. Layers of gutta-percha and cyanoacrylate adhesive systems are used for assembly the upper part. They are immersed for 30 seconds in 5,0 ml of sterile tryptic soy broth. It will serve to evaluate the external contamination. There is an evaluation of bacterial growth in plates, and after tubes with a cloudy broth, which is going to indicative if there is colonization, are excluded from further observation. Then, an evaluation of bacterial leakage through the implant-abutment interfaces is carried out, by immersion of the remaining assemblies in 5.0 mL of the same nutrient solution, in anaerobic conditions, at 35°C for 14 days. Every 24 hours, the solution clarity is evaluated. It gives indications on the possible bacterial penetration of the surrounding environment. If there is a presence of colonization, 0.1mL of the solution was plated on SB20 medium (bacitracin sacarose agar). Into the surrounding solution, it was found a growth of F. nucleatum, this fact confirmed that bacteria's inside the implant, escaped by the tested interface.

Koutouzis T. and collaborators paid more attention to dynamic loading, published the next protocol in Journal of Oral Implantology 2014. 20 implants of internal type Morse taper connection served; they are separated into 2 equal groups: evaluated with non-loading conditions, and they are undergoing dynamic loading conditions. The torque used was 25 Ncm. The FAI interface is covered and put in solution of bacteria.
The implants are subsequently attached to an autopolymerizing resin and then placed in test chambers. They are seated in dual axis chewing simulation in order to apply

dynamic loading. The FAI is immersed in a bacterial solution. A cyclic fatigue load was applied with a round stainless steel stylus with an angle of 30 °, a force of 50 N over 500 000 cycles at 1 Hz. After reaching a certain concentration, Escherichia coli DH5a diluted with luria broth and shaken at 37 ° C, and diluted again with a new luria broth 1: 100. The FAI of the implants is then submerged. Together, they are incubated for 5 days, at ambient temperature; the bacterial suspensions are changed every 18 hours with the same basic broth. Microbial samples are taken after the incubation time, using sterile cotton swabs, in the threaded part of the abutment. A negative and positive control is put in place to minimize the possibility of contamination. The negative controls the outer surface of the abutment area before immersing the implants in the bacterial solution. The positive control is the same but is carried out after. For the statistical analysis, two tests were used: the first one for an evaluation of differences between the two groups of regarding number of CFU for E.Coli, is the The Mann-Whitney U. The second test is for an evaluation differences in the number of implants exhibiting bacterial colonization of the FAI microgap between the two groups, and it's The Fisher exact test. A $P < 0.05$ was considered significant.

Khorshidi H. published in 2016 a study, where an 11-degree Morse Taper was compared to a butt joint connection. 10 implants of each type are used, with for the first a diameter of 4.2 mm and a length of 12 mm, and in the other hand a diameter of 4 mm and a length of 13 mm. The first step is to prepare the standard strain bacteria, there are 1683 specifications (PTCC) who are incubated in tryptic soy broth (TSB) with glycerol 15% and freezing in 70°C for a future use. The second step is to prepare the streptococcus mutans suspension with McFarland 0.5 turbidity. The saved bacteria are incubated in an incubator at 37 ° C with 5% CO2 and for 24 to 48 hours with 20 microliters in the blood agar environment with 5% sheep blood. Some of them are incubated with TSB at 2mm and reached a 0.5% McFarland turbidity. 2 microliters of suspension of Streptococcus mutans are injected under sterile conditions into the implants whose connection is tight at 30 Ncm. For the positive control group sample two tubes are prepared both with TBS nutritive solution and mixed with Streptococcus mutans 2 microliters's suspension. It proves that there is a growth of bacteria and they are present during the all processes. For the negative control group sample, two tubes are used and incubated with brain- heart infusion with sterile nutritious solution and confirmed with solution transparency. All implant-abutment complexes are separated

and immersed in a sterile nutrient solution during 1 minute. Tubes contaminated are removed from the study. They are then placed in sterile Eppendorf tubes with a nutrient solution of 150-200 cc up to the level of the connection. After that, during 14 days at 37°C, 20 tubes containing samples, 20 test tubes pollution control tubes of outer surface, 2 positive and 2 negative control samples were tested daily, through solution turbidity and then compared with the negative control sample. The colonization on blood agar environment is determined, as well as the existence or not of turbidity and bacterial colonization. The third step is to calculate colony. For that, 5 microns of turbidity solution incubated in a sheep blood agar plate during 24-48 hours. Then, a calculation is possible, for a number of bacteria estimate with the formulae: number of calculated colonies x thinness quotient x volume mL. The frequency of bacterial leakage is reported for both systems. The Kaplan-Meier survival curve is created and the chi-square test is analysed.

