UNESP - Universidade Estadual Paulista

"Júlio de Mesquita Filho"

Faculdade de Odontologia de Araraquara

Felipe Eduardo Pinotti

Avaliação da osseointegração de implantes com superfície hidrofílica em ratos com comprometimento sistêmico

Araraquara

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Tese apresentada á Universidade Estadual Paulista (Unesp), Faculdade de Odontologia, Araraquara para obtenção do título de Doutor em Odontologia, na área de Periodontia.

Orientadora: Profª. Drª. Rosemary Adriana Chiérici Marcantonio

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Felipe Eduardo Pinotti

Avaliação da osseointegração de implantes com superfície hidrofílica em ratos com comprometimento sistêmico

Comissão Julgadora

Tese para obtenção do grau de doutor em periodontia

Presidente e Orientador: Profª. Drª. Rosemary Adriana Chiérici Marcantonio

2° Examinador: Profª. Drª. Daniela Barcelos Zandim – FOAr - Brasil

3° Examinador: Prof. Dr. Rubens Spin Neto – Universidade Aarhus – Dinamarca

4° Examinador: Prof. Dr. Humberto Osvaldo Schwartz Filho – UFPR - Brasil

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DADOS CURRICULARES

Felipe Eduardo Pinotti

NASCIMENTO: 22.06.1992 – Araraquara SP

FILIAÇÃO: Wilson Francisco Pinotti Junior

Maria Teresinha Gomes da Silva Pinotti

2011 – 2015: Curso de Graduação em Odontologia

Faculdade de Odontologia de Araraquara – UNESP

2016 – 2018: Curso de Pós – Graduação em Odontologia – Área de Concentração: Periodontia

Nível: Mestrado

Faculdade de Odontologia de Araraquara – UNESP

2017 – 2019 : Curso de especialização – Periodontia

Faculdade de Odontologia de Araraquara – UNESP

2016 – Atual: Curso de Pós – Graduação em Odontologia – Área de Concentração: Periodontia

Nível: Doutorado

Faculdade de Odontologia de Araraquara – UNESP

DEDICATÓRIA

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À,

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“Seja você quem for, seja qual for a posição social que você tenha na vida, a mais alta ou a mais baixa, tenha sempre como meta muita força, muita determinação e sempre faça tudo com muito amor e com muita fé em Deus, que um dia você chega lá. De alguma maneira você chega lá.” Ayrton Senna.

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RESUMO

O objetivo deste estudo foi de avaliar o efeito de uma superfície modificada por jateamento e ataque ácido e mantida em solução isotônica (Superfície Hidrofílica) em comparação a uma superfície usinada sobre a osseointegração de implantes em animais com comprometimento sistêmico. Foram utilizados 128 ratos que foram divididos em 2 estudos. No primeiro estudo foram utilizados 64 ratos, que foram divididos em 4 grupos e avaliados em dois períodos experimentais (15 e 45 dias), com 8 animais em cada grupo (n=8). Os grupos foram divididos de acordo com o tipo de implante que foi instalado na tíbia dos animais e a condição sistêmica dos mesmos: Grupo Hidrofílico/Saudável (HS); Grupo Hidrofílico/Diabético (HD); Grupo Usinado/Saudável (US); Grupo Usinado/Diabético (UD). No segundo estudo foram utilizados 64 ratos, que foram divididos em 4 grupos com e avaliados em dois períodos experimentais (15 e 45 dias), com n=8. Os grupos foram divididos como o primeiro estudo: Grupo HS, Grupo Hidrofílico/Nicotina (HN), Grupo US e Grupo Usinado/Nicotina (UN). Em ambos os trabalhos foram realizadas análises: biomecânicas, histométricas e microtomográficas. No primeiro estudo foi verificado que os implantes com superfície hidrofílica apresentaram maiores torques de remoção, quantidade de osso ao redor dos implantes e maiores valores de %BIC e %BBT em animais normo e hiperglicêmicos. No segundo estudo os animais submetidos a administração de nicotina apresentaram melhores níveis de osseointegração quando os implantes utilizados apresentaram superfície hidrofílica do que superfície usinada. Conclui-se que implantes com superfície hidrofílica aceleram a osseointegração em animais com comprometimento sistêmico.

Palavras-chave: Osseointegração. Hiperglicemia. Nicotina

Pinotti FE. Evaluation of the osseointegration of implants with hydrophilic surfaces in rats with systemic involvement. [Tese de doutorado] Araraquara: Faculdade de Odontologia da UNESP; 2021

ABSTRACT

The aim of this study was to evaluate the effect of a surface modified by sandblasting and acid etching and kept in isotonic solution (Hydrophilic Surface) in comparison to a machined surface on the osseointegration of implants in animals with systemic involvement. 128 rats were used and divided into 2 studies. In the first study, 64 rats were used, which will be divided into 4 groups and evaluated in two experimental periods (15 and 45 days), with 8 animals in each group (n=8). The groups were divided according to the type of implant that was installed in the animals' tibia and their systemic condition: Hydrophilic/Healthy Group (HS); Hydrophilic/Diabetic Group (HD); Machined/Healthy Group (US); Machined/Diabetic Group (UD); In the second study, 64 rats were used, which were divided into 4 groups, and which were evaluated in two experimental periods (15 and 45 days), with n=8. The groups were divided according to the type of implant that was installed in the animals' tibia and their systemic condition: HS Group, Hydrophilic/Nicotine Group (HN), US Group and Machined/Nicotine Group (UN). In both studies, analyzes were performed: biomechanical, histometric, and microtomography. In the first study, is was found that implants with a hydrophilic urface had higher removal torque, amount of bone around the implants and higher values of %BIC and %BBT in normal and hyperglycemic animals. In the second study animals submitted to nicotine administration showed better levels of osseointegration when used implants with a hydrophilic surface than a machined surface. It is concluded that implants with a hydrophilic surface accelerate osseointegration in animals with systemic involvement.

Key-words: Osseointegration. Hyperglycemia. Nicotine

SUMÁRIO

1 INTRODUÇÃO

A utilização de implantes osseointegrados tem se tornado um dos principais tratamentos para o edentulismo, sendo atualmente indicada a diversas situações clínicas, justamente por conta da sua alta taxa de sucesso no tratamento reabilitador[1, 2] porém, alguns fatores provenientes do hospedeiro podem interferir diretamente na osseointegração dos implantes, dentre eles podemos destacar doenças sistêmicas como por exemplo a Diabetes Mellitus, e também, condições ambientais como por exemplo, o fumo.

A diabetes mellitus tipo é 2 uma doença sistêmica com sérias complicações que afeta a qualidade de vida dos pacientes. Uma dessas complicações está relacionada com a saúde oral, onde tem sido comprovado que a periodontite é uma das principais complicações do diabetes[3, 4]. Isso põe esses pacientes sob risco de perda dentária que pode ser posteriormente reposta com implantes dentários[5]. Além disso, tem sido relatado que a osseointegração dos implantes pode ser alterada em estados hiperglicêmico[6]. Estudos que avaliaram a taxa de osseointegração de implantes em pacientes diabéticos foram semelhantes em paciente com bom controle glicêmico, em comparação a pacientes com um mau controle glicêmico[7, 8]. Contudo, estes estudos também mostraram que a estabilidade do implante durante o período de cicatrização possui relação direta com o nível glicêmico dos pacientes, sendo que o paciente que possuíam pior controle glicêmico apresentavam implantes com menor estabilidade. Estudos pré-clinicos tem demonstrado que animais com diabetes apresentam um retardo na osseointegração em comparação a animais controle sem doença[9, 10], e estudos clínicos tem

demonstrado que o diabetes mellitus é um fator de risco para perda de implantes dentários[11, 12].

A nicotina presente no tabaco está entre as mais nocivas drogas à saúde humana, diversos estudos já relataram que a mesma causa uma série de alterações, tanto locais, como sistêmicas no organismo, entre elas, inibição de macrófagos, diminuição da quimiotaxia de células inflamatórias, diminuição da agregação plaquetária, que prejudicam a resposta imuno-inflamatória do hospedeiro[13-15]. Sabe-se que a nicotina também interfere na atividade de fibroblastos e osteoblastos, gerando uma menor formação óssea ao redor dos implantes, piorando sua osseointegração[16]. Um estudo pré-clínico mostrou que a nicotina interfere diretamente na osseointegração de enxertos autógenos, já que ratos sistematicamente modificados com nicotina, tiveram uma pior cicatrização do enxerto realizado em relação a ratos controle[17]. Uma revisão sistemática avaliou o contato osso implante de implantes colocados em animais que tiveram a injeção de nicotina, em comparação a animais saudáveis, como resultado, os autores mostraram que a nicotina compromete a osseointegração de implantes[18].

Uma abordagem utilizada para acelerar o processo de osseointegração é a utilização de implantes com superfície modificada[19]. Essas modificações físico-químicas de superfície visam aumentam a estabilidade do coágulo que serve de guia para o início do processo de osseointegração, promovendo um aumento da propriedade de osteocondução[20]. Dentre as características de superfície que aumentam a osteocondução dos implantes destaca-se as propriedades de topografia, composição química e molhabilidade[21].

A superfície modificada por jateamento e ataque ácido e mantida em solução de cloreto de sódio tem demonstrado acelerar e melhorar o processo de osseointegração[20, 22] e esses eventos tem sido relacionados a manutenção das camadas de óxidos promovido pela presença da solução de cloreto de sódio em contato com a superfície até o momento da instalação dos implantes, o que mantem a camada de óxidos e aumenta a molhabilidade dessa superfície[20]. Pesquisas tem demonstrado a superioridade dessa superfície em comparação a outros tipos de modificação de superfície que possuem apenas o jateamento e o ataque ácido[20, 22].

Baseado nos resultados na literatura que demonstram a influência do Diabetes melitus e da nicotina na osseointegração, achamos importante avaliar se a superfície modificada por jateamento e ataque ácido e mantida em solução de cloreto de sódio interfere na osseointegração de implantes colocados em animais com comprometimento sistêmico.

2 PROPOSIÇÃO

O objetivo geral desse estudo foi de avaliar o efeito de uma superfície de implante hidrofílica sobre a osseointegração em animais com comprometimento sistêmico.

Artigo 1: Implantes com superfície hidrofílica equiparam a osseointegração de implantes em ratos normo e hiperglicêmicos.

Os objetivos específicos desse estudo foram de avaliar em animais diabéticos, o efeito da superfície hidrofílica sobre:

1) O torque de remoção dos implantes;
2) A formação óssea ao redor dos Implantes;
3) O contato osso implante.

Artigo 2: Avaliação da osseointegração de implantes com superfície hidrofílica em ratos com alta dosagem de nicotina no sangue.

