VALENTIM ADELINO RICARDO BARÃO

Avaliações Biomecânicas e Biológicas Relacionadas às Próteses Implanto-Suportadas e aos Implantes Osseointegrados

Araçatuba – São Paulo

2011

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Avaliações Biomecânicas e Biológicas Relacionadas às Próteses Implanto-Suportadas e aos Implantes Osseointegrados

Tese apresentada à Faculdade de Odontologia do Campus de Araçatuba – Universidade Estadual Paulista "Júlio de Mesquita Filho"- UNESP, para obtenção do Título de DOUTOR EM ODONTOLOGIA (Área de concentração em Prótese Dentária).

Orientador: Prof. Adj. Wirley Gonçalves Assunção

Araçatuba – São Paulo

2011

Catalogação na Publicação (CIP)

Serviço Técnico de Biblioteca e Documentação – FOA / UNESP

B225a

Barão, Valentim Adelino Ricardo.
Avaliações biomecânicas e biológicas relacionadas às próteses implanto-suportadas e aos implantes osseointegrados / Valentim Adelino Ricardo Barão. - Araçatuba : [s.n.], 2011
245 f. : il. ; tab. + 1 CD-ROM

Tese (Doutorado) – Universidade Estadual Paulista, Faculdade de Odontologia, Araçatuba
Orientador: Prof. Dr. Wirley Gonçalves Assunção

1. Eletroquímica 2. Titânio 3. Implante dentário 4. Biomecânica 5. Análise de elemento finito

Black D32
CDD 617.69

Dados Curriculares

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DEDICATÓRIA

Dedicatória

À Deus

Tinha a certeza que o Senhor jamais me abandonaria, mas confesso que as vezes achei que estava desamparado. O Senhor me deu todas as forças e me encorajou de tal forma a concretizar meus objetivos pessoais e profissionais. Jamais posso me abster de agradecer Santa Rita de Cássia e Nossa Senhora Aparecida por todas as graças a mim consedidas. Serei eternamente seguidor de seus ensinamentos. Muito obrigado Senhor por nunca me abandonar. Creio em Ti e em Sua sabedoria.

Aos meus pais, Margarida e Adelino

Por me proporcionar esse momento. Pela dedicação e por todas as vezes que abdicaram de seus sonhos para a realização dos meus. Minha eterna gratidão.

A minha mãe, pessoa mais importante de minha vida. Tenho extrema admiração e orgulho pela mulher batalhadora e incanssável que a senhora se mostrou sempre ser mesmo diante de tantos obstáculos. Muitíssimo obrigado pelo amor, dedicação, sabedoria, confiança e palavras de incentivo a mim transmitidas.

Às minhas irmãs, Daniela e Renata

Pelo companherismo, solidariedade e carinho. Vocês são extremamente importantes em minha vida. Muito obrigado por sempre me apoiarem na concretezação de meus sonhos mesmo quando eu estava bem distante. No meu coração existe um lugar especial dedicado somente a vocês: minhas irmãs que tanto amo.

Às minhas avós, Avelina (in memorian) e Nair

Pela humildade, sabedoria e esforço. Muito obrigado pelas palavras sábias e pelo amor a mim transmitido.

AGRADECIMENTOS

ESPECIAIS

Agradecimentos Especiais

Ao meu orientador Prof. Adj. Wirley Gonçalves Assunção

Muito obrigado pela confiança nesses quase seis anos de convivência diária. Muito obrigado pelo suporte, orientação e incentivo mesmo quando eu achava que não seria capaz de concretizar certas tarefas. Obrigado por me dar o livre arbítrio em muitas decisões, que com certeza me fizeram crescer e amadurecer cientificamente e pessoalmente. Sou extremamente grato aos sábios ensinamentos, a grande amizade e a motivação que com certeza me fortaleceram. A você minha gratidão e profunda admiração.

Ao meu orientador estrangeiro Prof. Dr. Cortino Sukotjo

Da University of Illinois at Chicago. Muitíssimo obrigado pela oportunidade de concretizar o sonho de estudar nos EUA. Obrigado por dividir seus ensinamentos, pela grande amizade, pela brilhante orientação e por ser uma pessoa de grande coração. Admiro sua habilidade e rapidez em conduzir as pesquisas. Espero continuar contanto com sua ajuda e amizade. Meus sinceros agradecimentos.

Ao meu orientador estrangeiro Prof. Dr. Mathew T. Mathew

Da Rush University Medical Center. Agradeço seus grandes ensinamentos científicos na área de Eletroquímica, e pricipalmente pelo exemplo de como ser uma pessoa sensata e dedicada as pessoas. Me recordo com muito carinho das nossas conversas diárias que tanto me fizeram refletir e amadurecer. Muito obrigado por me dar serenidade, paciência e por sua valiosa amizade. Tenha minha grande admiração.

À Profa. Ass. Dra. Debora Barros Barbosa

Um exemplo de bondade e fé. Ainda me recordo da época da iniciação científica, que com certeza foi o início de minha jornada científica. Muitíssimo obrigado por ser a primeira a abrir as portas para mim. Agradeço o seu carinho, confiança, amizade e sinceridade. Obrigado por todas as palavras de incentivo durante as fazes difícies que passei. Aprecio muito a sua amizade sincera e espero contar com ela sempre.

À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)

Pela concessão da Bolsa de Doutorado (Processo 2007/58926-5) e pelo apoio financeiro (Processo 2010/09857-3), indispensáveis para a realização deste estudo. Obrigado por me incentivar sempre ao trabalho científico, e me permitir buscar incessantemente novas descobertas e realizações.

À Coordenação de Aperfeiçoamento de Nível Superior (CAPES)

Pela concessão de Bolsa de Estágio de Doutorando no Exterior (Doutorado Sandwich) (Processo BEX 4129/09-1) o que possibilitou a realização de um grande sonho em minha vida, desenvolver pesquisa em uma instituição nos EUA. Obrigado por esse grande incentivo.

AGRADECIMENTOS

Agradecimentos

À *Faculdade de Odontologia de Araçatuba - UNESP* pela oportunidade de realização do curso de Graduação, Mestrado e Doutorado.

À *University of Illinois at Chicago (UIC) – Department of Restorative Dentistry* pela oportunidade em desenvolver o estágio no exterior.

À *Rush University Medical Center – Department of Orthopedic Surgery* pela oportunidade em desenvolver os trabalhos de corrosão e tribocorrão.

À *coordenadora do Programa de Pós-Graduação em Odontologia,* da Faculdade de Odontologia de Araçatuba, da Universidade Estadual Paulista "Júlio de Mesquita Filho" *Profa. Adj. Maria José Hitomi Nagata,* pela gentileza, acessibilidade e caráter.

Ao *Prof. Ass. Dr. Paulo Henrique dos Santos* pela amizade, carinho e atenção. Muito obrigado pelas palavras de incentivo.

Ao *Prof. Adj. Eduardo Passos Rocha* por sempre se dispor, pela amizade e pelo exemplo de competência clínica.

À *Profa. Ass. Dra. e amiga Daniela Micheline dos Santos* pelo convívio, amizade e carinho durante todos esses anos. Agradeço a preocupação e incentivos constantes.

Ao *Prof. Adj. Marcelo Coelho Goiato* pela amizade, receptividade e oportunidades. Meu sincero agradecimento.

Aos meus orientadores das clínicas de Prótese Dentária e Materiais Dentários durante a Graduação *Prof. Titular Humberto Gennari Filho*, *Profa. Ass. Dra. Adriana Crsitina Zavanelli*, *Prof. Adj. Eduardo Passos Rocha, Profa. Adj. Maria Cristina Rosifini Alves Rezende* pelo incentivo clínico e por me despertarem o gosto pela carreira docência. Admiro sua competência.