2) DNA extraction

Microleakage has already been evaluated by few tests such as DNA extraction, which demonstrates that contamination occurs the inner parts of the implants to the external environment and vice versa. Host factors and oral bacteria have big influence on implant failure. Also, genetic materials allow detecting viable and nonviable microorganisms without discrimination by molecular methods. Each human is different, which can be associated with oral infectious diseases. (Do Nascimento C. et al., 2011; 2012; El Haddad E. et al., 2016).

This protocol was published in 2016, by El Haddad E. and collaborators.
For the test, four Nobel Biocare implants are used.
Implant preparation: This is an evaluation to see if there is a passage or contamination by the joint, and movement of bacteria inside the implant. The bacteria have a synthetic DNA target sequences. Inside the tubes, there are: 2 bacterial species: P. gingivalis and T. Forsythia and 2 plasmids (for antibiotic selection): Kanamycin and Ampicillin. A lysogeny broth is useful with a concentration of 50 ug/ml, at 37°C during 12-18h in a shaking incubator. This fluid is incorporated inside of the implants and than a heightening force of 35 N is applied. All implants are left in a microcentrifure tube for

48h ate 37°C, with a rest of the culture (lysogeny broth, bacteria, and antibiotics). Inside the implant and for the negative control, there is only lysogeny broth and antibiotics. After 48h, a paper prove is diving into all containing, implants inside and outside, as well in the negative control.

DNA extraction is used the GenElute TM Bacterial Genomic DNA kit. Lysozymes are incubated with the paper, and there is an addition of proteinase K, who is going to isolate DNA. Spin-column methods purify DNA at the end.

Real-time polymerase chain reaction: allows a quantification and quantitative analysis. The 16S rRNA sequences were used as a basis for the design of the oligonucleotides, which will serve for P. gingivalis and T. forsythia.A plasmid with specific DNA was used for quantitative analyzes, with duplex volumes. 50 nM of each primer and 200 nM of the probes were added.

Statistical analysis: The Student's t-test is applied to access a quantitative evaluation of the implant, inside and outside, leakage.

3) Stereomicroscope

The stereomicroscope technic, also called dissecting, allow a three-dimensional view of the specimen. Each eyes have a separate optical paths, that mean each one have an objective lenses and eyepieces different from each other. We see the same object in different ways. The light is very important with the stereomicroscope because, it's naturally reflected from the object. Thick or opaque samples are perfect for these types of illuminations. (Microscope Detective, 2017)

Smith Nicole A. and collaborators present this protocol in 2014.

Three organisms were selected because they mimesis the environment of the intraoral cavity and are involved in perimplantations and periodontitis. They are: the strain American Type Culture Collection (ATCC) 33277 of P. gingivalis, the ATCC strain intracommunity 25611 and the strain 10953 ATCC of nuclear AT (ATCC). A bacterial mixture in Todd-Hewitt broth supplemented with hemin and menadione (THB-HM) was prepared from cells in a growth phase. 46 implants are used, inoculated with 1 mL of the bacterial mix at apical end. 23 zirconia abutments and 23 titanium abutments are fixed to the implants. From there, they are divided into 4 groups, which differ, by the

applied tightening for: 2 groups of 10 sterile zirconia abutments and two others groups 10 sterile titanium abutments, one group of every implants type are screwed to 10 implant with a torque of 35 Ncm and the others groups with a torque of 20 Ncm. Every implant-abutment system is submerged in 1mL of THB-HM broth and microbes into glass test tubes and incubated in an anaerobic chamber at 37°C. In parallel, one negative control is composed with one of each groups and they are inoculated only with a sterile THB-HM broth for 72 hours and one positive, which is composed only with an abutment in titanium and a Zirconia abutment with a torque of 10 Ncm. Both inoculated with microbes. During the evaluation with a stereomicroscope, a possible correlation between the size of the microgap and the bacterial leakage (more or less extensive) was put forward. It has been taken into account, the shape, consequently the uniformity of the gap, the different torque values. The size of the microgaps was measured using the stereomiscrocope and counting CFUs access bacterial quantification. Analysis was done by ANOVA (a=0.05).

4) Scanning electron microscope

The most developed mechanical technic for the evaluation of the implant-abutment interface is the scanning electron microscope (SEM). To create an image, it will scan a focused electron beam over a surface. This one is going to sample; it will produce a signal, which produces signals, and give information about the tomography and composition of the surface. *Nano Science Instruments (2017). [On line]*.