Os objetivos específicos desse estudo foram de avaliar em animais com alta dosagem de nicotina no sangue, o efeito da superfície hidrofílica sobre:

4) O torque de remoção dos implantes;
5) A formação óssea ao redor dos Implantes;
6) O contato osso implante.

3 PUBLICAÇÕES

3 . 1 Artigo 1

Diabetes & Metabolism

Original paper: Implants with hydrophilic surfaces equalize the osseointegration of implants in normo- and hyperglycaemic rats

--Manuscript Draft--

Manuscript Number:	
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Corresponding Author:	Felipe Pinotti Araraquara, São Paulo BRAZIL
First Author:	Felipe Pinotti
Order of Authors:	Felipe Pinotti
	Mauricio Aroni
	Guilherme Oliveira
	Rosemary Adriana Chiérici Marcantonio
	Elcio Marcantonio Jr
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Abstract:	Aim The purpose of this study was to evaluate the effect of a surface modified by blasting and acid attack and maintained in an isotonic solution (Hydrophilic Surface, Acqua, Neodent, Brazil) compared to a machined surface on osseointegration in normo- and hyperglycaemic animals. Material and methods Sixty-four animals were allocated into 4 groups with 16 animals each, and they were subdivided into two experimental periods (15 and 45 days), with 8 animals in each group. The groups were divided according to the type of implant that was installed in the animals' tibia and the animals' systemic condition: CM - Machined implants placed in control animals; CH - Hydrophilic implants placed in control animals, CH - Machined implants placed in control animals; HH- Hydrophilic implants installed in animals with hyperglycaemia. The following analyses were performed: biomechanical (removal torque), microtomographic (evaluation of the bone volume around the implants- BV/TV), and histomorphometric (evaluation of bone-implant contact BIC% and of the bone formation area between the threads BBT%). Results It was found that the implants with hydrophilic surfaces presented higher removal torques and quantities of BV/TV% and higher BIC% and BBT% values in normo- and hyperglycaemic animals. The results of this study indicated that the hydrophilic surface accelerates the osseointegration process (~ 15% BIC/BBT at 15-day period), especially in animals with hyperglycaemia. Conclusion The hydrophilic surface equalled the osseointegration between normo- and hyperglycaemic animals, reversing the negative potential of hyperglycaemia on the osseointegration process.

– Artigo submetido no Periódico: Diabetes & Metabolism

Original paper: Implants with hydrophilic surfaces equalize the osseointegration of implants in normo- and hyperglycaemic rats

Felipe Eduardo Pinotti[a], Mauricio Andrés Tinajero Aroni[b], Guilherme José Pimentel Lopes de Oliveir**a**[c], Elcio Marcantonio Junior[a], Rosemary Adriana Chiérici Marcantonio[a]

[a] São Paulo State University (Unesp), School of Dentistry, Araraquara, Brazil.

[b] San Francisco de Quito University (UFSQ), Quito – Equador.

[c] School of Dentistry, Universidade Federal de Uberlândia, Uberlândia, Brazil

Correspondence:

Rosemary Adriana Chierici Marcantonio

Humaitá St., 1680. Zip-Code 14801-130, Araraquara, SP. Brasil

Phone: (16) 33016376/Fax: (16) 33016369

e-mail: adriana@foar.unesp.br

Abstract

Aim. - The purpose of this study was to evaluate the effect of a surface modified by blasting and acid attack and maintained in an isotonic solution (Hydrophilic Surface, Acqua, Neodent, Brazil) compared to a machined surface on osseointegration in normo- and hyperglycaemic animals.

Material and methods. - Sixty-four animals were allocated into 4 groups with 16 animals each, and they were subdivided into two experimental periods (15 and 45 days), with 8 animals in each group. The groups were divided according to the type of implant that was installed in the animals' tibia and the animals' systemic condition: CM - Machined implants placed in control animals; CH - Hydrophilic implants placed in control animals, CH - Machined implants placed in control animals; HH- Hydrophilic implants installed in animals with hyperglycaemia. The following analyses were performed: biomechanical (removal torque), microtomographic (evaluation of the bone volume around the implants- BV/TV), and histomorphometric (evaluation of bone-implant contact BIC% and of the bone formation area between the threads BBT%).

Results. - It was found that the implants with hydrophilic surfaces presented higher removal torques and quantities of BV/TV% and higher BIC% and BBT% values in normo- and hyperglycaemic animals. The results of this study indicated that the hydrophilic surface accelerates the osseointegration process (~ 15% BIC/BBT at 15-day period), especially in animals with hyperglycaemia.

Conclusion. - The hydrophilic surface equalled the osseointegration between normo- and hyperglycaemic animals, reversing the negative potential of hyperglycaemia on the osseointegration process.

Keywords: Diabetes, osseointegration, implant surface

Introduction

The use of osseointegrated implants is one of the main treatments for edentulism and is currently indicated for several clinical situations, precisely because of its high success rate in rehabilitation treatment [1, 2]; however, some factors acquired by the host during its lifetime can directly interfere with implant osseointegration (e.g., systemic diseases).

Diabetes mellitus is a systemic disease with serious complications that affects the quality of life of patients. One of these complications is related to oral health, since it has been reported that periodontitis is one of the main complications of diabetes [3, 4]. Therefore, these patients have a higher risk of tooth loss that can later be treated with the installation of dental implants [5]. Although it has also been reported that the process of implant osseointegration can be altered in hyperglycaemic states [6], studies that evaluated the rate of implant osseointegration in diabetic patients were similar for patients with good glycaemic control compared to patients with inadequate glycaemic control [7, 8].

However, other studies have demonstrated an adverse effect of diabetes on the osseointegration process, especially when the glycaemic control of the host is inadequate. Preclinical studies have shown that hyperglycaemic animals have a delay in osseointegration compared to normoglycaemic animals [9-12], and clinical studies have shown that diabetes mellitus is a risk factor for reducing the survival and success of dental implants [13-15].

The use of implants with modified surfaces is a good alternative to partially reverse the effect of systemic diseases on bone tissue by accelerating the osseointegration process [16]. These modified surfaces present some

physical and chemical modifications that will positively influence the stability of the blood clot, which is of paramount importance for the osseointegration process, thereby promoting an acceleration in the osteoconduction process [17]. Among the surface characteristics that interfere with the stability of the blood clot and consequently promote better osteoconduction of the implants, the topography, chemical composition and wettability deserve attention [18].

An implant surface modified by blasting and acid etching and then kept in sodium chloride solution has been widely studied and often demonstrates acceleration and improvement of the osseointegration process [17, 19]. A preclinical study showed that implants with a hydrophilic surface accelerated the minipig maxillary osseointegration process (49.30 vs. 29.42% at 2 weeks and 81.91 vs. 66.57% at 4 weeks) compared to hydrophobic implants [19]. A clinical study also showed that implants with hydrophilic surfaces enhance osseointegration compared with hydrophobic surfaces after 28 days of implant placement (48.3% vs. 34.2%) [20]. In addition, a preclinical study showed that implants with hydrophilic surfaces accelerated the osseointegration process in rat tibiae-grafted areas with different osteoconductive biomaterials [21], which are critical areas for the osseointegration process. Thus, the objective of this study was to evaluate the effect of an implant surface modified by blasting and acid etching and immersed in an isotonic solution (hydrophilic) compared to a machined surface on implant osseointegration in animals with induced hyperglycaemia. This study was performed to test the null hypothesis that implants with hydrophilic surfaces can match osseointegration in normo- and hyperglycaemic animals.

Methods

Ethics considerations, experimental design and groups

This study was approved by the Ethics Committee of Animal Use in Research (FOAr-UNESP - CEUA-44/2017 – ANEXO A). For the study, sixty-four male rats (*Rattus norvegicus*, albinus variation, Holtzman, of approximately 3 months of age, UNESP) were used. The animals were fed solid rat chow and had access to water *ad libitum* before and throughout the experimental period in an environment with controlled humidity, light and temperature cycles.

In this study, the effect of two implant surfaces on osseointegration in normo- and hyperglycaemic animals was evaluated. The 64 animals were randomly divided into 4 groups with 16 animals each, which were evaluated in two experimental periods (15 and 45 days), with 8 animals in each group. The groups were divided according to the type of implant that was installed in the tibia and the systemic condition of the animals: CM - Machined implants (Neodent®, Curitiba, PR, Brazil) placed in control animals; CH - Hydrophilic implants with the surface modified by oxide blasting and acid etching and then kept in sodium chloride solution (Surface ACQUA, Neodent®, Curitiba, PR, Brazil) placed in control animals, HM - Machined implants placed in hyperglycaemic animals; HH- Hydrophilic implants installed in hyperglycaemic animals.

Hyperglycaemia induction

To induce hyperglycaemia, after a 16-hour fasting period, except for water *ad libitum,* the animals were subjected to intraperitoneal administration of 50 mg/kg streptozotocin dissolved in citrate buffer pH 4.5 thirty days before the surgical procedure. The animals that were kept in a normoglycaemic state received a

saline injection intraperitoneally of the same volume. After 24 hours of induction, evidence of hyperglycaemia was obtained by analysing the glycaemic rate. Animals with glycaemia greater than 300 mg/dl were considered hyperglycaemic. Weekly glycaemic level assessments were carried out throughout the study to ensure this condition was maintained [22].

Surgical procedure

The animals were anaesthetized with an intramuscular injection of a combination of ketamine (Agener União Ltda, São Paulo, SP, Brazil -dosage of 0.08 ml/100 g of body mass) with xylazine (Rompum, Bayer SA, São Paulo, SP, Brazil - dosage of 0.04 ml/100 g of body weight). After confirmation that the animals were fully anaesthetized, the trichotomy was performed in the region of the animals' tibiae.

An incision of approximately 10 mm was made over the tibial tuberosity, and delicate tissue dissection was performed. Then, the surgical site was prepared for implant placement by means of a progressive sequence of drills (spear drill; 2.0 mm spiral drill - Neodent®; Curitiba, PR, Brazil) to accommodate a 4 x 2.2 mm titanium implant (implants with a machined surface or a hydrophilic surface- Acqua surface, Neodent®, Curitiba, PR, Brazil). The perforations were carried out with the aid of an electric motor, adjusted to 1200 rpm, under abundant irrigation with sterile saline solution. The implants were placed with the aid of a digital key (Hexagonal digital key 1.2 mm - Neodent, Curitiba, PR, Brazil) [21].

After the implant placement, the tissues were sutured internally with 5.0 resorbable thread (Vicryl Ethicon, Johnson & Johnson, São José dos Campos,

Brazil) and externally with 4.0 silk thread (Ethicon, Johnson & Johnson, São José dos Campos, Brazil). The animals received a single dose of penicillin associated with streptomycin at a dosage of 0.1 ml/kg of body mass (Multibiotic Small, Vitalfarma, São Sebastião do Paraíso, MG, Brazil) and ketoprofen at a dosage of 0.1 ml/kg of body mass (Ketoflex; Mundo Animal, São Paulo, Brazil) intramuscularly (APÊNDICE A).