Aos *demais professores, técnicos e funcionários* do Departamento de Materiais Odontológicos e Prótese da Faculdade de Odontologia de Araçatuba, da Universidade Estadual Paulista "Júlio de Mesquita Filho" pela convivência e pelo carinho e amizade.

Aos *funcionários da Pós-graduação da Faculdade de Odontologia de Araçatuba - UNESP* pela paciência, disponibilidade, alegria e admirável interesse em nos ajudar sempre.

Aos *funcionários da Biblioteca da Faculdade de Odontologia de Araçatuba – UNESP* pela disponibilidade e carinho.

À *Profa. Judy Yuan* da University of Illiois at Chicago, pela bondade, presteza, prestatividade, amizade e sinceridade. Sou muito grato a toda a sua ajuda principalmente na análise estatística e sugestões nas pesquisas desenvolvidas.

À *Profa. Ana Karina Bedran Russo* da University of Illinois at Chicago, pela receptividade, carinho, amizade e sábios ensinamentos. Muito obrigado por ter me apresentado ao Prof. Sukotjo.

Aos *Profs Damian Lee*, *Maria Therese Galang*, *Kent L Knoernschild* pelo convívio, amizade e importantes ensinamentos. Meu sincero agradecimento.

A *todos os alunos e funcionários do Department of Restorative Dentistry da University of Illinois at Chicago* pelo convívio diário, receptividade e carinho.

Aos *Profs Markus A Wimmer e Michel Laurent* da Rush University Medical Center, pela receptividade, valiosos comentários e sugestões científicas, amizade e oportunidade. Minha gratidão.

Aos amigos da Rush University Medical Center, *Christopher Nagelli*, pela alegria, companheirismo e sincera amizade; *Robert Trombley (Bob)*, *Felix Liedtke*,

Patrick Werner, *Bryan Schlink*, *Diego Orozco*, *Valentina Ngai*, *Carol Pacione*, *Robin Pourzal*, *Luis Gallardo*, *Hannah Lundberg*, *Erica Dahlmeier*, *Andrew Morrison*, *Jonathan Stoia*, *Christopher Knowlton* e *Peter Simon* por tornarem meus dias mais alegres e prazeirosos.

Ao meu amigo *Aldiéris Alves Pesqueira* pela agradável convivência durante esses 10 anos dividindo apartamento. Muito obrigado pela sincera amizade, conselhos, ajuda e pelo inegualável senso de humor que me faz sorrir diariamente.

À minha amiga *Juliana Aparecida Delben* pela sincera amizade e companheirismo. Pessoa que eu admiro muito pela determinação, inteligência e busca incessante de novas fronteiras.

Aos meus sinceros amigos *Aline Satie Takamyia*, pela bondade e serenidade, *Aline Ursula Rocha Fernandes*, pelo sorriso expontâneo, *Amália Moreno*, pela inegualável energia e espontaneidade, *Amilcar Chagas Freitas Júnior*, pelo empenho e dedicação, *Ana Cristina dos Santos Padilha*, pela receptividade e bondade, *Ana Kelly Garcia Galo*, pelo carinho, *Ana Paula Martini*, pela educação e prestatividade, *Bruna Cristina Zuccolotti*, pela espontaneidade e alegria, *Douglas Roberto Monteiro*, pela generosidade, alegria, educação e inestimável amizade, *Eduardo Faco*, pelo humor, *Érica Alves Gomes*, pela elegância, simpatia e generosidade, *Érika Oliveira de Almeida*, pela força de vontade e superação, *Erivan Gualberto Júnior*, pela firmeza e persistência, *Juliana Jorge*, pelo convívio

harmonioso, *Leonardo Perez Faverani*, pela educação, sensatez e gentileza, *Lisiane Cristina Bannwart*, pela alegria de viver e extroversão, *Lucas Fernando Tabata*, pelo bom humor e apoio, *Marcela Filié Haddad*, pela alegria, bondade, generosidade, paciência e persistência, **Marcelle Danelon**, pelo carinho e bondade, *Mayara Ferreira*, pela agradável convivência, *Mirela Shinorara*, pelos sensatos conselhos, alegria e disponibilidade, *Murillo Rezende*, pela convivência, *Paulo Henrique Manzato Gomes*, pelos momentos agradáveis, paciência e humor, **Renata Junqueira da Fonseca**, pela responsabilidade e alegria, *Rodolfo Bruniera Anchieta*, pela prestatividade e educação, *Rosse Mary Falcón Antenucci*, pelo bom humor, esforço e responsabilidade, *Simone Watanabe*, pelo carinho e dedicação e **Vanessa Peret Jabob**, pela elegância e carinho.

As amizades que encontrei em Chicago: *Maria Runa* (minha irmazinha Portuguesa), por ser minha companheira de moradia, pela verdadeira amizade, ajuda e companheirismo, *Luisana Echandi*, pela agradável convivência e amizade, *Jorgina Pereira*, pelo humor peculiar, hospitalidade e ajuda, *Simone Ballack*, pelos momentos inegualáveis de muita alegria e diversão durante as sessões de cinema e compras, *Edilson Lima e Vanessa Luiz*, por alegrar os meus domingos, *Nodesh Shyamsunder*, pela verdadeira amizade, companheirismo e agradável convivência, *Jasleen Raina*, pela generosidade e receptividade e *Isacc* pela preocupação e sensatez.

A todos os *meus amigos e familiares* que sempre torceram pelo meu sucesso, muito obrigado.

Epígrafe

A coisa mais importante é ter as mãos limpas

Mathew T Mathew

RESUMO GERAL

Barão VAR. **Avaliações Biomecânicas e Biológicas Relacionadas às Próteses Implanto-Suportadas e aos Implantes Osseointegrados** [tese]. Araçatuba: Faculdade de Odontologia da Universidade Estadual Paulista; 2011.

Resumo Geral

Objetivos: (1) Investigar o papel de diferentes níveis de pH da saliva artificial (3; 6,5 e 9) no comportamento corrosivo do titânio comercialmente puro (cp-Ti) e da liga Ti-6Al-4V (Capítulo 1); (2) Avaliar a influência do processo de corrosão na afinidade do lipopolissacarídeo (LPS) de *Escherichia coli* para o cp-Ti e o Ti-6Al-4V (Capítulo 2); (3) Investigar a natureza tribocorrosiva do titânio em saliva artificial em presença de LPS (Capítulo 3); (4) Comparar o efeito de diferentes designs de overdentures implanto-retidas e prótese total fixa implanto-suportada na distribuição de tensões em mandíbula edêntula pelo método de elementos finitos tridimensional (Capítulo 4).

Materiais e métodos: Testes eletroquímicos como potencial de circuito aberto, espectroscopia de impedância eletroquímica, e teste potenciodinâmico foram conduzidos. Para o teste de afinidade ao LPS, os espécimes foram tratados com LPS (1,5; 15 e 150 μg/ml) durante 24 horas para avaliar a aderência de LPS. Os discos foram transferidos a cada 24 horas para solução fresca de água livre de LPS até completar 72 horas, para investigar a liberação de LPS. No teste de tribocorrosão, o pH da saliva (6,5), a concentração de LPS (0, 0,15, 15 and 150

µg/ml), a duração do deslizamento (2000 ciclos), freqüência (1,2 Hz) e carga (20 N) foram usados para mimetizar o processo mastigatório. Finalmente, modelos tridimensionais de uma mandíbula edêntula foram construídos. No grupo OR a mandíbula foi restaurada com overdenture retida por implantes não esplitados com sistema de retenção O'ring; nos grupos BC-C e BC as mandíbulas foram restauradas com overdentures retidas por implantes esplintados com sistema de retenção barra-clipe associado ou não a cantilevers distais, respectivamente; no grupo FD a mandíbula foi restaurada com prótese total fixa suportada por implantes. As tensões de von Mises (σ_{vM}), máxima (σ_{max}) e mínima (σ_{min}) tensões principais foram obtidas.