The protocol is from M. Prado A. and collaborators in 2016.
10 Morse taper implants were necessary to perform the first experimentation. Morse taper abutments were assembled with a torque of 15Ncm. One of the essential elements is human saliva. Collected, from healthy young adults without oral diseases, who didn't take antibiotics in the last 6 months. 5 µl were added to 2 ml brain heart infusion (BHI) medium. The implants-abutments systems were immersed in 24 well plates, for 72 hours, at 37 ° C and under microaerophilic conditions. SEM analyses the morphologic aspects of the implant internal connection surface. An optical profilometry will perform an evaluation of the inner surfaces of the implant, cut in section. Three areas of the implant were measured: border, middle and apical. The results of the arithmetic

roughness (Ra) and the maximum distance from the peak to the valley (Rt) were statistically analysed by a Kruskal-Wallis and Mann Whitney test, with a significance level of $P < 0.05$.

A protocol by Pereira J. et alli published in 2015, 5 external hexagon abutment were tightened with a torque of 32 Ncm to implants. In an other hand, 20 pure titanium grave IV square samples are used in order to verify the influence of the surface roughness on the biological adhesion of the implant. They were divided into two equal groups: half were polished through a SiC papers down to 2400 mesh reaching a Ra roughness of 0.1 µm mimicking abutment surfaces, and other half, underwent an SLA treatment, the surfaces were glit-blasted with alumina particles. They also undergo a cleaning in propanol and in distilled water. A solution of KrolI serves for the immersion of the samples and then cleaned again, conserved in a desiccator, sterilized by autoclaving. Only then they have a contact with the biofilms. 10 mL human saliva is collected with the same condition then before, and diluted in phosphate solution (PBS) for a value of 1: 5. An ELISA spectrophotometer is used to measure the optical density, which after adjustment is 0.5. The initial 5µl suspension was mixed with the brain and heart infusion (BHI) and 5% of sucrose. Both the square titanium and implant-abutment were immersed in 2ml of BHI medium with cell suspensions in 24 wells at 37 ° C for 96h with micro-aerophilic conditions. The growth is daily evaluated. All square Ti samples are washed twice with PBS in order to detach the biofilm and displaced in new wells-plates. The old ones are incubated to serve to the determination of the OD at 630 nm. After, it was made a CFU enumeration. A Pro Meter is used for surface analysis in order to obtain values of the roughness Ra of the square samples of titanium. A SEM is used for microscopic analyses of the square Ti and implant-abutment surfaces. Before the analysis with SEM, gold was used for coating. A one-way analysis of variance (ANOVA), is used for statistical analysis with $p < 0.05$.

The aim of the Kano S-C et alii's protocol published in 2007, is a comparison between 4 groups of abutments to create a classification of the implant-abutment interface taking into consideration the horizontal and vertical gaps. It was used: 48 external hexagonal implants and 48 external hex compatible abutments, divided in fours groups of 12 specimens: machined titanium abutments, used as a control, premachined cast-on palladium abutments having a metal base and a plastic sleeve cast-on with palladium

alloy, plastic burnout abutments cast with nickel chromium alloy, abutments cast with cobalt chromium alloy. Implant-abutments systems are polished and shaped, after cleaning. The technique used is the conventional lost-wax casting. To obtain an average value, each location was measured 3 times. To orient the specimens during the process, index points and the abutment were useful. The eyepiece cross-hair reticule used as a reference, its was defined two lines: X and Y, one passed through the horizontal platform of the implant and the other one, crossed the X line at the most external point of the horizontal platform of the implant. The point (X = 0, Y = 0) is the point where we start measured, it is defined by the intersection between X and Y lines. The first misfit is called A, it is defined as the vertical gap measured from the zero point on the other side of the implant and the same area of the abutment. The other, called B was defined as the horizontal gap from the zero point to the external contour of the abutment. There are two situations: a horizontal overcontour (B>0) is when the abutment is wider than the implant; and a undercontour (B<0), when the abutment was narrower in diameter than the implant. So as a classification who take in consideration both horizontal and vertical components, it will be as: Type I - Considered ideal - No horizontal or vertical gap could be measured (A = 0 ; B = 0), Type II: Only horizontal misfit; the abutment can be undercontoured (B < 0) or overcontoured (B > 0), Type III: Only vertical misfit (A > 0), Type IV: Both horizontal and vertical misfit are funded. At the end, 96 measurements are effectued for each group, knowing that both types of gaps are measured in 8 locations on each of 12 specimens. After that, means and standard deviation are calculated, with an analysis of variance (ANOVA) and Tukey multiple-comparisons post-hoc analysis, with P<0.05.