At 15 and 45 days after the surgical procedure, the animals were sacrificed through an overdose of anaesthetic. The tibiae were randomly separated, one of which was used for microtomographic and histomorphometric analyses, while the other was used for biomechanical evaluation. This selection occurred randomly.

Biomechanical analysis

After euthanasia, the tibiae selected for biomechanical evaluation were stabilized in a small vice, and a hexagonal wrench was connected to both the implant and the torque wrench (Tohnichi, model ATG24CN-S, Tokyo, Japan - with a graduated scale of 0.05 N/cm, measuring the strength of 3 to 24 Ncm). Anti-clockwise movement was performed to unscrew the implant. The maximum peak required to move the implant was noted as the removal torque value (Ncm).

µCT analysis

The tibiae selected for this analysis were fixed in 4% paraformaldehyde for 48 hours and subsequently stored in 70% alcohol. To perform the microtomographic evaluation, the tibiae were scanned by a microtomograph (Skyscan, Aatselaar, Belgium) with the following parameters: camera pixel:

12.45; X-ray tube power: 65 kVP; X-ray intensity: 385 µA; integration time: 300 ms; filter: Al-1 mm; and voxel size: 18 μm^3. After scanning the samples, the images were reconstructed, spatially repositioned and analysed with specific software (NRecon, Data Viewer, CTAnalyser, Aatselaar, Belgium). As the implants placed on the animals' tibias did not have a cover screw, in some cases, bone formation occurred within the prosthetic platform, and since this did not interfere with the bone formation data around the implants, it was necessary to create a region of total interest (ROI) covering the entire region of the implant, which was defined as a circular region 0.5 mm around the entire diameter of the implant. This ROI was defined as the total volume (0.5 mm margin around the implants - 4.5 mm x 3.2 mm), and the second ROI was defined to remove the volume from the implant neck. With the results obtained in the two ROIs, it was possible to define the volume of bone tissue in the ROI using the formula Total Volume - Platform Volume = Volume of bone tissues. The threshold used in the analysis was 25-90 shades of grey, and the values of the volume of mineralized tissue around the implants were obtained as a percentage (BV/TV%). A trained examiner who was blinded to the experimental groups performed this analysis (FEP).

Histomorphometry analysis

After µCT scanning, the tibiae with the implants were processed by incubation in a graded series of alcohol dehydration (60 - 100%) and subsequently infiltrated and polymerized in light-cured resin (Technovit 7200 VLC, Kultzer Heraus GmbH & CO, Wehrheim, Germany). Using the cut, wear and polish system, the tibia-implant set was processed to obtain non-descaled histological cuts (Exakt Apparatebeau, Hamburg, Germany). The final cuts were

approximately 45 µm thick, stained with Stevenel blue associated with acid fuchsin and analysed. The sections were photographed under an optical microscope (DIASTAR - Leica Reichert & Jung products, Wetzlar, Germany) at 100X magnification. Histomorphometric evaluations were performed with ImageJ software (San Rafael, CA, USA). The percentages of bone-implant contact (% BIC) and bone area between turns (% BBT) were assessed separately in the first three threads of the implants. These analyses were performed by a blinded and trained examiner (FEP).

Statistical analysis

All data generated by the analyses in this study were distributed according to the normal distribution as determined by the Shapiro-Wilk normality test. The data were evaluated using the two-way parametric ANOVA test complemented by the Tukey test, which took into account the treatment variables and experimental period. GraphPad Prism 6 software (San Diego, CA, USA) was used for statistical analysis of this study, and all statistical tests were applied at a 5% significance level.

Results

Biomechanical analysis (Removal torque)

There was a progressive increase in the removal torque of the implants in all groups over the 45-day period compared to the 15-day period, with the exception of the HH group ($p < 0.001$). Implants with hydrophilic surfaces showed higher values of removal torque than implants with machined surfaces in the control group at the 15-day period ($p < 0.001$) and in the group of hyperglycaemic animals at both evaluation periods ($p < 0.001$). The removal

torque was higher in animals in the control group than in animals in the hyperglycaemic group ($p < 0.001$), with the exception of the HH group compared to the CH group at the 15-day time point. The mean and standard deviation of the biomechanical analysis of the implants in all groups and evaluation periods are shown in Table 1.

µCT analysis (BV/TV)

There was a progressive increase in BV/TV values (%) in all groups at the 45-day period compared to the 15-day period ($p < 0.001$). Implants with hydrophilic surfaces showed higher BV/VT values (%) than machined-surface implants in all groups ($p < 0.001$), with the exception of the CM group compared to the CH group at the 45-day time point. The hyperglycaemic animals had lower BV/TV values (%) than the animals in the control group ($p < 0.001$). The mean and standard deviation data from the BV/TV analysis (%) of the implants in all groups and evaluation periods are shown in Table 2.

Histomorphometry

BIC (%) and BBT (%)

There was a progressive increase in the BIC (%) and BBT (%) in all groups at the 45-day period compared to the 15-day period ($p < 0.001$). Implants with hydrophilic surfaces showed higher BIC (%) and BBT (%) values than machined surface implants in all groups ($p < 0.001$), with the exception of the CM group compared to the CH group at the 45-day period. The hyperglycaemic animals had lower BIC (%) and BBT (%) values than the control group animals with machined implants ($p < 0.001$); however, there were no differences in osseointegration between control and hyperglycaemic animals at the 45-day

period when implants with hydrophilic surfaces were placed. The mean and standard deviation of the histometric analysis of BIC (%) and BBT (%) in all groups and evaluation periods are shown in Tables 3 and 4, respectively.

Discussion

In general, it was verified in this study that implants with hydrophilic surfaces promoted improvement in the osseointegration process compared to implants with machined surfaces and that hyperglycaemia impairs the osseointegration process. However, an interesting finding in this study is that the use of hydrophilic implants equalized the osseointegration process between normo- and hyperglycaemic animals, which, clinically, could mean reducing the importance of glycaemic control for the osseointegration process when using implants with hydrophilic surfaces, which can increase the predictability of treatment of diabetic patients with dental implants.

It was observed in the biomechanical analysis that the secondary stability of the implants was time-dependent and that implants with a hydrophilic surface had higher removal torque values than implants with a machined surface, a finding similar to that observed in other preclinical studies [21]. In addition, implants installed in normoglycaemic animals showed greater removal torque at 45 days than implants installed in hyperglycaemic animals, even in animals where hydrophilic implants were installed. Although hydrophilic implants accelerate the osseointegration process [17, 20, 21], it is important to emphasize that the bone remodelling process is continuous and that long periods of hyperglycaemia are associated with deficiencies in bone turnover due to the influence of the action of the terminal products of advanced glycation

that impair the function of osteoblasts [23, 24], increase the bone tissue reabsorption process [25, 26] and impair the formation of bone tissue matrix [27].

In contrast, microtomographic and histometric analysis showed that there were no differences in osseointegration and bone formation around the implants between normal and hyperglycaemic animals that were subjected to the installation of hydrophilic surfaces. These similar results in normo- and hyperglycaemic animals specifically associated with the use of implants with a hydrophilic surface may be due to findings from previous studies that demonstrated that these surfaces have a high degree of wettability [17, 28], increase the expression of biomarkers for bone formation [28], increase the adhesion of undifferentiated mesenchymal cells [29], and stimulate differentiation and osteoblastic function [30]. These properties of the hydrophilic surfaces are related to the improvement in the osseointegration pattern found in preclinical [17, 19, 31] and clinical studies in healthy individuals [20, 32]. Furthermore, implants with hydrophilic surfaces accelerate secondary stability in humans [32, 33], which can speed up the completion of rehabilitation procedures.

The findings of this study may have valuable applicability in patients with diabetes mellitus, which affects a significant percentage of adults [34, 35] and is considered an important risk factor for the loss of implants in function or for osseointegration failures [36]; however, these effects are dose dependent. In fact, the study by Oates et al., 2009 [8] that assessed the stability of implants in healthy patients and type II diabetic patients after 2 and 6 weeks of implant placement showed that the patient's hyperglycaemia was directly correlated

with the osseointegration of the implant, and a higher rate of glycated haemoglobin was related to lower stability of the implant. It is important to note that after obtaining osseointegration, even if the implants are installed in compensated diabetic patients, the glycaemic control of these patients may vary during life, and invariably at some point, the implant surface may be challenged by the accumulation of terminal products from advanced glycation [37, 38].

This study has some limitations that must be considered when interpreting our data. Rough surfaces have been shown to accelerate osseointegration in general, and although preclinical studies demonstrated that hydrophilic surfaces improve the osseointegration process [19, 21], a clinical study by Khandewall et al., 2014 showed that hydrophilic and hydrophilic roughness implants were no different in achieving osseointegration in diabetic patients, and the real importance of hydrophilic surfaces in this context was limited in our study by the control group receiving an implant with an untreated machined surface [6]. Another factor to consider is that this study evaluated only the moment of implant installation until the osseointegration phenomenon was obtained. The challenge of this implant to survive under ongoing conditions of hyperglycaemia will continue because implants installed in diabetic patients have shown a greater possibility of being affected by peri-implant disease and have reduced success compared to implants installed in healthy patients [34, 39], and this information should be obtained regarding the use of implants with a hydrophilic surface in the future.

Conclusion

Implants with a hydrophilic surface were observed to accelerate the osseointegration process in normo- and hyperglycaemic animals. This surface was also able to equalize this phenomenon among normo- and hyperglycaemic animals, reversing the negative potential of hyperglycaemia on the osseointegration process.

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Tables

Table 1 Mean and standard deviation data from the removal torque analysis (Ncm2) of
the implants in all groups and evaluation periods.

Grupos x Período	15 dias	45 dias
SM	7.71 ± 1.38^{B}	$21.43 \pm 2.69^{A*}$
SH	17.63 ± 2.50^{A}	$21.88 \pm 2.80^{A*}$
HM	3.42 ± 2.07^{C}	$11.75 \pm 2.18^{B*}$
HH	14.29 ± 3.86^{A}	$17.25 \pm 2.49^{B}*$

Different letters represent differences between groups in each experimental period; * Higher torque than in the 15-day period - Two-way Anova complemented by the Tukey test ($p < 0.05$).

Table 2 Mean and standard deviation data from the BV / TV analysis (%) of the implants
in all groups and evaluation periods.