Resultados: A saliva ácida aumentou a taxa de corrosão do cp-Ti e da liga Ti-6Al-4V e promoveu maior aderência de LPS na superfície dos discos de Ti ($P<0,05$). A liga Ti-6Al-4V exibiu maior afinidade ao LPS ($P<0,05$). O LPS afetou o comportamento tribocorrosivo de ambos os tipos de Ti. O LPS estatisticamente acelerou a troca iônica entre o Ti e a saliva, e reduziu a resistência da superfície do Ti à corrosão ($p<0,05$). O deslizamento reduziu a proteção da superfície do Ti. No geral, a liga Ti-6Al-4V exibiu melhor comportamento corrosivo, mas ambos os tipos de Ti mostraram perda de peso similares ($p>0,05$). O LPS sigificantemente aumentou a perda de peso do cp-Ti ($p=0,041$), e sua rugosidade ($p<0,001$). O grupo BC-C exibiu os maiores valores de tensões enquanto que o grupo FD mostrou as menores tensões nos implantes/componetes protéticos. No grupo das overdentures, o uso de implantes não esplintados reduziu as tensões nos implantes/componentes protéticos e tecidos de suporte.

Conclusões: O nível de pH da saliva influenciou no comportamento corrosivo do cp-Ti e da liga Ti-6Al-4V, no qual o pH ácido acelerou a taxa e a cinética de corrosão. O LPS afetou negativamente o comportamento tribocorrosivo do Ti. Clinicamente, a corrosão/desgaste do titânio e sua afinidade ao LPS pode influenciar na inflamação peri-implantar e no prognóstico do implante. O uso de prótese total fixa implanto-suportada e prótese total removível retida por implantes não esplinatados para reabilitar mandíbula edêntula reduziu as tensões no tecido ósseo peri-implantar, mucosa e implantes/componentes protéticos.

Palavras-chave: Eletroquímica; Titânio; Implante dentário; Biomecânica; Análise de elemento finito

GENERAL ABSTRACT

Barão VAR. **Biomechanical and Biological Evaluations of Implant-Supported Prosthesis and Osseointegrated Implants** [Thesis]. Araçatuba: Univ Estadual Paulista; 2011.

General Abstract

Objectives: (1) To investigate the role of different levels of pH of artificial saliva (pHs 3, 6.5 and 9) under simulated oral environment on the corrosion behavior of commercially-pure titanium (cp-Ti) and Ti-6Al-4V alloy (Chapter 1); (2) To assess the influence of corrosion process on *Escherichia coli* lipopolysaccharide (LPS) affinity for cp-Ti and Ti-6Al-4V alloy (Chapter 2); (3) To evaluate the tribocorrosive (corrosion/wear) nature of titanium in artificial saliva (pH 6.5) with LPS (Chapter 3); and (4) To compare the effect of different designs of implant-retained overdentures and fixed full-arch implant-supported prosthesis on stress distribution in edentulous mandible by using a three-dimensional finite element analysis.

Materials and methods: Standard electrochemical tests, such as open circuit potential, electrochemical impedance spectroscopy, and potentiodynamic tests were conducted in a controlled environment. For LPS affinity test, specimens were treated with LPS (1.5, 15 and 150 μg/ml) for 24 hours to evaluated LPS adherence. Discs were then transferred every 24 hours to fresh LPS-free water, up to 72 hours, to investigate LPS elution. In the tribocorrosion test, the pH of

saliva (6.5); LPS concentration (0, 0.15, 15 and 150 µg/ml), sliding duration (2000 cycles), frequency (1.2Hz) and load (20 N) parameters mimicked the mastication process. Finally, tridimensional models of an edentulous mandible were constructed. In the OR group, the mandible was restored with an overdenture retained by unsplinted implants with O'ring attachment; in the BC-C and BC groups, the mandibles were restored with overdentures retained by splinted implants with bar-clip anchor associated or not with distally placed cantilevers, respectively; in the FD group, the mandible was restored with a fixed full-arch implant-supported prosthesis. The von Mises stress (σ_{vM}), the maximum (σ_{max}) and minimum (σ_{min}) principal stresses were obtained.

Results: Acidic saliva increased the corrosion rate of cp-Ti and Ti-6Al-4V alloy, and promoted greater LPS adherence to Ti surfaces ($P<.05$). Ti-6Al-4V alloy exhibited greater LPS affinity ($P<.05$). LPS affected the tribocorrosive behavior of both Ti types. LPS statistically accelerated the ion exchange between Ti and saliva, and reduced the resistance of the Ti surface against corrosion ($p<.05$). Sliding decreased the protectiveness of Ti surface. In general, Ti-6Al-4V alloy exhibited better corrosion behavior, but both Ti types showed similar total weight loss ($p>.05$). LPS significantly increased the cpTi weight loss ($p=.041$), and its roughness ($p<.001$). BC-C group exhibited the highest stress values while FD group showed the lowest one in the implant/prosthetic components. Within overdenture groups, the use of unsplinted implants reduced the stress level in the implant/prosthetic components and supporting tissues

Conclusions: The pH level of artificial saliva influenced the corrosion behavior of cp-Ti and Ti-6Al-4V alloy in that lower pH accelerated the corrosion rate and kinetics. LPS negatively affected the corrosion/wear behavior of Ti. Clinically, corrosion/wear of Ti and its surface affinity for LPS could influence periimplant inflammation and implant prognosis. The use of fixed implant dentures and removable dentures retained by unsplinted implants to rehabilitate completely edentulous mandible reduced the stresses in the periimplant bone tissue, mucosa and implant/prosthetic components.

Key-words: Eletrochimica; Titanium; Dental Implant; Biomecanics; Finite element analysis

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Lista de Abreviaturas

Ti	= Titanium
Al	= Aluminum
V	= Vanadium
Ni	= Nickel
Co	= Cobalt
Fe	= Iron
C	= Carbon
Nb	= Niobium
Zr	= Zirconium
KCl	= Potassium chloride
NaCl	= Sodium chloride
$CaCl_2$	= Calcium chloride
H_2O	= Water
NaH_2PO_4	= Sodium phosphate
Na_2S	= Sodium sulfide
cp-Ti	= Commercially-pure titanium
TiAlV	= Titanium-Aluminium-Vanadium
TiO_2	= Titanium oxide
O_2	= Oxigen
H_2	= Hydrogen

N_2	= Nitrogen
Z'	= Real component of impedance
Z"	= Immaginary component of impedance
Z	= Total impedance
SCE	= Saturated calomel electrode
OCP	= Open circuit potential
I_{corr}	= Corrosion current density
I_{pass}	= Passivation current density
E_{corr}	= Corrosion potential
E_{pass}	= Passivation potential
R_{sol}	= Resistance of solution
CPE	= Constant-phase element
C_{dl}	= Capacitance of double layer
R_p	= Polarization resistance
$C_{dl\ in}$	= Inner layer capacitance of double layer
$R_{p\ in}$	= Inner layer polarization resistance
$C_{dl\ out}$	= Outer layer capacitance of double layer
$R_{p\ out}$	= Outer layer polarization resistance
XPS	= X-ray photoelectron spectroscopy
EDS	= Energy-dispersive spectroscopy
PS	= Potentiostatic test
LPS	= Lipopolissacharide
EIS	= Electrochemical Impedance Spectroscopy