5) Finite element modelling

The finite element modelling called FEA, is a series of numerical methods. For this reason, it's always complicated to practice an analytical solution. It helps to solve complicated geometric problems by dividing, not find a solution function for the entire domain; it formulates solution functions for each finite element and combines them properly to obtain a solution to the whole body. For this division, a creation of a mesh is needed. The all process is called "discretization"; it will define nodes and boundary conditions. Geng Y. et alii. (2008)

Macedo J.P. et alii. in 2017 present a protocol based on three-dimensional CAD models of Morse taper and external hexagon implants and abutment. Images were then constructed in order to be able to carry out a finite element analysis. There are two-dimensional planes. We can notice that there is a defined region enclosed and the third one, the length of the bone is observable with the 3D section of the mandibule. The implants have a perfect placement, exactly at the same distance from the coronal section of the cortical bone. A mesh generation algorithm has been used including in the contours of the mandible section and the implant; it involves the FEM deep drawing software. Each region independent of the system has its own colour allowing immediate visualization and validation before mesh generation. The dark blue represents the cortical bone, the green is the trabecular bone with extrusion of 10 mm, red, purple and light bubble are the structures of the implants at a 90 ° revolution as a function of the central axis. The mesh will adapt to the curves, with an adaptation of the dimensions, which allows an optimization of the dimension and the quality of the mesh. This corresponds to the geometrical characteristics of the interfaces and the zones of curvature. The conditions are limited, in order to simulate for the FE analysis. For the sensitivity of the results, a convergence analysis is operated. The structural material has two mechanical properties: isotropic and homogeneous. It was considered happening a full osseointegration. Three characteristics are taken into account, they are based on boundary conditions and external loads: Plane Z=0: The condition of symmetry was attribute to all nodes at this plane. Plane Z=1: All nodes at this face are motionless. Plane Y=0: Lower face of the model. Then, 150 N are applied to an external load, on the axial and oblique directions with the last on an angle of 45° by the buccal side.
Unfortunately, it was not accomplished a statistical analysis.

6) Radiographic analysis

One of the mechanical technics to evaluate the implant-abutment interface is with radiographic analysis. Few studies talk about an evaluation without cutting or coating and exposing the object, a new way to evaluate the object in natural conditions.

The first processus study is by Scarano A. and collaborators published in 2016.

The highlight of this study is the technique as a non-destructive, non-invasive, and tri-dimensional way. There are 4 groups of 10 implants each: Group I are a screw-retained internal hexagon abutment*;* Group II are Morse Cone taper internal connection*;* Group III are Morse Cone taper internal connection*;* Group IV are screwed trilobed connection.

The Sky scan 1072 is use for practiced five x-ray microtomography consecutive. All of the implant systems must have microgaps over and along the interface. All parameters adopted are the same for each group. We found: a rotation step of 0.45 degrees; a total rotation angle is 180 degrees; a power source equal to 100 KV/98 microA ; the filter thickness has to be 1 mm (Al) and for the last one, the magnification at X30 and cross-section, pixel size of 9.77 mm. A 3-D model is fabricated for each implant by software reconstruction.

It is the same pattern as the previous study in Meleo D. published in 2012. For the evaluation, it is used 3 different types of conical connection implant systems: Ankylos connection, Straumann connection and Bicon connection. All the parameters are equals but this time, magnification and cross-section are different than before. Sample 1 has a magnification at 30X against 26X for sample 2 and sample 3. While cross section pixel size is 9.77 µm for the first sample and for the others, it's 11.27 µm. CTan replicates the perfect three-dimensional model of each implant. It permits to observe internal and external components without destruction or alteration of samples. 10 μm gaps have been observable with acquisition resolutions. We identified L0 as the level of initial contact between implant and abutment. L1 corresponding to the section where can be observed a micro-gap between two near surfaces, at the end of the connection seal. A calculation is effectuated between L0 and L1 by CTan software, to measure the lateral surface of truncated cone.

In the Scarano A et alii's protocol published in 2016, all the steps are the same than others, this time it's a comparative between internal hexagon and Cone Morse.

The evaluation is realized with 20 implants. After the production of a three-dimensional model of each implant, a statistical evaluation is executed with the Analysis of Variance technique. Statistical significance is set at $P < 0.05$.