Grupos x Período	15 dias	45 dias
SM	48.12 ± 1.51^{C}	$70.21 \pm 0.92^{A*}$
SH	63.57 ± 2.12^{A}	$70.78 \pm 0.98^{A*}$
HM	31.33 ± 2.05^{D}	$61.11 \pm 1.06^{C*}$
HH	59.32 ± 2.62^{B}	$67.31 \pm 1.60^{B*}$

Different letters represent differences between groups in each experimental period; * Higher BV/ TV than in the 15-day period - Two-way Anova complemented by the Tukey test ($p<0.05$)

Table 3 Mean and standard deviation data from the BIC analysis (%) of the implants in
all groups and evaluation periods.

Grupos x Período	15 dias	45 dias
SM	40.49 ± 5.18^{B}	82.60 ± 4.37^{A*}
SH	54.26 ± 6.59^{A}	85.06 ± 5.52^{A*}
HM	21.93 ± 8.37^{C}	69.42 ± 5.52^{B*}
HH	38.35 ± 5.36^{B}	81.01 ± 2.19^{A*}

Different letters represent differences between groups in each experimental period; * Higher BIC
than in the 15-day period - Two-way Anova complemented by the Tukey test ($p < 0.05$)

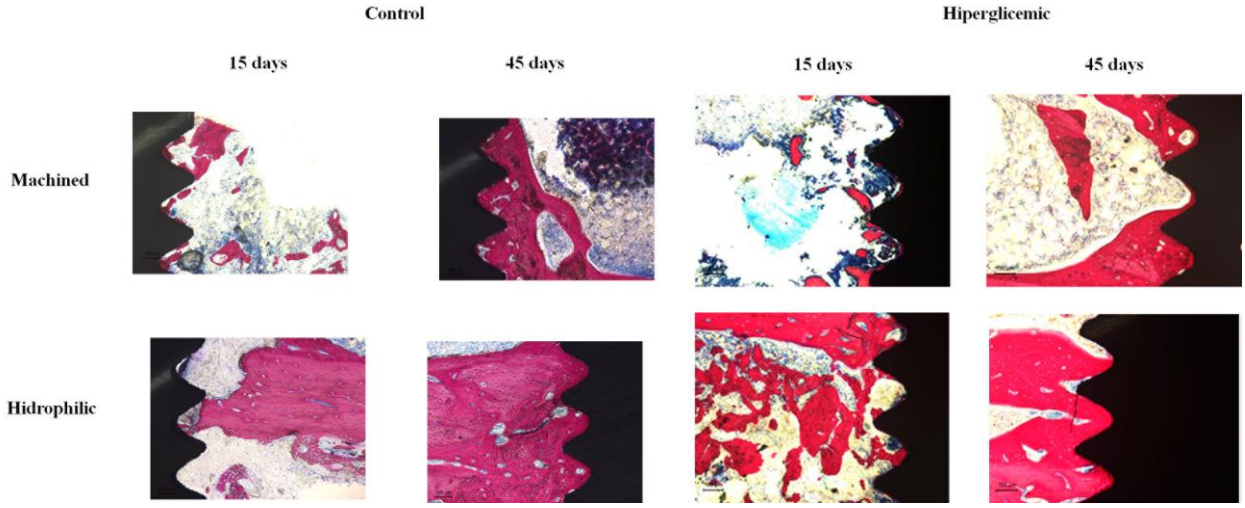
Table 4 Mean and standard deviation data from the BBT analysis (%) of the implants in
all groups and evaluation periods.

Grupos x Período	15 dias	45 dias
SM	34.79 ± 5.19^{B}	81.88 ± 3.10^{A}
SH	50.57 ± 5.28^{A}	83.22 ± 3.83^{A}
HM	11.01 ± 5.10^{C}	69.07 ± 4.32^{B}
HH	33.88 ± 4.98^{B}	79.21 ± 3.39^{A}

Different letters represent differences between groups in each experimental period; * Higher BIC than in the 15-day period - Two-way Anova complemented by the Tukey test ($p < 0.05$)

Figure Captions

Fig.1 Representative images of the histological noncalcified sections of all the groups
and periods of evaluation.



3. 2 Artigo 2

Brazilian Oral Research

Osseointegration of implants with hydrophilic surfaces in rats with high serum levels of nicotine.

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– Artigo submetido no periódico: Brazilian Oral Research

Original paper: Evaluation of the osseointegration of implants with hydrophilic surfaces in rats with high serum levels of nicotine.

Felipe Eduardo Pinotti[a], Mauricio Andrés Tinajero Aroni[b], Guilherme José Pimentel Lopes de Oliveira[c], Bruno Graciliano Silva[a], Elcio Marcantonio Junior[a], Rosemary Adriana Chiérici Marcantonio[a]

[a] São Paulo State University (Unesp), School of Dentistry, Araraquara, Brazil.

[b] San Francisco de Quito University (UFSQ), Quito – Equador.

[c] School of Dentistry at the Universidade Federal de Uberlândia, Brazil

Author for correspondence:

Rosemary Adriana Chierici Marcantonio

Humaitá St., 1680. Zip-Code 14801-130, Araraquara, SP. Brasil

Phone: (16) 33016376 / Fax: (16) 33016369

e-mail: adriana@foar.unesp.br

Abstract

The aim of this study was to evaluate the effect of a surface modified by sandblasting and acid etching and maintained in an isotonic solution (Hydrophilic Surface, Acqua, Neodent, Brazil), compared to that of a machined surface, on osseointegration in animals subjected to the administration of nicotine. Sixty-four rats were used and divided into 4 groups according to the type of implant they received and whether nicotine was administered: MH - Installation of machined implants in healthy animals; MN - Installation of machined implants in animals subjected to nicotine administration; HH - Installation of implants with hydrophilic surfaces in healthy animals; and HN - Installation of implants with hydrophilic surfaces in animals subjected to nicotine administration. The animals were euthanized 15 and 45 days after implant placement (n = 8). Osseointegration was assessed by means of biomechanical analyses (removal torque), microtomography (volume of bone around the implants- %BV/TV) and histomorphometry (bone-implant contact -%BIC and bone area between implant threads -%BBT). Implants with hydrophilic surfaces showed higher values of removal torque, %BV/TV, %BIC, and %BBT than implants with machined surfaces. Animals subjected to nicotine administration showed better levels of osseointegration when the implants used exhibited a hydrophilic surface.

Keywords: Implant surface, osseointegration, smoke.

Introduction

Oral rehabilitation with osseointegrated implants has achieved good outcomes in several clinical conditions regardless of the size of the rehabilitation [1, 2]; however, some host factors may directly interfere with host bone tissue metabolism, which can negatively affect the implant osseointegration process and impair long-term maintenance of the implants [3-5].

Smoking is one of the most important risk factors related to tooth loss [6, 7]. Previous clinical studies have shown that smokers are approximately 2 to 3.6 times more likely to lose teeth [6, 8, 9]. Thus, this population would have a greater need to replace lost dental elements with osseointegrated implants. However, this same habit is also related to problems in achieving good osseointegration, as well as in relation to the host's resistance to the microbial challenge that can result in peri-implant diseases [10, 11]. These outcomes are related to the effects of smoking on the inhibition of macrophages, decreased chemotaxis of inflammatory cells, and decreased platelet aggregation, which impair the host's immune-inflammatory response [12-14].

Among the numerous toxic substances present in cigarettes, nicotine stands out for being the substance related to addiction among its consumers [15], as well as to important changes related to the healing of tissues [16, 17]. Nicotine interferes with the activity of fibroblasts [17] and osteoblasts [16], generating less bone formation around the implants and decreasing their osseointegration [18].

One approach used to accelerate the osseointegration process in individuals with challenging systemic conditions is the modification of the

implant surfaces [19]. These physical-chemical surface modifications aim to increase the stability of the blood clot that serves as a guide for the beginning of the osseointegration process, promoting an increase in their osteoconduction properties [20]. A surface modified by sandblasting and acid etching and maintained in sodium chloride solution has been shown to accelerate and improve the osseointegration process [20-22]; these events have been related to the maintenance of the oxide layers promoted by the presence of the sodium chloride solution being in contact with the surface until the moment of implant installation, which maintains the oxide layer and increases the surface wettability [20].

Thus, the objective of this study was to evaluate the effect of an implant surface modified by sandblasting, acid etching and maintained in an isotonic solution (hydrophilic) compared to a machined surface implant on osseointegration in animals subjected to nicotine administration.

Materials and methods

Study design and ethical considerations

This study was approved by the Ethics Committee of Animal Use in Research (FOAr-UNESP - CEUA-44/2017 – ANEXO B). In this study, sixty-four male rats (*Rattus norvegicus*, albinus variation: Holtzman) of approximately 3 months of age, with a body mass between 200-300 grams, were kept in the Vivarium of the Faculty of Dentistry of UNESP in Araraquara (FOAr-UNESP). The animals were fed solid commercial rat chow and had access to water *ad libitum* before and throughout the experimental period in an environment with controlled water, light and temperature.

The 64 animals were randomly divided into 4 groups with 16 animals each, which were evaluated in two experimental periods (15 and 45 days), with 8 animals in each group. The groups were divided according to the type of implant that was installed in the tibiae and whether the animals were subjected to challenge with nicotine: MH: Systematically healthy animal subjected to installation of an implant with a machined surface (Neodent®, Curitiba, PR, Brazil); MN: Animal received an implant with a machined surface (Neodent®, Curitiba, PR, Brazil) and was subjected to nicotine challenge; HH: Systematically healthy animal subjected to implant installation with a hydrophilic surface created by sandblasting and acid etching and maintained in a sodium chloride solution (ACQUA Surface, Neodent®, Curitiba, PR, Brazil); HN: Animal received an implant with a hydrophilic surface (ACQUA Surface, Neodent®, Curitiba, PR, Brazil).

Nicotine administration

Nicotine administrations were performed through daily subcutaneous applications every 12 hours in the dorsal region of the animals, starting 30 days before the surgical procedure. The animals received a diluted solution of nicotine of 5 mg/ml, which was administered a dose of 3 ml/kg (Okamoto et al., 1994). The animals in the control group received administrations of saline solution at the same frequency as the animals subjected to the challenge with nicotine. The nicotine and saline solution administrations were maintained until the animals were euthanized.

Surgical Procedure

The animals were anaesthetized by a combination of ketamine (Agener União Ltda, São Paulo, SP, Brazil) at a dosage of 0.08 ml/100 g of body mass with xylazine (Rompum, Bayer SA, São Paulo, SP, Brazil) at a dose of 0.04 ml/100 g of body weight. Subsequently, the animals were subjected to trichotomy of the inner region of the right and left hind legs.

An incision of approximately 10 mm was made, in plane, over the tibial tuberosity. After delicate dissection, the region was prepared to install the implants through the application of a progressive sequence of drillers (2.0 mm spiral drill- Neodent®; Curitiba, PR, Brazil) to accommodate a 4 mm height and 2.2 mm diameter titanium implant (Machined and Acqua Implants, Neodent®, Curitiba, PR, Brazil). All drilling was carried out with the aid of an electric motor, adjusted to 1200 rpm, under abundant irrigation with sterile saline solution. The implant was installed with the help of a digital key (Hexagonal digital key 1.2 mm - Neodent, Curitiba, PR, Brazil).