SEM	= Scanning electron microscopy
Mm	= Millimiter
MPa	= Megapascal
N	= Newton
µm	= Micrometer
Nm	= Nanometer
%	= Porcentage
G	= Gram
$\mathbf{K_{wc}}$	= Total weight loss due to wear and corrosion (tribocorrosion)
$\mathbf{K_c}$	= Total weight loss due to corrosion
$\mathbf{K_w}$	= Total weight loss due to (mechanical) wear
$\mathbf{K_{wo}}$	= Wear rate in the absence of corrosion
$\mathbf{\Delta K_w}$	= Effect of corrosion on the wear rate
$\mathbf{K_{co}}$	= Corrosion rate in the absence of wear
$\mathbf{\Delta K_c}$	= Effect of wear on the corrosion rate.
M	= Atomic mass
I	= Total current
T	= Total exposure time
N	= Number of electrons involved in the corrosion process
F	= Faraday's Constant
V	= Volt
Q	= Charge passed through the working electrode

g/mol	= Gram per mol
EU	= Endotoxin unit
EU/ml	= Endotoxin unit per milliliter
µl	= Microliter
A/cm^2	= Area per centimeter square
cm^3	= Centimiter cubic
µg/ml	= Microgram per milliliter
e^-	= Electron
M^+	= Metal ions
3-D	= Three-dimensional
ASTM	= American Society for Testing of Materials
Vs	= Versus
Ra	= Roughness average
RMS	= Root mean square
Min	= Minute
g/l	= Gram per milliliters
NaOH	= Sodium hydroxide
cm^2	= Centimiters square
Ml	= Milliliters
^oC	= Degree Celcius
KHz	= KiloHertz
mHz	= Millihertz

Hz	= Hertz
mV/sec	= Millivolts per second
S	= Second
F	= Faraday
E. coli	= *Escherichia coli*
LAL	= *Limulus* amoebocyte lysate
i.e.	= In others words
e.g.	= Exempli gratiã (for exemple)
wt%	= Percentage of total weight
FEA	= Finite Element Analysis
MEF	= Método de Elementos Finitos
σ_{vM}	= von Mises stress
σ_{max}	= Maximum principal stress
σ_{min}	= Minimum principal stress
T	= Temporal
LP	= Lateral Pterygoid
MP	= Medial Pterygoid
M	= Masseter

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INTRODUÇÃO GERAL

1 Introdução Geral

A melhora da saúde e do bem estar dos seres humanos é a principal consideração de qualquer pesquisa. Pesquisadores da área médica têm voltado sua atenção para a odontologia já que a saúde oral é vital para o indivíduo manter uma vida normal. Na realidade, a completa saúde do sistema oral é influenciada por diversos fatores como a condição/estrutura do dente natural, a manutenção freqüente do sistema oral, a saúde do sistema digestivo e os hábitos alimentares [1]. Entretanto, uma vez que o sistema oral é prejudicado, o cirurgião dentista é responsável por substituir ou reparar os danos dentários a fim de manter o sistema funcionalmente ativo.

O uso dos implantes osseointegráveis tem sido difundido amplamente [2] como uma alternativa cada vez mais importante para determinados tratamentos dentários convencionais. O titânio comercialmente puro é o material mais comumente utilizado para a fabricação dos implantes dentários por apresentar uma boa resistência mecânica, ser resistente à corrosão, causar reação inflamatória mínima e apresentar um módulo de elasticidade semelhante ao tecido ósseo [3, 4]. No entanto, estudos recentes têm mostrado que o titânio pode degradar/corroer quando exposto a substâncias químicas, tais como ácido, flúor e saliva [4-12]. O processo de corrosão pode afetar a biocompatibilidade e a função dos implantes dentários, podendo levar a sua falha [8].

Durante a mastigação, o implante dentário é exposto a ações mecânicas (movimentos de atrição), mudanças de temperatura, variações na pressão de oxigênio e variações químicas do ambiente (e.g. mudanças de pH), sendo submetido a um processo contínuo e complexo de degradação [11, 13]. A presença de eletrólitos na saliva pode criar danos adicionais por induzir uma fenda de corrosão, sendo essa encontrada em muitos implantes dentários [8]. A saliva ácida, resultante de infecções ou de certos alimentos, também pode contribuir para a corrosão dos implantes dentários [4, 8]. Também tem sido relatado que as reações de corrosão podem ocorrer em superfícies metálicas expostas a tecidos e fluidos. Esses produtos da corrosão podem induzir reações inflamatórias, levando à liberação de mediadores inflamatórios como macrófagos, o que resultaria em reabsorção óssea [8, 14]. Dessa forma, no Capítulo 1 avaliamos o papel de diferentes pHs da saliva no comportamento corrosivo do titânio comercialmente puro e a liga Ti-6Al-4V em um ambiente oral simulado.

Apesar de diversos estudos terem focado na corrosão química da superfície do titânio, poucos avaliaram o efeito combinado da ação mecânica e química desse processo, o que se assemelha à condição clínica real. Uma nova área de investigação multidisciplinar denominada *tribocorrosão* pode resolver essas questões. Por definição, tribocorrosão é uma transformação irreversível de um metal/liga resultante de interações físico-química e mecânica simultâneas em um contato tribológico. A combinação de processos como deslizamento, rolamento, atrição, impacto, erosão, etc, juntamente com ataque químico ou

eletroquímico (presença de saliva) pode resultar em várias formas de degradação desde a perda de material até delaminação e rachaduras. Além disso, quando a tribocorrosão ocorre em meio biológico, como no sistema bucal, a presença de espécies biológicas aumenta a complexidade para o entendimento da cinética de degradação dos materiais e da interação de ambos os mecanismos de condução [15].

Estes testes laboratoriais são importantes para compreender o comportamento dos materiais dentários antes da sua utilização clínica. Além disso, esses estudos vão ajudar na seleção de materiais adequados e no desenvolvimento de guias e protocolos de saúde para o cirurgião dentista e para os pacientes [12, 16].

Na cavidade oral, além das forças mecânicas, os implantes também estão expostos a agentes agressivos como o biofilme bacteriano e a saliva [7]. Este ambiente é particularmente favorável para a biodegradação dos metais devido a suas propriedades térmica, iônica, microbiológica e enzimática [17].

Bactérias periodontopatogênicas ocorrem mais comumente nos tecidos periimplantares com inflamação [18]. Estas bactérias podem contribuir para periimplantites em pacientes parcialmente e completamente desdentados [19].

Bactérias gram-negativas produzem endotoxina, que é um lipopolissacarídeo (LPS) presente nas paredes celulares bacterianas [18, 20]. Existe considerável evidência da ação do LPS na patogênese da periodontite crônica. Esta toxina, que é encontrada em grandes quantidades na superfície dos dentes periodontalmente afetados, tem um efeito sobre determinadas células,

tais como macrófagos, linfócitos, fibroblastos e osteoblastos [21]. No sulco natural, estes efeitos podem contribuir para a inflamação, osteoclasia, inibição do crescimento celular e cicatrização demorada [22-24]. Assim como no sulco natural, o LPS produzido pela *P. gingivalis* e outras bactérias gram-negativas na região periimplantar é um fator que influencia a inflamação periimplantar e o prognóstico do tratamento com implante [18].

A inflamação periodontal pode resultar de uma interação entre materiais restauradores e macromoléculas de bactérias gram-negativas como o LPS no sulco natural [18]. Quirynen et al. [25] observaram que os fatores de risco associados com periimplantites parecem estar relacionados com a composição do ambiente bacteriano ao redor do implante e a capacidade das bactérias em aderir ao material do implante. Estudos *in vitro* [22-24] têm mostrado a afinidade do LPS de *Porphromonas gingivalis* e *Escherichia coli* para materiais de próteses fixas. Esta afinidade mostrou-se relacionada a fatores como o tipo de LPS, o tipo de material, as propriedades de superfície e o pH. A afinidade do LPS para os materiais, avaliada pela adesão e liberação das moléculas do LPS na superfície do material restaurador, pode influenciar a concentração da toxina dentro do sulco. Se a interação entre um material e o LPS aumentar os níveis de toxinas no fluido gengival e tecidos circundantes, a resposta biológica poderá ser desfavorável [18].