For the protocol written by Rack A. and his collaborators in 2010, new type of protocol carries out the evaluation. The implant is 4.1 mm in diameter and has a conical connection. The tightening force of screw was 25 Newton and a crestal bone level of 3 mm is simulated below the shoulder of the implant. A ball will support a static force

(nominal 0 N, 30 N, 60 N, 100 N), which is supervised by a digital force gauge, named SH-500, with an angle of 90 °. The Synchrotron-based micro-imaging called BAMline of the third-generation synchrotron light source is used to make the measurements. It has an insertion device, which has filtered 0.2 mm of Cu and 0.2 mm of Be of the wavelength of the white radiation while passing through a X-ray photon selector monochromator (double layer) with energy of 50 keV. The resulting photon flux density is of the order of 10*10 photons s^{-1} mm^{-2} with an energy bandwidth of 1.7%. The luminescence image of a scintillator screen is optically coupled to a camera via a diffraction limited visible optical light that serves as a basis to obtain the radiographic projection images. A visible light microscope is used to read the luminescent image of a single 50 μm CdWO4 crystal adhered to an unpurified $Y_3\ Al_5\ O_{12}$ (YAG) substrate of 500 μm thick, which is intended to be a scintillator screen. The thickness scintillator proves that the spatial resolution of the detection system is, more or less, 4 μm. (Koch et al. 1998). The visible light optics is protected by the thickness of the scintillator and substrate against the radiation caused by the high energy X-ray beam applied. The energy of the X-ray photon, the spatial resolution of the configuration of the indirect detector and the coherence properties of the BAM line are responsible of the presence of contrast edge despite the relatively large propagation distance.

7) Spectrophotometric analysis

Spectrophotometric analysis is a method based on the measurement of the concentration of solutes into a solution, by absorption of the light by the solution. This method takes advantage of the dual nature of light: first- particle nature which gives rise to the photoelectric effect and second- A wave nature which gives rise to the visible spectrum of light. It will measure the amount of light transmitted or absorbed by the solution. Every atomic or molecular approach has a specifics characteristic of phenomena and absorption spectra. By this fact, every concentration can be found, and calibration curve is made. (University of California Santa Barbara website, 2017).

The protocol described is from Journal of Dental Biomechanics, by Berberi A. et alii. in 2014.

Different suspensions of 0.3 to 0.5 µL helped to inoculate the implants. More specifically, Rhodamine B (RhB), it is a marker detectable because its wavelength is 535 nm, has a high solubility in water, and reacts well with photogenerated oxyradicals.
20 OsseoSpeed implants were used to perform this test. They were divided into 4 groups as: in group A -TiDesign as abutments types, in group B -Natea, Group C- Dual and group D- Implanet. For the leakage evaluation, 0.1 g of RhB is dissolved in 20 mL of distilled water, in order to form a RhB 10^{-2} M solution; and then, disposed into falcon tube. The RhB was used to create a calibration curve to accurately quantify the leakage observed in each groups. Therefore, the absorbance spectra have an extreme accuracy and are captured by Vision Collect a wavelength monitoring. Each concentration has its own fluorescence which the spectrometer is able to obtain due to a previously calibration. In order not to damage the samples and to avoid any risk of contamination, particular attention has been paid to the separation of the various elements.
The next step is the kinetic quantification of leakage, by adding 0.1 µL to the internal volume value. The implant-abutment is placed into 15 mL tubes with 2mL of distilled water, shacked for 48 hours and protected from the sun. A spectrophotometric analysis was carried out, using a spectrophotometer, by extracting 1 mL of distilled water from each group. Two analyses are made after 1h and 48h. The fluorescence of the water is measured and the concentration of RhB in the water and consequently the leakage volume of RhB by the formulae: Initial concentration × Initial volume = Final concentration × Final volume. Statistical analysis is realised by GraphPad prism. One-way analysis of variance (ANOVA) was used, and statistical significance was set at 0.05.

III. Results

Despite of all protocols and mechanicals technics used, results showed us that the presence of bacterial leakage in every implant-abutment systems is inevitable, a consequence of not having a perfect fit between the implant and the abutment. All results are resumes in table 1.
Annexe – Table 1.

IV. Discussion

The contaminations of the fixture and abutment components, and transmission of microorganism from the oral environment during the implant installation, are among others responsible for peri-implantitis. Bacteria infiltration can be realised from the external source to the inner area of the implant, and vice versa. Micro gaps between the fixture and the abutment could facilitate the migration of bacteria. (Do Nascimento et al., 2008). There are three consequences to the formation of micro gap: improper stress distribution, micro movement at the interface, and chronicle inflammation at the interface (Smith N.A. et al., 2014).