After the implants were installed, the tissues were sutured in layers internally with resorbable 5.0 thread (Vicryl Ethicon, Johnson & Johnson, São José dos Campos, Brazil) and externally with 4.0 silk thread (Ethicon, Johnson & Johnson, São José dos Campos, Brazil). The animals received, in a single dose, penicillin associated with streptomycin at a dosage of 0.1 ml/kg of body mass (Multibiotic Small, Vitalfarma, São Sebastião do Paraíso, MG, Brazil) and ketoprofen at a dosage of 0.1 ml/kg of body mass (Ketoflex; Mundo Animal, São Paulo, Brazil) intramuscularly. Then, 15 and 45 days after the surgical procedure, the animals were euthanized by an anaesthetic overdose. The tibiae were randomly separated, one of which was used for microtomographic and

histomorphometric analyses, while the other was used for biomechanical analysis (APÊNDICE A).

Biomechanical analysis

After euthanasia, the tibiae were stabilized in a small vice. A hexagonal wrench was connected to both the implant and a torque wrench (Tohnichi, model ATG24CN-S, Tokyo, Japan - with a graduated scale of 0.05 N/cm, measuring the strength from 3 to 24 Ncm), and anti-clockwise movement was performed with the objective of unscrewing the implant. The maximum peak required to move the implant was noted as the removal torque value (Ncm).

Microtomographic analysis

Tibiae that did not undergo biomechanical analysis were fixed in 4% paraformaldehyde for 48 hours and later stored in 70% alcohol. The samples were scanned by a microtomograph (Skyscan, Aatselaar, Belgium) with the following parameters: camera pixel: 12.45; X-ray tube power: 65 kVP, X-ray intensity: 385 µA, integration time: 300 ms, filter: Al-1 mm and voxel size: 18 μm^3. The images were reconstructed, spatially repositioned and analysed by specific software (NRecon, Data Viewer, CTAnalyser, Aatselaar, Belgium). The region of interest (ROI) was defined as a circular region 0.5 mm around the entire diameter of the implant. This ROI was defined as the total volume (0.5 mm margin around the implants - 4.5 mm x 3.2 mm). As the implants did not receive a cover screw in some cases, bone formation within the prosthetic platform may have occurred. However, this bone formation does not interfere with the analysis of the volume of mineralized tissue around the implant, and a second ROI was defined to remove the volume from the platform. With the

results obtained in the two ROIs, it was possible to define the volume of bone formation using the formula Total Volume - Platform Volume = Volume of mineralized tissues (%BV/TV). The threshold used in the analysis was 25-90 shades of grey, and the values of the volume of mineralized tissue around the implants were obtained as a percentage [23]. A trained examiner who was blinded to the experimental groups performed this FEP analysis.

Histomorphometric analysis

After scanning, the tibiae were dehydrated in a graded series of ethanol (60 - 100%) and infiltrated and polymerized in light-cured resin (Technovit 7200 VLC, Kultzer Heraus GmbH & CO, Wehrheim, Germany). The blocks containing the implant and bone tissue were cut at a central point using a cut and wear system (Exakt Apparatebeau, Hamburg, Germany). The sections were approximately 45 µm thick, stained with Stevenel blue associated with acid fuchsin and analysed under an optical microscope (DIASTAR - Leica Reichert & Jung products, Wetzlar, Germany) at 100X magnification. Histomorphometric evaluations were performed with ImageJ software (San Rafael, CA, USA). The percentages of bone-implant contact (% BIC) and the bone area between turns (% BBT) were evaluated separately in the first three threads of the implants. These analyses were performed by a blinded and trained examiner (FEP).

Statistical analysis

The distribution of the data generated in this study was assessed using the Shapiro-Wilk normality test. All data in this study were distributed according to normality; thus, parametric tests were applied for inferential analysis of the data. Comparison between the groups was performed using the one-way ANOVA test

complemented by the Tukey test. The unpaired t-test was used to perform the comparison between different periods within each group. GraphPad Prism 6 software (Sam Diego, CA, USA) was used to perform the statistical analysis, and all statistical tests were applied at a significance level of 5%.

Results

Biomechanical analysis

There was a progressive increase in the removal torque of the implants in all groups, with the exception of the HN group, over the 45-day period compared to the 15-day period ($p < 0.001$). Implants with hydrophilic surfaces showed higher removal torque values than implants with machined surfaces in healthy animals at the 15-day time point (7.33 ± 1.03 Ncm vs. 18.00 ± 2.44 Ncm) ($p < 0.001$) and in the nicotine groups at both evaluation time points (3.28 ± 1.97 Ncm vs. 15.43 ± 3.55 Ncm at 15 days; 11.43 ± 1.81 Ncm vs. 17.88 ± 2.10 Ncm at 45 days) ($p < 0.001$). In addition, animals subjected to nicotine administration showed lower values of removal torque than healthy animals with implants with machined surfaces (3.28 ± 1.97 Ncm vs. 7.33 ± 1.03 Ncm at 15 days; 11.43 ± 1.81 Ncm vs. 21.67 ± 2.87 Ncm at 45 days) versus implants with hydrophilic surfaces (17.88 ± 2.10 Ncm vs. 22.43 ± 2.50 Ncm at 45 days) (Figure 1).

Microtomographic analysis

There was a progressive increase in BV/TV% in all groups over the 45-day period compared to the 15-day period ($p < 0.001$). Implants with hydrophilic surfaces showed higher BV/TV % than machined surface implants in both

healthy animals (48.07 ± 1.65% vs. 63.82 ± 2.16% at 15 days) and in animals subjected to nicotine administration (31.46 ± 1.70% vs. 62.61 ± 1.36% at 15 days; 63.58 ± 2.52% vs. 69.38 ± 1.93% at 45 days) ($p < 0.001$). The animals subjected to the challenge with nicotine showed lower values of BV/TV% than the healthy animals when the machined implants were compared (31.46 ± 1.70% vs. 48.07 ± 1.65 at 15 days; 63.58 ± 2.52% vs. 70.18 ± 1.01% at 45 days) ($p < 0.001$), but there were no differences in BV/TV% between healthy and nicotine animals when the hydrophilic implant surfaces were evaluated (Figure 1).

Histomorphometric analysis

There was a progressive increase in the %BIC and %BBT values in all groups at the 45-day period compared to the 15-day period ($p < 0.001$). In general, the implants with hydrophilic surfaces showed higher of %BIC (39.92 ± 5.32% vs. 55.16 ± 6.57% at 15 days in healthy animals; 21.80 ± 5.14% vs. 39.25 ± 4.46% at 15 days and 69.76 ± 5.72% vs. 80.33 ± 1.94% at 45 days in animals subjected to nicotine treatment) and %BBT (33.53 ± 4.06% vs. 50.15 ± 5.56% at 15 days in healthy animals; 11.22 ± 4.90% vs. 32.28 ± 5.24% at 15 days and 68.78 ± 6.11% vs. 80.25 ± 3.87% at 45 days in animals subjected to nicotine treatment) values than the machined-surface implants in all groups ($p < 0.001$). Animals subjected to nicotine application showed lower values of %BIC (21.80 ± 5.14% vs. 39.92 ± 5.32% at 15 days and 69.76 ± 5.72% vs. 81.69 ± 3.98 at 45 days with implants with a machined surface; 39.25 ± 4.46% vs. 55.16 ± 6.57% at 15 days for implants with a hydrophilic surface) and %BBT (11.22 ± 4.90% vs. 33.53 ± 4.06% at 15 days and 68.78 ± 6.11% vs. 81.03 ± 2.34 at 45 days for implants with a machined surface; 32.28 ± 5.24% vs. 50.15 ± 5.56% at 15 days

for implants with a hydrophilic surface) than the healthy animals in all groups ($p < 0.001$) (Figure 1). Figure 2 shows representative images of the nondecalcified histological sections in all groups and experimental periods.

Discussion

Despite the efforts of different national health systems around the world to raise public awareness of the harmful effects of smoking on general health, it is estimated that the population of active smokers worldwide is approximately 1.1 billion people [24] and that approximately 50% of people who try to quit smoking fail in that attempt [25]. Thus, the treatment of smoking patients is common in dental practice, because smokers require more treatment to replace lost teeth than healthy patients [11, 26]. The results of this study were encouraging regarding the use of implants with hydrophilic surfaces due to the benefit of the osseointegration process in relation to implants with machined surfaces in healthy rats and rats that were subjected to nicotine administration. Furthermore, the osseointegration process was similar between healthy and nicotine-challenged animals when hydrophilic surface implants were installed.

The analysis of the removal torque, % BV/TV, %BIC, and %BBT demonstrated that the animals subjected to nicotine application presented less implant locking, less bone around the implants, less bone-implant contact, and less bone between the threads of the implants. These findings corroborate previous results presented in the literature that demonstrated that nicotine and smoking impair the bone healing process in bone fractures [27], in skin [28] and in mucous membrane wounds [29] due to a series of changes, both local and systemic in the body, including macrophage inhibition, decreased inflammatory

cell chemotaxis, and decreased platelet aggregation, which impair the host's immune-inflammatory response [12-14].

Specifically, in bone tissue, nicotine has been shown to reduce osteogenesis by inhibiting the expression of BMP2 and VEGF [16] and increasing vasoconstriction [30], in addition to impairing the quality of blood clot formation [28]. Previous studies have shown how the aforementioned effects negatively interfere with bone repair in different experimental models. A preclinical study showed that nicotine administration to rats harmed bone repair in areas grafted with autogenous bone [31]. A systematic review of preclinical studies has shown that animals subjected to nicotine administration presented a reduced bone-implant contact of implants compared to healthy animals [5]. On the other hand, a preclinical study demonstrated that there was no deleterious effect of nicotine on the osseointegration process. However, the administration of this medication began just after implant placement, and this study design limits the applicability of these findings since patients will not suddenly initiate smoking habits after the surgical procedure if they do not have this habit before the intervention [32].