As modificações de superfície dos materiais de titânio devido ao ataque eletroquímico podem ser um fator importante para a afinidade ao LPS. Portanto,

no Capítulo 2 investigamos a influência do processo corrosivo na afinidade do LPS de *Escherichia coli* para o titânio comercialmente puro e a liga Ti-6Al-4V.

Sabendo que os implantes dentários são submetidos a diferentes fatores adversos de origem mecânica, química e microbiológica na cavidade oral, o que poderia comprometer a sua longevidade, no Capítulo 3 simulamos esses três fatores a fim de investigar o comportamento tribocorrosivo (corrosão/desgaste) do titânio comercialmente puro e da liga Ti-6Al-4V.

Como citado anteriormente, os implante dentários são submetidos a ações mecânicas durante a mastigação. Estudos relacionados à biomecânica [26-29] têm mostrado que uma das principais causas da reabsorção óssea esteja ligada ao excesso de carga sobre o implante, já que este, quando submetido a cargas funcionais, transmite as tensões geradas diretamente ao osso [30]. Alguns fatores podem influenciar na transmissão dos esforços ao tecido ósseo, como o tipo de carga, as propriedades dos materiais do implante e da prótese, a geometria e o tipo de superfície do implante, a qualidade e quantidade do tecido ósseo, a natureza da interface osso-implante e o tipo de prótese [31].

Estudos relacionados à bioengenharia, ou seja, a aplicação dos conhecimentos da área de engenharia na odontologia, e mais especificamente na implantodontia, tem destacado a análise do comportamento biomecânico dos implantes osseointegrados e suas próteses [26-29, 32-43]. Com ferramentas da engenharia é possível avaliar as tensões geradas nos implantes, bem como avaliar as deformações dos componentes protéticos, sendo possível quantificá-las. Tais dados têm extrema importância para o desenvolvimento de novas

técnicas e aprimoramento das características dos próprios implantes, de forma a suprir as necessidades clínicas. Clinicamente não é possível estudar a distribuição de tensões em nível ósseo, apenas em nível dos *abutments*, através da análise com *strain gauges*, por exemplo. Entretanto, existem outras metodologias que se baseiam em simulações, como a fotoelasticidade e o método de elementos finitos (MEF), que possibilitam melhor compreensão do mecanismo de transmissão e distribuição das tensões das cargas funcionais ao osso via implantes [32].

O MEF apresenta vantagens em relação às outras duas metodologias citadas no que diz respeito à possibilidade da individualização das estruturas nos modelos (fibromucosa, osso cortical, osso medular, implantes, componentes protéticos e prótese), e na análise dos resultados que se apresentam na forma de mapas de tensões, com escala de cor que evidencia as áreas de maior e menor tensão nas estruturas. O mesmo não é possível com a fotoelasticidade, que se baseia na construção de um modelo fotoelástico, no qual o osso cortical e o osso medular são representados por uma única resina fotoelástica, não havendo diferenciação entre eles. Além disso, a fibromucosa é representada, geralmente, por uma camada de material de moldagem resiliente sobre o modelo fotoelástico, o que não permite a observação das tensões. Como os implantes e componentes utilizados nos estudos fotoelásticos são reais, não é possível também a análise das tensões em seu interior, que tem grande relevância clínica e científica, o que apresentaria grande interesse no

desenvolvimento de novos desenhos e materiais que favoreçam a biomecânica [44].

Diversas são as formas de reabilitação protética para mandíbula edêntula, sendo o tratamento clássico, a confecção de próteses totais convencionais [45]. Outra opção seria a colocação de três a seis implantes na região anterior da mandíbula, usados para suporte de próteses totais tipo protocolo, podendo ainda, optar-se pela instalação de dois a quatro implantes na mesma região para retenção de uma overdenture [33].

Como não existe consenso sobre um tratamento padrão para pacientes edêntulos mandibulares e a literatura a respeito da análise comparativa entre overdentures e próteses do tipo protocolo se limita a estudos clínicos relacionados ao sucesso, custo do tratamento e satisfação do paciente, no Capítulo 4 comparamos o efeito de diferentes designs de overdentures implanto-retidas e prótese total fixa implanto-suportada (protocolo) na distribuição de tensões em mandíbula edêntula por meio do método de elementos finitos tridimensional (MEF-3D).

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CAPÍTULO 1 *

Estabilidade do cp-Ti e da Liga Ti-6Al-4V para Implantes Dentários em Função do pH Salivar – um Estudo Eletroquímico

Stability of cp-Ti and Ti-6Al-4V Alloy for Dental Implants as a Function of Saliva pH – an Electrochemical Study

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Artigo aceito para publicação no *Clinical Oral Implants Research*, e suas normas de publicação encontram-se disponíveis no anexo B.

Estabilidade do cp-Ti e da Liga Ti-6Al-4V para Implantes Dentários em Função do pH Salivar – um Estudo Eletroquímico

2.1 Resumo

Objectivos: Investigar o papel de diferentes níveis de pH da saliva artificial em ambiente oral simulado no comportamento corrosive do titânio comercialmente puro (cp-Ti) e da liga Ti-6Al-4V. Atenção especial é dada no entendimento das mudanças na cinética de corrosão e na caracterização de superfície do Ti por meio da espectroscopia de impedância eletroquímica (EIS).

Materiais e métodos: Cinquenta e quatro discos de titânio (15 mm de diâmetro e 2 mm de espessura) foram divididos em 6 grupos (n=9) em função do pH salivar (3, 6,5 e 9) e tipo de Ti. Os espécimes foram mecanicamente polidos de acordo com procedimentos metalográficos. Testes eletroquímicos padrões tais como potencial de circuito aberto, EIS, e teste potenciodinâmico foram conduzidos em um ambiente controlado. Os dados foram avaliados por meio da ANOVA de dois fatores, teste múltiplo de Tukey e teste independente de T (α=0,05). As superfícies do Ti foram examinadas por meio da microscopia de interferometria de luz branca e pela microscopia eletrônica de varredura.

Resultos: O pH salivar afetou significantemente o comportamento corrosivo de ambos os tipos de Ti. Em pH baixo foi observado uma aceleração da troca iônica entre o Ti e a saliva e uma redução na resistência à corrosão da superfície do Ti ($P<0,05$). A taxa de corrosão aumentou significantemente em meio ácido ($P<0,05$). Os diferentes tipos de Ti tiveram um comportamento corrosivo

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semelhantes. A microscopia de interferometria de luz branca mostrou maior alteração de superfície do Ti em pH baixo. A microscopia eletrônica de varredura não mostrou alterações detectáveis. Não foi observado corrosão por pites em nenhum grupo.

Conclusões: O nível de pH da saliva influencia no comportamento corrosive do cp-Ti e da liga Ti-6Al-4V, no qual o pH ácido acelera a taxa e a cinética de corrosão. Os produtos de corrosão podem reduzir a taxa de sobrevida dos implantes dentários.

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Stability of cp-Ti and Ti-6Al-4V Alloy for Dental Implants as a Function of Saliva pH – an Electrochemical Study

2.2 Abstract

Objectives: To investigate the role of different levels of pH of artificial saliva under simulated oral environment on the corrosion behavior of commercially-pure titanium (cp-Ti) and Ti-6Al-4V alloy. Special attention is given to understand the changes in corrosion kinetics and surface characterization of Ti by using electrochemical impedance spectroscopy (EIS).