In Pereira J et al. (2015), Koutouzis et al. (2014) and Prado A. M. et al. (2016), the protocols described are more realistic regarding conditions of charges and in the salivary medium. In the first one, it was demonstrated that the surface roughness has a noticeable influence on the multi-species biofilm growth. It supposes to mean, that the formation of the biofilm and low values of surface roughness are linked. Perfect environment in the initial microbial adhesion have retentive areas. Onto the areas, pH can reduce, which can induce corrosion on the dental materials, on metallic surface (Prado A. M., 2016). In the second, it was reveal that dynamic loading of dental implants has an effect on bacterial penetration down to the threaded portion of the FAI. The precision of the fit between the implant and the abutment, and the torque forces will influence the degree of bacterial penetration to the internal parts, under non-loading conditions. The main factors for determining the stability of the abutment are the tightening and loosening torques of the abutment screw. The tightening which results in a symmetric stress distribution in the connexion, and in titanium alloy abutment produce abrasion and plastic deformation (Prado A. M., 2016). In Smith N. A. (2014), charges conditions are more studied. In the titanium abutment-implant system, the smallest micro gap was found, with a size of 2 μm, and the biggest of 26.7 μm in the zirconia abutment-implant system. Even in the smallest case, each bacterium is really smaller then this size. For each implant systems and abutment types, there is a size of micro gap and in which of them they are different bacterial leakages. Do Nascimento C. et alii. (2007) and Berberi A. et alii. (2014), are more concentrated on the salivary medium, a big variety of microorganisms are capable to penetrate along the implant. In witch, there is *Bacteroides*, *Fusobacterium*, *Peptostreptococcus* micros, are responsible for peri-

implantitis. It have been proved that the design of the internal connexion has an important role in inducing leakage results, and that Astra Tech Implant System has a real compactness and firmness compared to others (Berberi A. et alii., 2014).

According to Deborah Meleo (2012), conic connections are more geometrically suitable than a flat one, Tube-in-tube conical shapes have a better seal against bacteria and best mechanical stability. In screwed retained implant-abutment connections, bacteria penetrate in vivo and in vitro inside the internal hollow portion. The Morse cone seems to have a better seal than others. The length of the join could help to the bacterial penetration. Cone Morse taper connections seem to be more tight and stable than flat-to-flat connections (Scarano A. et al., 2015). With or without loading conditions, bacterial leakage can be avoided on Cone Morse taper implant (Tripodi et al. sit in Khorshidi et alii., 2016). For the bone volume subjected to low stress magnitude, compared to external hexagon implants, the Morse taper implant systems revealed better biomechanical behavior (Macedo J. et al. 2017).

At the end, the ideal would be to have no gaps. The most common type gap are vertical and horizontal gaps. Horizontal are always bigger than vertical and it can result in less stability and a larger area for bacterial accumulation (Kano S. C. et alii. 2007).

V. Conclusion

This systematic review can conclude that:

- The seal at the interface reduces significantly intraoral bacteria penetration.
- It does not exist, an ideal fixture-abutment system.
- Uniform protocols are needed for a better future evaluation.
- The manufacturer-recommended torque value must be respected; smaller micro gaps are observed in higher torque value.
- The connection geometry to the fixture-abutment complex influences the mechanical properties of an implant system.
- More studies are required to achieve for knowledge of a better geometric shape of implants systems.

VI. References bibliographic

Berberi A. et alii. (2014). Leakage evaluation of original and compatible implant–abutment connections: In vitro study using Rhodamine B, *Journal of Dental Biomechanics*, 5: pp. 1–7.

Do Nascimento C. et alii. (2008). Bacterial leakage along the implant–abutment interface of premachined or cast components, *International Journal of Oral and Maxillofacial Surgery*, 37: pp. 177–180.

Do Nascimento C. et alii. (2012). Bacterial leakage along the implant– abutment interface: culture and DNA Checkerboard hybridization analyses, *Clinical Oral Implants Research,* 23: pp. 1168–1172.

El Haddad E. et alii. (2016). Implant-abutment leaking of replace conical connection nobel biocare implant system. An in vitro study of the microbiological penetration from external environment to implant-abutment space, *Oral and Implantology Journal*, 9(2): pp. 76–82.

Geng Y. et alii. (2008) Application of the Finite Element Method in Implant Dentistry, advanced topics, In Science And Technology In China, chapiter 4 “Finite Element Modelling in Implant Dentistry” pp. 81-89.