Another important finding in this study was the improvement in osseointegration provided by implants with a hydrophilic surface, which reversed the harmful effects of nicotine, especially at the longest experimental period (45 days). This good outcome of hydrophilic surfaces is related to their high degree of wettability [20, 33], which increases adhesion, differentiation, and osteoblastic activity [34]. Indeed, preclinical studies have shown that hydrophilic surfaces improve osseointegration in native [20-22] and grafted bone [23]. Hydrophilic surfaces improved the osseointegration of implants

placed in rabbit tibiae 28 days after the surgical procedure compared with a hydrophobic surface [20], improved the osseointegration of the tibiae of rats grafted with osteoconductive bone substitutes 15 and 45 days after implant placement [35], and improved the osseointegration 90 days after implant placement in diabetic pig calvaria compared with hydrophobic surfaces [36]. In healthy humans, an improvement in the osseointegration process has also been demonstrated in implants with hydrophilic surfaces compared to implants with hydrophobic surfaces after 2-4 weeks of implant placement [22].The quest to improve the osseointegration process in smoking patients can not only start with interventions to improve the surfaces of the implants but also with approaches to induce the patient to quit smoking to benefit the osseointegration process [37, 38]. A preclinical study showed that when rats that inhaled cigarette smoke received implants in their tibia, the cessation of this inhalation during the healing period was sufficient to improve the osseointegration process [37]. Other clinical studies have shown that quitting smoking benefits the treatment of periodontal disease [39] and improves periodontal parameters in these populations [40]. However, the effect of quitting smoking on osseointegration in humans lacks further information and confirmation.

This study has as a limitation in that only nicotine was used to disrupt the osseointegration process to mimic the condition of active smokers. However, several other toxic substances can be formed during smoking, and the effect of all of these associated substances can be even more harmful to the osseointegration process that was demonstrated in this study. Other experimental models that mimic the smoking habit simulate the inhalation of smoke, which also has limitations in mimicking a passive smoker condition [41, 42]. Despite these limitations, the model used mimics the systemic effects caused by smoking in the osseointegration process, as demonstrated in a previous systematic literature review [5]. Another limitation of this study is that a comparison of the hydrophilic surface with other types of commercially available surfaces has not been performed, and this topic requires further investigation.

Conclusion

The hydrophilic surface accelerated osseointegration in both groups of rats (healthy and nicotine) in the early period of evaluation. The hydrophilic surface also equalized the osseointegration in nicotine-exposed animals compared with healthy animals after 45 days of implant placement.

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Figures Legends

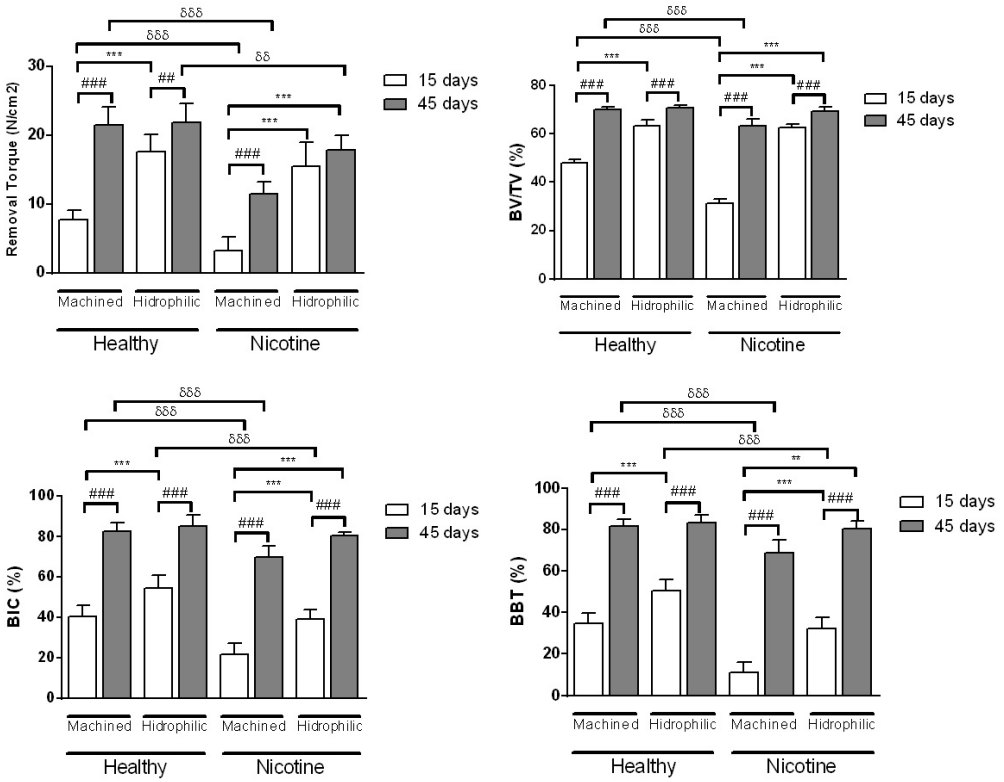

Figure 1: Representative graphs showed the mean and standard deviation of all the analysis performed in this study. **p<0.01; ***p<0.001- Differences between the implant's surfaces- One-way anova complemented by Tukey.; δδδ p <0.001 -Differences between the control and nicotine groups – One-way anova complemented by Tukey; ##p<0.01; ###p<0.001- Intragroup differences regarding the experimental period – Unpaired t-test.

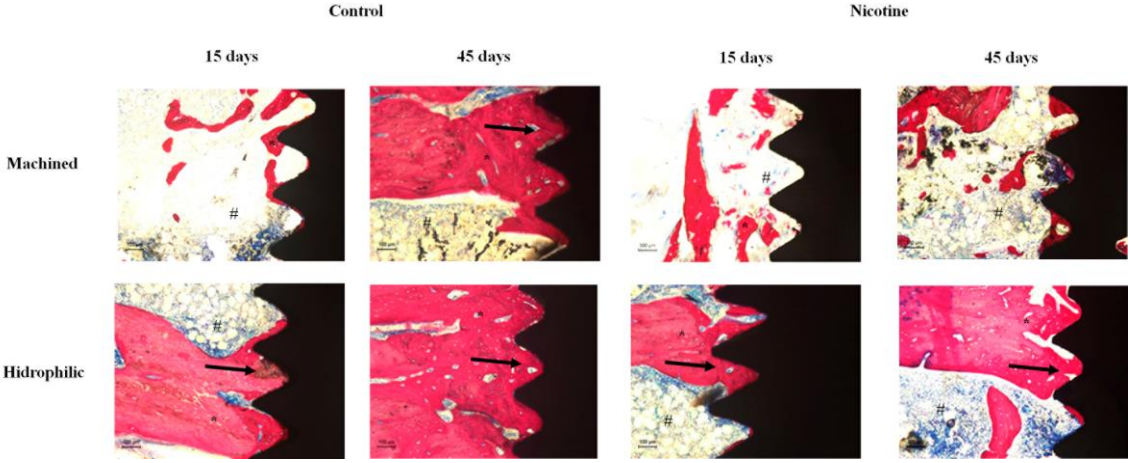

Figure 2: Representative images of the nondecalcified histological sections in all groups and experimental periods.

4 CONSIDERAÇÕES FINAIS

O objetivo deste estudo foi avaliar o efeito de uma superfície modificada por jateamento e ataque ácido e mantida em solução isotônica (Superfície Hidrofílica) em comparação a uma superfície usinada sobre a osseointegração de implantes em animais com comprometimento sistêmico. De uma forma geral, implantes com a superfície hidrofílica tiveram uma osseointegração mais acelerada do que implantes de superfície usinada em animais com comprometimento sistêmico, como podemos ver pela diferença significativa entre os animais principalmente nos períodos de 15 dias.

Em relação ao estudo 1, onde foi avaliada a osseointegração em animais normo e hiperglicêmicos, de uma forma geral verificou-se a superioridade da superfície hidrofílica em relação a superfície usinada, e que a hiperglicemia em si ela pode prejudicar o processo de cicatrização, porém, neste mesmo trabalho pode-se observar que os grupos onde foram utilizados os implantes com superfície hidrofílica não houve diferenças na osseointegração entre os animais normo e hiperglicêmicos, reduzindo portanto a importância do controle glicêmico em relação a osseointegração quando se utiiza implantes hidrofílicos, aumentando assim a previsibilidade de sucesso para o tratamento de implantes dentários neste tipo de paciente.

Os resultados que foram apresentados neste trabalho corroboram com outros estudos já publicados na literatura, onde tem sido relatado que a osseointegração dos implantes pode ser alterada em estados hiperglicêmicos[6].

Estudos pré-clínicos publicados, corroboram com nossas análises, os mesmos, demonstraram que animais com diabetes induzida apresentaram um retardo na osseointegração em comparação a animais controle sem doença, sendo que o atraso na osseointegração ocorre devido a produtos provenientes da hiperglicemia[9, 10]. Estudos que avaliaram a taxa de osseointegração de implantes em pacientes diabéticos demonstraram resultados semelhantes entre paciente com bom controle glicêmico e paciente saudável[7, 8], contudo, estes estudos também demostraram que a estabilidade do implante durante o período de cicatrização possui relação direta com o nível glicêmico dos pacientes, sendo que o paciente que possuíam pior controle glicêmico apresentavam implantes com menor estabilidade.

Como citado anteriormente no artigo proposto, a similaridade na osseointegração de animais normo e hiperglicêmicos, se deve ao fato da utilização da superfície hidrofílica e dentre suas principais características devemos destacar seu alto grau de molhabilidade[24,28], este alto grau de molhabilidade irá aumentar a expressão de biomarcadores ósseos, adesão de células mesenquimais e também estimular o processo osteoblástico[20,21], que são de suma importância para a formação óssea ao redor do implante e consequentemente sua osseointegração. Nossos resultados demonstraram dados importantes, embora em animais, em relação a condições de alto índice de nível glicêmico onde os implantes com superfície hidrofílica apresentarem resultados iguais aos do grupo controle e melhor osseointegração do que implantes de superfície usinada em ambos os períodos experimentais.

No estudo 2, teve-se como objetivo avaliar a osseointegração de implantes em animais com alta dosagem de nicotina, os resultados obtidos

assim como no estudo 1, também demostraram uma superioridade da superfície hidrofílica quando comparada a superfície usinada.

Atualmente, pode-se encontrar uma vasta literatura que avalia o efeito da nicotina em relação a osseointegração de implantes, demonstrando que ela causa uma série de alterações, tanto locais, como sistêmicas no organismo, entre elas, inibição de macrófagos, diminuição da quimiotaxia de células inflamatórias, diminuição da agregação plaquetária, que prejudicam a resposta imuno-inflamatória do hospedeiro[13-15], além disso, sabe-se que a nicotina também interfere na atividade de fibroblastos e osteoblastos, gerando menor formação óssea ao redor dos implantes e piorando sua osseointegração[16].

Estes fatos podem nos elucidar a respeito dos resultados encontrados no nosso trabalho, onde a osseointegração dos grupos nicotina, foi significativamente menor do que nos grupos controle, resultados estes que corroboram com os estudos de Yamano et al., 2010 e Ghanem et al., 2017 , em um estudo pré-clínico em ratos, demostraram que a nicotina interfere diretamente na osseointegração de enxertos autógenos, os animais sistematicamente modificados com nicotina tiveram uma pior cicatrização do enxerto realizado em relação aos do grupo controle. Jensen et al., 2015, publicaram uma revisão sistemática que avaliou o contato osso/implante de implantes colocados em animais que tiveram a injeção de nicotina em comparação a animais saudáveis. Como resultado, os autores demostraram que a nicotina possue um efeito direto na piora da osseointegração de implantes, fato este também demostrado no nosso trabalho nos grupos onde não foi utilizado os implantes hidrofílicos.