Materials and methods: Fifty-four Ti discs (15-mm diameter, 2-mm thickness) were divided into 6 groups (n=9) as a function of saliva pH (3, 6.5 and 9) and Ti type. Samples were mechanically polished using standard metallographic procedures. Standard electrochemical tests, such as open circuit potential, EIS, and potentiodynamic tests were conducted in a controlled environment. Data were evaluated by two-way ANOVA, Tukey multiple comparison test, and independent *t*-test (α=.05). Ti surfaces were examined using white-light-interferometry microscopy and scanning electron microscopy (SEM).

Results: Saliva pH level significantly affected the corrosion behavior of both Ti types. At low pH, acceleration of ions exchange between Ti and saliva, and reduction of resistance of Ti surface against corrosion were observed (P<.05). Corrosion rate was also significantly increased in acidic medium (P<.05). Similar corrosion behavior was observed for both Ti types. The white-light-interferometry images of Ti surfaces show higher surface changes at low pH

level. SEM images do not show detectable changes. No pitting corrosion was observed for any groups.

Conclusions: The pH level of artificial saliva influences the corrosion behavior of cp-Ti and Ti-6Al-4V alloy in that lower pH accelerates the corrosion rate and kinetics. The corrosion products may mitigate the survival rate of dental implants.

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2.3 Introdução (Introduction)

Commercially pure titanium (cp-Ti) and Ti alloys (such as Ti-6Al-4V) have been widely used to fabricate dental implants due to their resistance to corrosion and biocompatibility (Cortada et al. 2000; Schiff et al. 2002; Mabboux et al. 2004; Vieira et al. 2006). When exposed to the environment, a stable and dense oxide layer (TiO_2) forms on the surface of Ti, which plays a role in Ti corrosion resistance (Huang 2003). Nevertheless, when Ti is subjected to acid, fluoride and saliva, the protectiveness of the oxide film can be lost and a corrosion process is initialized (Nikolopoulou 2006; Correa et al. 2009). This may affect the biocompatibility and function of dental implants and possibly lead to failure of the implant in the oral cavity (Nikolopoulou 2006).

In the oral environment, dental implants are exposed to several adverse factors such as change of temperature and oxygen level (due to the food and changes in the saliva), masticatory force, and chemical components which contribute to the degradation process of implants (Nakagawa et al. 2002). In addition, the eletrolytes from saliva can induce crevice corrosion in the peri-prosthetic space of the implant (Nikolopoulou 2006). The pH of saliva varies in areas around dental implants. Food such as milk calcium-fortified foods and nuts can alkalify the oral pH (Murrell et al. 2010). Several situations can acidify the pH of saliva such as infections, certain foods (sugary food, pickled foods, sour candies, fruits, soft drinks and juices – pH around 2.5-3.5), mouthwash products (Murrell et al. 2010, Dong et al. 1999, Gregory-Head et al. 2000), smoking,

chronic/systemic diseases and medication, which may also contribute to the corrosion of dental implants (Nikolopoulou 2006; Vieira et al. 2006; Correa et al. 2009). As a consequence of those corrosion products, inflammatory mediators produced by macrophages are released, contributing to bone loss (Nikolopoulou 2006; Denaro et al. 2008). This issue has been investigated in depth in orthopedics. In dentistry, literature that describes the clinical implications of the corrosive nature of dental implants is limited. Hence, there is an urgent need to conduct ~~a~~ research to gather indepth knowldege in the area.

Some studies have evaluated the corrosion profile of Ti and Ti-based alloys in presence of fluoride (Nakagawa et al. 1999; Schiff et al. 2002; Huang 2003; Correa et al. 2009; Mereci et al. 2009), albumin (Huang 2003), bacteria (Laurent et al. 2001; Souza et al. 2010), inflammatory and hyperglycemic conditions (Lin & Bumgardner 2004; Messer et al. 2009; Messer et al. 2010) and lipopolysaccharide (Barao et al. 2011). In general, those situations accelerated the corrosion process of Ti and Ti alloys. Recent studies have evaluated the effects of acidic pH of electrolyte solution on titanium corrosive behavior (Souza et al. 2009; Mereci et al. 2009). Souza et al. (2009) observed that acidic pH of Ringer solution (pH 5.5) decreased the resistance of corrosion of Ti-13Nb-13Zr alloy. This trend was not observed for Ti-6Al-4V alloy. Mereci et al. (2009) showed increased corrosion of Ti-Ta alloy and Ti-6Al-7Nb alloy in the presence of acidified artificial saliva (pH 2.5).

The literature on the effect of pH on the corrosion behavior of Ti is limited, and no study has investigated the effect of a wide pH range of artificial

saliva that replicate the intraoral condition on corrosive kinetics of titanium. The current and potential gradients induced by pH changes lead to enhancement of corrosion on same areas of the implant (Souza et al. 2009). Therefore, in this study, the role of different pHs (3.0, 6.5 and 9.0) on the corrosive behavior of cp-Ti and Ti-6Al-4V alloy in an oral environment was investigated. The specific aim of this study was to understand the corrosion kinetics and surface morphology of Ti by using electrochemical impedance spectroscopy (EIS) as well as basic corrosion tests. The research hypothesis was that acidic saliva decreases the corrosion resistance of both Ti types. In addition, the authors hypothesized that the corrosion rate and corrosion kinetics of cp-Ti and Ti-6Al-4V alloy are not different.

2.4 Materiais e métodos (Materials and methods)

Specimen Preparation for Electrochemical Test

In the present study, a total of 54 cp-Ti and Ti-6Al-4V alloy discs, 15-mm diameter and 2-mm thickness, were milled from titanium rods purchased from commercial company through a supplier Mac-Master Carr, Elmhurst, IL, USA. The samples were divided into 6 groups (n=9) as a function of different artificial saliva pH (3, 6.5 and 9) and Ti type. Before the tests, all samples were polished using grinding papers (#320, #400, #600, #800) (Carbimet 2, Buehler, Lake Bluff, IL, USA), and polishing cloth (TextMet Polishing Cloth, Buehler) with diamond paste (MetaDi 9-micron, Buehler), and lubricant (MetaDi Fluid, Buehler). Polishing cloth (Chemomet I, Buheler) with colloidal silica polishing suspension (MasterMed, Buehler) was used as a final polishing procedure in order to obtain a mirror surface of the Ti discs (Roughness average (Ra) = 12.443±3.797 nm and Root Mean Square (RMS) roughness = 15.980±3.944 nm for cp-Ti; Ra = 5.022±1.552 nm and RMS = 6.773±1.858 nm for Ti-6Al-4V alloy). Samples were ultrasonically cleaned (FS 20, Fisher Scientific Inc., Pittsburg, PA, USA) with deionized water (10 min) and 70% propanol (10 min), and finally dried with hot air of 250 ^{o}C. The nominal chemical composition of cp-Ti and Ti-6Al-4V alloy is shown in Table 1. The composition of artificial saliva (Liu et al. 2007) was KCl (0.4 g/L), NaCl (0.4 g/L), $CaCl_2 \cdot 2H_2O$ (0.906 g/L), $NaH_2PO_4 \cdot 2H_2O$ (0.690 g/L), $Na_2S \cdot 9H_2O$ (0.005 g/L), and urea (1 g/L). Different pH values were achieved by adding lactic

acid (acidic) or NaOH (basic) in an appropriate amount and evaluated in a pH meter (Accumet AB15, Fisher Scientific Inc.).