Kano S.-C. et alii. (2007). Classification System to Measure the Implant-Abutment Microgap, *The International Journal of Oral & Maxillofacial Implants,* 22: pp. 879–885.

Khorshidi H. et alii (2016). In Vitro Evaluation of Bacterial Leakage at Implant-Abutment Connection: An 11-Degree Morse Taper Compared to a Butt Joint Connection, *International Journal of Biomaterials*, vol. 2016, pp. 1–5.

Koutouzis T. et alii. (2011). Bacterial Colonization of the Implant– Abutment Interface Using an In Vitro Dynamic Loading Model, *Journal Periodontol*, 82: pp. 613-618.

Koutouzis T. et alii. (2014). The Effect of Dynamic Loading on Bacterial Colonization of the Dental Implant Fixture–Abutment Interface: An In Vitro Study, *Journal of Oral Implantology*, 40(4): pp. 432-437.

Macedo J.P. et alii. (2017). Finite element analysis of stress extent at peri-implant bone surrounding external hexagon or Morse taper implants, *Journal of the Mechanical Behavior of Biomedical Materials, 71*, pp. 441–447.

Meleo D. et alii. (2012). Fixture-abutment connection surface and micro-gap measurements by 3D micro-tomographic technique analysis, *Ann Ist Super Sanità*, 48 (1): pp. 53-58.

Microscope Detective. [On line]. Available with http://www.microscope-detective.com/contact.html#sthash.MnUqqDvQ.dpbs [consulted the eight of June 2017]

Nano Science Instruments (2017). [On line]. Available with http://www.nanoscience.com/technology/sem-technology/. [Consulted the eight of June 2017].

Pereira J. et alii. (2015). Relation between Dental Implant Joint Surfaces and Biofilm Formation, *Dentistry*, 5, pp. 296.

Prado M. A. et alii. (2016). Biofilm Affecting the Mechanical Integrity of Implant-Abutment Joints, *International Journal of Prosthodont,* 29, pp. 381–383.

Rack A. et alii. (2010). In vitro synchrotron-based radiography of micro-gap formation at the implant–abutment interface of two-piece dental implants. *Journal of Synchrotron Radiation*, 17, pp. 289–294.

Scarano A. et alii. (2016). Implant-Abutment Contact Surfaces and Microgap Measurements of Different Implant Connections Under 3-Dimensional X-Ray Microtomography, *Implant Dentistry*, 25(5), pp. 656-662.

Scarano A. et alii. (2016). Evaluation of Microgap With Three- Dimensional X-Ray Microtomography: Internal Hexagon Versus Cone Morse, *The Journal of Craniofacial Surgery*, 27: pp. 682–684.

Smith Nicole A. et al. (2014). Evaluation of the sealing capability of implants to titanium and zirconia abutments against Porphyromonas gingivalis, Prevotella intermedia, and Fusobacterium nucleatum under different screw torque values, *The Journal of Prosthetic Dentistry*, 112: pp. 561-567.

Tripodi D. et alii. (2012). An In Vitro Investigation Concerning the Bacterial Leakage at Implants With Internal Hexagon and Morse Taper Implant-Abutment Connections, *Implant Dentistry*, 21(4), pp.335-339.

University of California Santa Barbara website , (2017). [On line]. Available with http://www2.bren.ucsb.edu/~keller/courses/esm223/Spectrometer_analysis.pdf [Consulted the eight of June 2017].

VII. Annexes

Tabela 1:

Do Nascimento C. et alii. 2008.	2 types of abutments : premachined and cast , 10 implants for each. Outside contamination – 1 for each groups Remaining for experiment – 9 of each groups Bacterial leakage/ incubation duration (h) – 1 for premachined at 24h, 1 from cas tat 48h.
Koutouzis T. et alii. 2014	The conditions of charges are : 50 N , 30°, 500 000 cycles. All positive controls developed multiple CFU , in group 1 and 2. 1 of the 20 implants of group 1 and 4 of the 10 implants of group 2 had FAI microgaps colonized by E. Coli. It shows that for the samples taken from the threaded portion of the abutment , group 1 exhibited significantly lower numbers of CFU for E. Coli than group 2.
Khorshidi H. Et alii. 2016	Present of number of sample in each groups was 10. In butt joint connections samples : 7 out of 10 observed singns of leakage ð first on the 3rd day, second on the 8th day and five during the 13th day. Some colonies were separated 2 days before turbidity.
El Haddad E. et alii. 2016.	Bacterias are found in the inner side, in all implants with a median percentage of 10.9%. In internally and externally cases, bacterias grew during the first 48 hours and then dye, potentialment because of nutrient consumption. But the difference between both is significant - outside quantification it a lot more example N1 is 484 597 vs 3 519.
Smith Nicole A et alii. 2014	Microgaps at zirconia abutment-interface: - 14.3 ±4.4 with a torque of 20Ncm - 10.5 ± 1.4 with a torque of 35Ncm Microgaps at titanium abutment-interface: - 5.3 ± 1.9 with a torque of 20Ncm - 5.2 ± 2.2 with a torque of 35Ncm The screw torque has no effect on titanium A/I (P = 0.875) but has effect on ziconia A/I (P=0.017). For the last group, microgaps are smaller when the torque is 35Ncm.