Um fato importante deste trabalho, e que alavanca uma ótima perspectiva para o futuro, são os resultados obtidos a partir da comparação com os grupos onde foram utilizados os implantes hidrofílicos, em que apresentaram melhores resultados de osseointegração em relação aos implantes lisos, em animais com comprometimento sistêmico. Estes resultados podem estar relacionados ao fato das características do implantes hidrofílico, que devido as propriedades de topografia, composição química e molhabilidade podem promover aumento na osteocondução dos implantes[21]. Essas modificações físico-químicas de superfície visam aumentam a estabilidade do coágulo que serve de guia para o início do processo de osseointegração, promovendo um aumento da propriedade osteocondução[20].

5 CONCLUSÃO

Pode-se concluir que implantes com superfície hidrofílica aceleram a osseointegração de implantes em animais com comprometimento sistêmico.

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APÊNDICE A

Artigo 1 – Implantes com superfície hidrofílica equiparam a osseointegração de implantes em ratos normo e hiperglicêmicos.

O estudo foi aprovado na Comissão de Ética no Uso de Animal da FOAr-UNESP (CEUA-19/2016).

Distribuição dos animais e grupos

Neste estudo foram utilizados 64 ratos machos (*Rattus Norvegicus*), variação albinus, Holtzman, de aproximadamente 3 meses de idade, com massa corporal entre 200-300 gramas, que foram mantidos no Biotério da Faculdade de Odontologia da UNESP de Araraquara (FOAr-UNESP), alimentados com ração sólida e com acesso a água ad libitum, antes e durante todo o período experimental, em ambiente com água, luz e temperatura controladas.

Os animais foram divididos aleatoriamente em 4 grupos com 16 animais cada, que foram avaliados em dois períodos experimentais (15 e 45 dias - 8 animais/grupo/período). Os grupos foram divididos de acordo com o tipo de implante que foi instalado na tíbia e com a condição sistêmica dos animais:

Grupo Hidrofílico/Saudável: Animal sistematicamente saudável e instalação de implante com superfície modificada por jateamento de óxidos e ataque ácido e mantida em solução de cloreto de sódio (Implante ACQUA, Neodent®, Curitiba, PR, Brasil)

Grupo Hidrofílico/Diabético: Animal com indução de diabetes e instalação de implante com superfície modificada por jateamento de óxidos e ataque ácido e mantida em solução de cloreto de sódio (Implante ACQUA, Neodent®, Curitiba, PR, Brasil)

Grupo Usinado/Saudável: Animal saudável e instalação de implante com superfície usinada (Neodent®, Curitiba, PR, Brasil)

Grupo Usinado/Diabético: Animal com indução de diabetes e instalação de implante com superfície usinada (Neodent®, Curitiba, PR, Brasil)

Indução da diabetes

Para indução do diabetes, após um período de 16 horas de jejum, exceto água ad libitum, os animais foram submetidos à administração, por via intraperitoneal de estreptozotocina 50 mg/kg dissolvida em tampão citrato pH 4,5, 30 dias antes do procedimento cirúrgico. Os animais que foram mantidos sem diabetes receberam injeção de solução salina via intraperitoneal no mesmo volume. Após 24 horas da indução foi executada a comprovação do quadro diabético por meio da análise da taxa glicêmica na qual foram considerados diabéticos os animais com glicemia superior a 300mg/dl. Avaliações semanais do nível glicêmico foram realizadas durante todo o estudo para assegurar esta condição[23].

Após a confirmação do estado hiperglicêmico, os animais do grupo diabetes compensado receberam injeções subcutâneas, duas vezes ao dia, de 2.5-3U de insulina (Humulin NPH U-100, Lilly, Fegershein, France) para controle da glicemia durante todo período experimental. Os animais saudáveis receberam as mesmas injeções porém com solução salina.

Artigo 2 – Avaliação da osseointegração de implantes com superfície hidrofílica em ratos com alta dosagem de nicotina no sangue

O estudo foi aprovado na Comissão de Ética no Uso de Animal da FOAr-UNESP (CEUA-44/2017).

Distribuição dos animais e grupos

Neste estudo foram utilizados 64 ratos machos (*Rattus Norvegicus*), variação albinus, Holtzman, de aproximadamente 3 meses de idade, com massa corporal entre 200- 300 gramas, que serão mantidos no Biotério da Faculdade de Odontologia da UNESP de Araraquara (FOAr-UNESP), alimentados com ração sólida e com acesso a água ad libitum, antes e durante todo o período experimental, em ambiente com água, luz e temperatura controladas.

No estudo foi avaliado o efeito de duas superfícies de implantes sobre o reparo ósseo em animais com alto índice de nicotina. Os 64 animais foram divididos aleatoriamente em 4 grupos com 16 animais cada, que foram avaliados em dois períodos experimentais (15 e 45 dias), com 8 animais em cada grupo. Os grupos foram divididos de acordo com o tipo de implante que foi instalado na tíbia e da condição sistêmica dos animais:

Grupo Hidrofílico/Saudável: Animal sistematicamente saudável e instalação de implante com superfície modificada por jateamento de óxidos e ataque ácido e mantida em solução de cloreto de sódio (Implante ACQUA, Neodent®, Curitiba, PR, Brasil)

Grupo Hidrofílico/Nicotina: Animal com administração subcutânea de nicotina e instalação de implante com superfície modificada por jateamento de

óxidos e ataque ácido e mantida em solução de cloreto de sódio (Implante ACQUA, Neodent®, Curitiba, PR, Brasil)

Grupo Usinado/Saudável: Animal sistematicamente saudável e instalação de implante com superfície usinada (Neodent®, Curitiba, PR, Brasil)

Grupo Usinado/Nicotina: Animal com administração subcutânea de nicotina e instalação de implante com superfície usinada (Neodent®, Curitiba, PR, Brasil)

Aplicação da Nicotina

Para indução da nicotina, foram realizadas aplicações subcutâneas a cada 12h na região dorsal dos animais dos grupos Nicotina, por um período de 30 dias antes do procedimento cirúrgico e mantidas até a eutanásia do animal. Os animais receberam uma solução diluída de nicotina na proporção de 5mg/ml, que foi administrada uma dose de 1mg/kg do animal (Okamoto et al., 1994), os grupos controle (saudável), receberam administrações de soro fisiológico a cada 12h, no mesmo período que os animais do grupo nicotina.

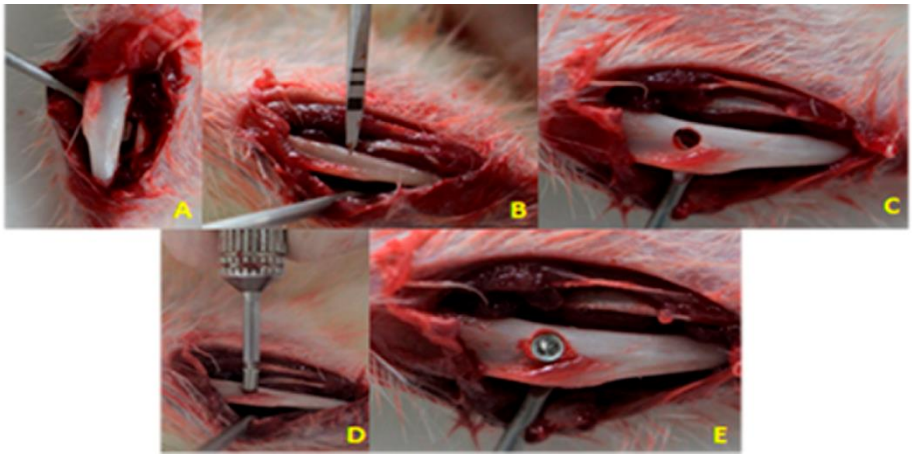
NOS DOIS ESTUDOS FORAM UTILIZADAS AS MESMAS METODOLOGIAS PARA COLOCAÇÃO DOS IMPLANTES E PARA AS ANÁLISES:

Procedimento Cirúrgico

Os animais foram anestesiados por uma combinação de Quetamina (Agener União Ltda, São Paulo, SP, Brasil) na dosagem de 0,08 ml/100g de massa corporal com Xilazina (Rompum, Bayer S.A., São Paulo, SP, Brasil) na dosagem de 0,04 ml/100g de massa corporal. Posteriormente, os animais foram submetidos à tricotomia da região interna das patas posteriores direita e esquerda.

Uma incisão de aproximadamente 10mm foi realizada, em planos, sobre a tuberosidade da tíbia. Após dissecção delicada, a região foi preparada para instalação dos implantes por meio da aplicação de uma sequência progressiva de fresas (fresa lança; fresa espiral de 2.0 mm – Neodent®; Curitiba, PR, Brasil) para instalar um implante de titânio com 4 mm de altura por 2.2 mm de diâmetro (Implantes Usinado e Acqua, Neodent®, Curitiba, PR, Brasil). Todas as perfurações foram realizadas com auxílio de um motor elétrico, ajustado a 1200 rpm, sob abundante irrigação com solução salina estéril. O implante foi instalado com a ajuda de uma chave digital (Chave digital hexagonal 1.2mm – Neodent, Curitiba, PR, Brasil) (Figura 1 D-E).

Figura 1 - Figura demonstrativa das etapas da cirurgia realizadas nas tíbias dos animais.



(A) –Tibia do animal após dissecção delicada dos tecidos; (B) – Preparação para instalação dos implantes por meio da aplicação de uma sequência progressiva de fresas; (C) – Região preparada após sequencia de fresas; (D) – Instalação do implante utilizando uma chave digital hexagonal de 1.2mm; (E) – Implante em posição.
Fonte: Elaboração própria.

Após a instalação dos implantes, os tecidos foram suturados por planos: internamente com fio reabsorvível 5.0 (Vicryl Ethicon, Johnson & Johnson, São José dos Campos, Brasil) e externamente com fio de seda 4.0 (Ethicon, Johnson & Johnson, São José dos Campos, Brasil). Os animais receberam, em dose única, penicilina associada à estreptomicina na dosagem 0.1 ml/kg de peso (Multibiótico Small, Vitalfarma, São Sebastião do Paraíso, MG, Brasil) e 0.1 ml/kg de peso de cetoprofeno (Ketoflex; Mundo Animal, São Paulo, Brasil) por via intramuscular.