Electrochemical Tests

A custom made acrylic electrochemical cell was used to conduct the tests (Figure 1). All the measurements were performed in a standard 3-electrode setting according to the American Society for Testing of Materials (ASTM) guidelines (G61 and G31-72). A saturated calomel electrode (SCE) was used as the reference electrode, graphite rod as the auxiliary electrode, and the exposed surface of the Ti as the working electrode (the exposed area was 1.77 cm^2). A potentiostat (G300, Gamry Inc., Warminster, PA, USA), linked to a computer for data acquisition, was used to perform the corrosion measurements. A total volume of 10 ml of electrolyte was used for each corrosion experiment. After mounting the sample into the cell, approximately 4 hours were allowed to achieve electrochemical stabilization of the system. The temperature of the test solution was maintained at 37 ± 1°C to mimic the oral environment.

Initially, open circuit potential (OCP) was monitored during a period of 3600 seconds to evaluate the potential and to stabilize the system; then, EIS test was conducted to investigate the electrochemical double layer formation and the properties of the oxide film formed on the Ti surface (corrosion kinetics). Through the EIS results, electrochemical process can be represented by an equivalent electrical circuit consist of the capacitance and resistance that assist in quantifying the corrosion process. The EIS measurements were performed in

the frequency range from 100 KHz to 5 mHz, with AC sine wave amplitude of 10 mV applied to the electrode at its corrosion potential. These values were used to determine the real (Z') and imaginary (Z") components of the impedance, which were plotted with the Nyquist plot, or the total impedance (|Z|) and phase angle. Finally, the samples were cycle polarized from -0.8 V to 1.8 V and from 1.8 V to -0.8 V at a scan rate of 2 mV/sec.

Corrosion parameters were obtained from the potentiodynamic polarization curves. Tafel's method was used to investigate the corrosion rate of Ti such as corrosion current density (I_{corr}). The passivation current density (I_{pass}) corresponds to the current value in the transition from the active region to the passive state of Ti. EIS results were used to model the corrosion process (kinetics) and to understand the properties of the oxide film formed on the Ti surface, using a simple equivalent circuit (Randle's circuit). For EIS data simulations (capacitance of double layer - C_{dl}, and polarization resistance – R_p), the Zview2 software (Scribner Associates Inc., Southern Pines, NC, USA) was used.

Surface characterization

A white light interferometry microscope (Zygo New View 6300, Zygo Corporation, Middlefield, CT, USA) was used to capture three-dimensional (3-D) changes between non-corroded and corroded Ti surfaces. In order to understand the mechanical changes on the Ti surface produced by the corrosion, two parameters of surface roughness (Ra and RMS) were investigated before

(baseline) and after corrosion using a white light interferometry microscope (Zygo New View 6300, Zygo Corporation). A scanning electron microscope (SEM) (Joel JSM-6490 LV, Oxford Instruments, Oxford, UK) was used for further surface characterization. Energy-dispersive spectroscopy (EDS) was used for chemical characterization of the Ti surface.

Statistical Analysis

The corrosion data were tested separately for I_{corr}, I_{pass}, C_{dl} and R_p. For each parameter, two-way ANOVA design was conducted to explore the effects of both Ti type (factor 1, two levels) and saliva pH (factor 2, three levels), as well as their interaction on corrosive behavior of the Ti. Subsequent pairwise comparisons within groups were made using Tukey's post hoc analysis. Two-sample independent t-test was performed to compare the surface roughness between cpTi and Ti-6Al-4V. The level of statistical significance was considered to be 0.05 (Statistical Package for the Social Sciences, version 17.0; SPSS Inc, Chicago, IL, USA).

2.5 Resultados (Results)

Cycle polarization curves and evolution of electrochemical parameters

The cyclic polarization curves of both Ti types were similar and revealed an active-to-passive transition in artificial saliva at different pH levels (Figure 2a). From the negative hysteresis, both cp-Ti and Ti-6Al-4V alloy are not susceptible to pitting and crevice corrosion in any pH of artificial saliva (Figure 2a, curved arrow). Surface examination of the discs after testing confirmed that pitting and crevice corrosion had not occurred. A passive stabilization region was observed for all groups (Figure 2a, straight arrow). Pitting potential was slightly higher for Ti-6Al-4V alloy.

Based on these curves, electrochemical parameters such as I_{corr} and I_{pass} were obtained and are presented in Figure 2 (b-c). I_{corr} and I_{pass} expressed the corrosion rate of a material. The higher the values of I_{corr} and I_{pass} were, the higher the corrosion rate. According to two-way ANOVA the interaction effect between Ti type and saliva pH was not statistically significant for I_{corr} ($P>0.05$) and I_{pass} values ($P>0.05$). On the other hand, the saliva pH significantly affected I_{corr} and I_{pass} values of both Ti types ($P<0.05$). For both Ti types, I_{corr} significantly decreased with the increased pH (cpTi, $P<0.001$; Ti-6Al-4V, $P<0.001$), indicating that at low pH the corrosion rate of Ti was significantly higher. Regardless of pH value, no significant difference of corrosion rate was observed between both Ti types ($P>0.05$) (Figure 2b). The I_{pass} tended to reduce at near to neutral and basic pHs (Figure 2c), corroborating with the I_{corr} results.

EIS measurements

The Nyquist (Figure 3a) and Bode plots (Figure 3b) provide the variation of impedance as a function of frequency of the electrochemical double layer formed at the interface of Ti-solution during corrosion process (corrosion kinetics). The Nyquist plots demonstrate the electrochemical resistance of Ti surface. Acidic pH decreased the semicircular diameter of capacitance loop, which means poor corrosion resistance for both cp-Ti and Ti-6Al-4V alloy. In the phase angle of Bode plots, just one time constant was observed for all groups, which indicates the presence of compact, homogeneous and protective passive film. At high frequency, both the phase angle and impedance were low. By decreasing the frequency, the impedance steadily increased with the increment in phase angle. At low frequencies the impedance tended to stabilize, and the phase angle for Ti in acidic media exhibited the lowest angle value, which characterize poorest corrosion resistance of Ti in saliva at low pH.

A simple equivalent circuit (Randle's circuit) (Figure 4a), which compromises the polarization resistance in parallel with the capacitance of double layer, in series with the resistance of solution (R_{sol}), was used for the EIS model. Using such modeling approach, it is possible to develop an electrical equivalent circuit to the electrochemical reactions at the metal-solution interface. R_{sol} is the uncompensated resistance of the electrolyte between the working and the reference electrode; R_p is the polarization resistance or the charge transfer resistance at the working electrode/electrolyte interface, related to the rate of corrosion reactions at the passive domain; C_{dl} is the specific

double-layer capacitance at the working electrode/electrolyte interface. Due to the inhomogeneous passive layer at the material surface, the capacitance is represented by a constant-phase element (CPE) as an alternative to an ideal capacitance element. Impedance experimental data were fitting with chi-square error <0.001 (Valero Vidal & Igual Muñoz 2008). Several studies (Maurer et al. 1993; Narayanan et al. 2007) have reported the effectiveness of EIS models in estimating the protectiveness of the oxide film formed on the Ti surface as a function of saliva pH levels. The evolution of C_{dl} and R_p are presented in Figure 4 (b-c). C_{dl} and R_p indicate the corrosion kinetics. Lower value of C_{dl} and higher value of R_{dl} indicated the lower corrosion resistance.

Two-way ANOVA revealed that the interaction effect between Ti type and saliva pH, and main effect of Ti type factor were not statistically significant for C_{dl} ($P>0.05$) and R_p ($P>0.05$). On the other hand, the pH of saliva significantly influenced the corrosion kinetics of Ti ($P<0.05$, ANOVA). The C_{dl} was significantly higher at acidic pH for both Ti types (cpTi, $P<0.001$; Ti-6Al-4V, $P=0.004$) (Figure 4b). The R_p was significantly lower at low pH for both Ti types (cpTi ,$P<0.001$; Ti-6Al-4V, $P<0.001$) (Figure 4c). Similar corrosion kinetics behavior was observed for cp-Ti and Ti-6Al-4V alloy (Figures 4b-c).