M. Prado A. et alii. 2016	Mean removal torques values are lower in group B (contact with biofilm) than in group A (free from biofilm). plastic deformation and abrasion marks are detected. On morse taper connection surface, mean roughness are higher in group B than group A.
Pereira J. et alii. 2015.	The biomass density is higher on SLA titanium surface than in polished titanium surface for 24h of growth. It's mean that the roughness of the surface is influenceable on the ability of multi-species biofilm formation. The number of CFU of multi-species is higher on SLA surfaces than on polished Ti squere surface.
Kano S.-C. et alii. 2007.	For the vertical misfit, no significant difference were found, but for the horizontal misfit, machined titanium abutments presented significantly higher horizontal misfit compared to other groups ($P < .001$). cast NiCr abutments had significantly smaller horizontal misfit than premachined caston abutments ($P < 0.001$). Like classification system for all sites measured at the implant-abutment interface - 23% had an ideal relationship, - 34% had a horizontal discrepancy - 4% had a vertical discrepancy - 39% had both vertical and horizontal discrepancies.
Macedo J.P. et alii. 2017.	At the cortical bone surrounding the external hexagon implant joint for both loading conditions, axial and oblique shows the highest values of von Mises stress. At the most coronal section of the cortical bone and also at the interface between cortical and trabecular bone are regions were is the highest stresses concentrated. A higher volume of stressed peri-implant trabecular bone : Morse taper implant than around external hexagon implant.
Scarano A. et alii. 2016.	Group I: Presence of a lot of gaps with a size of 52.3 ± 4.5 mm at the screw-abutment interface, 50 ± 5.2 mm at the internal portion of the implant and the threads of the screw. There is no perfect adaptation of the implant and abutment. The internal volume was 9.304 mm^3. Group II : Gaps with 2 to 4 mm size are presents. but no detectable gaps at the conical connection between implant and abutment. The internal space volume was 5.014 mm^3

	Group III : there is no microgap , or detectable separation, or line visible. The internal space volume was 5.231 mm^3. Group IV : The contact between implant and abutment was perfect, its extension was 560 mm. Gaps are present with a maximum size of 235 mm. The internal volume was 6.396 mm^3.
Rack A. et alii. 2010.	In all stages with different values for the applied force F (nominal 0 N, 30 N, 60 N, 100 N), a micro-gap between the abutment and the implant can be detected. - F = 0 N : shows a micro-gap , the size is > 1 mm and <<4 mm - F = 30 N : the micro-gap becomes clearly visible, - F = 60 N : the size is more ou less 11 mm, the surfaces of the implant and the abutment are almost parallel - F = 100 N, the micro-gap shows a non-parallel shape: at the upper end, the gap's size is ± 22 mm, at the lower end the size is ± 15 mm
Berberi A. et alii. in 2014.	The calibration curve absorbance is linear. At 1 and 48 h, leakage volume and percentage of each combination are measured: Group A- 1.48% (SD) = 0.0022 µL) 5.56% (SD = 0.0074 µL); Group B- 27.92% (SD = 0.0382 µL), 39.80% (SD = 0.0192 µL); Group C - 10.59% (SD = 0.0116 µL), 19.31% (SD = 0.0193 µL); Group D- 51.03% (SD = 0.0625 µL), 66.71% (SD = 0.0725 µL). $0.001 < P < 0.0001$. Standard deviation (SD) - very low.

Table 1: Results of all protocols.

Do Nascimento C. et alii. 2008; Koutouzis T. et alii. 2014 ; Khorshidi H. Et alii. 2016 ; El Haddad E. et alii. 2016 ; Smith Nicole A et alii. 2014 ; M. Prado A. et alii. 2016 ; Pereira J. et alii 2015 ; Kano S.-C. et alii. 2007; Macedo J.P. et alii. 2017; Scarano A. et alii. 2016 ; Rack A. et alii. 2010; Berberi A. et alii. 2014.