Nos períodos de 15 e 45 dias após os procedimentos cirúrgicos de instalação dos implantes, os animais foram submetidos a eutanásia por aprofundamento da dose de anestésico. As tíbias foram randomicamente separadas, sendo que uma delas foi utilizada para as análises

microtomográficas e histomorfométrica, enquanto que a outra tíbia foi utilizada para a análise biomecânica.

Avaliação Biomecânica

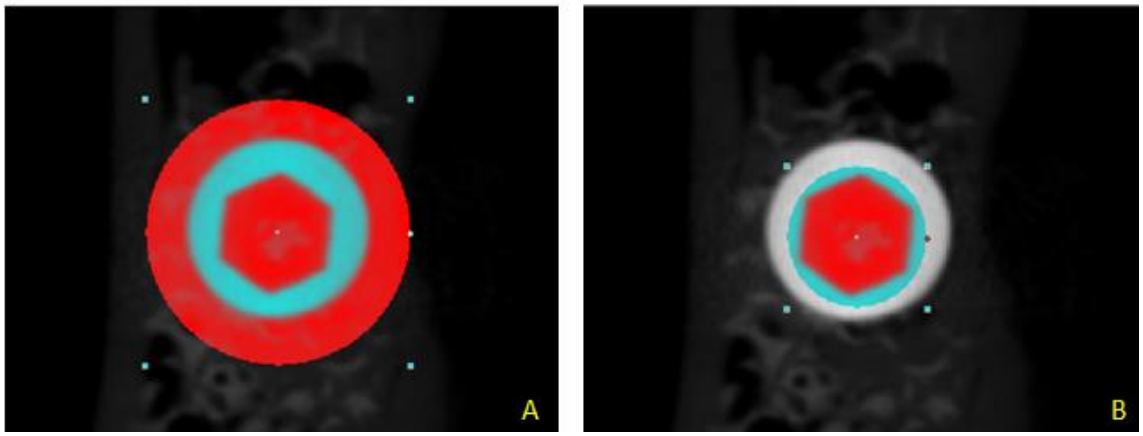
Após a eutanásia as tíbias foram estabilizadas em uma pequena morsa. Uma chave hexagonal foi conectada tanto no implante como no torquímetro (Tohnichi, modelo ATG24CN-S, Toquio, Japão - com escala graduada de 0.05 N/cm, medindo a força de 3 a 24 Ncm) e foi realizado um movimento anti-horário com o objetivo de desrosquear o implante. O pico máximo necessário para movimentar o implante foi anotado como o valor do torque de remoção.

Avaliação microtomográfica

As tíbias que não passaram pela análise biomecânica foram fixadas em paraformaldeido a 4% por 48 horas e posteriormente armazenadas em Álcool 70º. Antes das peças serem processadas para inclusão em resina, foram escaneadas por um microtomógrafo (Skyscan, Aatselaar, Bélgica) com os seguintes parâmetros: Pixel da câmera: 12.45; Potência do tubo de raio-x: 65 kVP, intensidade do raio-x: 385 µA, tempo de integração: 300 ms, filtro: Al-1 mm e tamanho do voxel: 18 μm^3. As imagens foram reconstruídas, reposicionadas espacialmente e analisadas por softwares específicos (NRecon, Data Viewer, CTAnalyser, Aatselaar, Bélgica). A região de interesse (ROI) foi definida como uma região circular com 0,5 mm em torno de todo o diâmetro do implante. Esse ROI foi definido como Volume Total (0,5mm de margem ao redor dos implantes- 4,5 mm x 3,2 mm). Como os implantes colocados não receberam o *cover screw* em alguns casos pode ocorrer a formação óssea dentro da plataforma protética. Para que esta formação óssea não interfira com

a análise do volume de tecido mineralizado ao redor do implante foi definido um segundo ROI para remoção do volume da plataforma (Figura 2). Com os resultados obtidos nos dois ROIs, foi possível definir o volume de formação óssea utilizando a fórmula: Volume Total – Volume Plataforma = Volume de tecidos mineralizados. O *threshold* utilizado na análise foi de 25-90 tons de cinza, e os valores do volume do tecido mineralizado ao redor dos implantes foi obtido na forma de porcentagem. Um examinador treinado e cego para os grupos experimentais executou essa análise.

Figura 2 - Figura representativa dos ROIs utilizados para realização da análise microtomográficas.



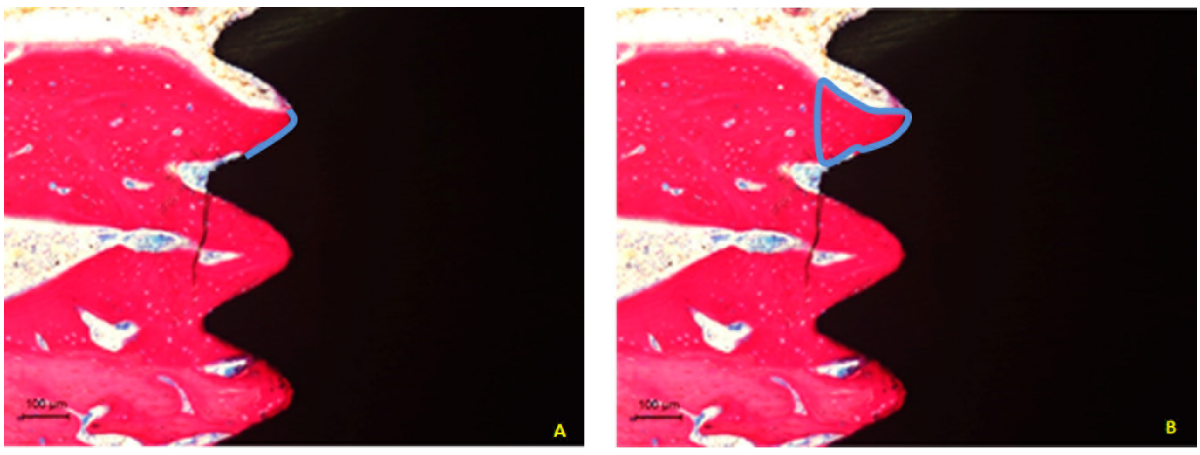
(A) – ROI maior (área óssea ao redor do implante) e (B) – ROI menor (área interna da plataforma).
Fonte: Elaboração própria.

Avaliação Histomorfométrica

Após o escaneamento, as tíbias foram desidratadas em uma série crescente de etanóis (60 – 100%) e infiltradas e polimerizados em resina fotopolimerizável (Technovit 7200 VLC, Kultzer Heraus GmbH & CO,

Wehrheim, Alemanha). Os blocos contendo o implante e o tecido ósseo foram cortados em um ponto central usando um sistema de corte e desgaste (Exakt Apparatebeau, Hamburgo, Alemanha). As secções apresentaram aproximadamente 45 µm de espessura e foram coradas com azul de Stevenel associado a fucsina ácida e analisadas em um microscópio óptico (DIASTAR – Leica Reichert & Jung products, Wetzlar, Alemanha) com o aumento de 100X. As avaliações histomorfométricas foram realizadas com o software para análise de imagem (Image J, San Rafael, CA, EUA). As porcentagens de contato osso-implante (%BIC) e de área óssea entre espiras (%BBT) foram avaliadas separadamente nas seis primeiras roscas dos implantes. Essas análises foram realizadas por um examinador cego e treinado.

Figura 3 – Figura representativa das anaálises histomorfométricas.



(A) Figura representativa mostrando como foi mensurado o contato osso implante (BIC) na análise histométricas. A imagem em azul demonstra a região medida de contato osso/implante. (B). Figura representativa mostrando como foi mensurado a área óssea dentro das roscas (BBT) do implante na análise histométrica, a área delimitada em azul demonstra a região analisada da formação óssea entre as rosca do implante (Aumento original 100 X- Azul de Stevenel´s com fucsina ácida).

Fonte: Elaboração própria.

Análise estatística

Os dados numéricos gerados foram submetidos ao teste de Normalidade de Shapiro-Wilk para avaliar se respeitaram a distribuição de acordo com o teorema da distribuição central. Caso os dados sejam normais, os testes paramétricos de Anova complementado pelo teste de Tukey foi utilizado para a análise inferencial dos dados. Caso contrário, o teste não paramétrico de Kruskall-Wallis complementado pelo teste de Dunn foi utilizado para a análise dos dados.

ANEXO A

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UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Araraquara
FACULDADE DE ODONTOLOGIA

CERTIFICADO

Certificamos que a proposta intitulada ***"AVALIAÇÃO DA OSSEOINTEGRAÇÃO DE IMPLANTES AQUA EM RATOS COM DIABETES INDUZIDA"***, registrada com o nº 19/2016, sob a responsabilidade do(a) **Prof(a). Dr(a). Élcio Marcantonio Júnior** – que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela **COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DA FACULDADE DE ODONTOLOGIA DE ARARAQUARA** em reunião de 27/03/2017.

Finalidade	( ) Ensino (X) Pesquisa Científica
Vigência do Projeto	Fevereiro/2019
Espécie/linhagem	Rato/Rattus Novergicus, variação albinus, Holtzman
Nº de animais	96
Peso/Idade	200-300 g – 3 meses
Sexo	Macho
Origem	Biotério de Criação da Faculdade de Odontologia de Araraquara

Profa. Dra. CARINA APARECIDA FABRÍCIO DE ANDRADE
Coordenadora da CEUA

Comissão de Ética no Uso de Animais – CEUA
Rua Humaitá nº 1.680 – Centro – CEP 14801-903 – Caixa Postal nº 231 – ARARAQUARA – SP
1º andar – fone (16) 3301-6459 / fax (16) 3301-6433 – e-mail: ceua@foar.unesp.br – home page: http://www.foar.unesp.br

ANEXO B

unesp

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Araraquara
FACULDADE DE ODONTOLOGIA

CERTIFICADO

Certificamos que a proposta intitulada ***"AVALIAÇÃO DA OSSEOINTEGRAÇÃO DE IMPLANTES AQUA EM RATOS COM INDUÇÃO DE NICOTINA"***, registrada com o nº 44/2017, sob a responsabilidade do(a) **Prof(a). Dr(a). Élcio Marcantonio Júnior** – que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela **COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DA FACULDADE DE ODONTOLOGIA DE ARARAQUARA** em reunião de 23/02/2018.

Finalidade	( ) Ensino (**X**) Pesquisa Científica
Vigência do Projeto	Dezembro/2020
Espécie/linhagem	Rato Holtzman
Nº de animais	64
Peso/Idade	200-300 g – 3 meses
Sexo	Macho
Origem	Biotério Central da Faculdade de Odontologia de Araraquara – UNESP

Profa. Dra. CARINA APARECIDA FABRICIO DE ANDRADE
Coordenadora da CEUA

Araraquara, 24/09/2021

Felipe Eduardo Pinotti