Surface characterization

The white light interferometry microscope images of Ti surfaces clearly showed higher surface changes at low pH level when compared to the non-corroded surface. Qualitatively, at basic pH, the Ti surface was close to the non-

corroded surface. Both Ti types exhibit similar visual observation (Figure 5). The surface roughness of cp-Ti was not affected by the pH of saliva (P=0.207 for Ra; P=0.207 for RMS). On the other hand, significant effect was observed for Ti-6Al-4V alloy (P<0.001 for Ra; P=0.001 for RMS). Both Ra and RMS roughness values were higher at acidic pH. Cp-Ti exhibited greater roughness value when compared to Ti-6Al-4V alloy before and after corrosion (Control: P<0.001; pH 3: P<0.001; pH 6.5: P=0.003; pH 9: P<0.001) (Table 2). SEM images did not show clear evidence of changes in the Ti surface as the result of exposure to artificial saliva at different pH levels (Figure 6). No pitting corrosion was observed for any groups, which correlates with the cyclic polarization curves. The α + β microstructure of the Ti-6Al-4V alloy was clearly visible on all SEM images throughout all conditions. Examples of EDS results are provided in Figure 7 (a-b). EDS mapping confirmed the presence of Ti and Carbon (C) in cp-Ti, while in Ti-6Al-4V alloy, presence of Ti, Aluminum (Al), Vanadium (V), C and Iron (Fe) was observed. The presence of C and Fe may be a result of any contamination and probably background noise from the specimen holder. The presence of Al may explain the light spots observed in SEM images. Comparing the spectra of cp-Ti and Ti-6Al-4V alloy after corrosion experiment (Figure 7) with the nominal chemical composition of both titanium types before corrosion (Table 1), revealed that the wt% of C increased after corrosion. C level increased from 0.006% to 1.79% for cp-Ti and from 0.004% to 3.03% for Ti-6Al-4V alloy. The wt% of other elements remained similar.

2.6 Discussão (Discussion)

The role of pH on the corrosion behavior

From the results of this study, it is clear that the corrosion behavior of Ti was affected by the pH levels of the surrounding artificial saliva ($P<0.05$, Two-way ANOVA). The research hypothesis that acidic saliva decreases the corrosion resistance of both Ti types and that no significant difference would be observed between cp-Ti and Ti-6Al-4V alloy was accepted.

The corrosion rate of both Ti types increased in acidic artificial saliva as observed in the I_{corr} and I_{pass} values. According to Assis et al. (2006) this phenomenon can be explained by the presence of some irregularities and porosities of the oxide layer formed on the Ti surface. In the present study, the white light interferometry images demonstrated that the oxide film loses its smoothness mainly at pH 3, which increased the surface area and consequently increased the corrosion. Therefore, the protective oxide film is degraded in acidic solution (Nakagawa et al. 1999; Souza et al. 2009).

Acidic saliva statistically accelerated the ions exchange between Ti and saliva, and reduced the resistance of Ti surface against corrosion as evidenced by the C_{dl} and R_p results, respectively. Therefore, the protectiveness of the oxide film is reduced at low pH (Souza et al. 2009) and may explain the high corrosion rate observed at pH 3. Souza et al. (2009) also found a poor corrosion resistance of Ti alloy (Ti-13Nb-13Zr and Ti-6Al-4V) in Ringer solution at low pH. An electrochemical mechanistic model is illustrated further.

In the present study, no significant different on the corrosion behavior between cp-Ti and Ti-6Al-4V alloy was observed. Similar results were observed by Schiff et al. (2002) who compared the corrosion resistance of NiTi, NiTiCo, and Ti-6Al-4V alloys with cp-Ti in Fusayama Meyer artificial saliva at pHs 2.5 and 5.3. Authors also noted a reduction of surface resistance against corrosion for all materials in acidified saliva, which correlates with the results of the current study.

Formation of passive film in cp-Ti and Ti-6Al-4V alloy

Ti is an electrochemically active material. However, when exposed to air, an oxide layer is formed on its surface which confers protection against corrosion to this metal (passivation) (Huang 2003). When Ti is subjected to chemical attack, acid solutions, and fluoride, the oxide layer can breakdown and corrosion takes place (Zavanelli et al. 2000; Huang 2003). For both Ti types, Ti oxide in different crystalline structure (anatase and rutile) is formed on Ti surface. The possible corrosion mechanisms during the formation of the passive film are illustrated in Figure 8. Electrons (e^-) are attracted from saliva by the metal ions (M^+) of Ti during the electrochemical reaction at the interface of exposed surface and solution. The current study demonstrates that the formation and structure of passive layer is influenced by the pH of the surrounding media, as observed by classical Pourbaix diagram (Mudali Kamachi & Raj 2008). The results from potentiodynamic tests and EIS tests indicate the intense nature of electrochemical reaction at low pH or acidic environment, which may explain the

greater corrosion observed in the present study at pH 3.0. Promisingly, there is no evidence of depassivation or repassivation mechanisms at high anodic potential of +1.8 V vs SCE in both materials at the three pH levels.

In the cyclic polarization measurements, the region of constant current with increasing potential suggests that both Ti types were passive and it is a typical electrochemical behavior of titanium. In addition, the negative hysteresis on the reverse scan as the current decrease suggests the passive layer did not breakdown in any situation. These results correlated with the SEM images, since pits formation was not observed on the Ti surface.

The results from Nyquist and Bode plots suggest that the oxide film formed on the surface of both Ti types is less protective in acidic medium. The decrease of R_p and increase of C_{dl} for both Ti types in saliva at low pH are related to the formation and growth of the passive film, which indicate the film compactness (Li & Zuo 2008). The changes of the components values in the Randle's circuit may reveal the protective effect of the passive film. According to the R_p and C_{dl} results, it can be stated that the protective effect of the passive film on both Ti types was decreased with decreasing pH value. These may explain the higher corrosion rate of Ti at low saliva pH. In addition, the similarity in the EIS spectra for cp-Ti and Ti-6Al-4V alloy can be due to their similar chemical compositions, and the variation in microstructure of these materials does not promote significant differences in corrosion behavior.

Practical and clinical implications of the study

In summary, the electrochemical tests performed in the present study revealed that acidic saliva was found to have a negative effect on cp-Ti and Ti-6Al-4V alloy corrosion resistance, causing changes in the protective passive layer of both materials.

Apparently, the elements present on both Ti surfaces were similar pre and post corrosion process except for the carbon content (evaluated by EDS). The electrochemical reaction between Ti and media (saliva) increased at low pH as observed in the Nyquist plots, which may lead to the release of corrosion products into the body system over time (Chaturvedi 2009). These corrosion products can promote oral and extra oral manifestations such as discoloration of soft tissues, allergic reactions (i.e. edema, stomatitis, gingivitis), and eczematous rashes (Chaturvedi 2009). The released ions from Ti inhibit the growth of hydroxyapatite crystals, which may lead to bone destruction (osteolysis), and loss of implant stability. Also, corrosion can reduce the fatigue life and ultimate strength of the material, which lead to mechanical failure of dental implants (Chaturvedi 2009).

Furthermore, corrosion induced by low saliva pH level tended to increase the roughness values of both types of Ti. In dentistry, roughness is an important property of adhesion and colonization of bacteria (Morgan & Wilson. 2001); therefore, a greater biofilm accumulation may be expected on the Ti surface after corrosion mainly at pH 3, which may lead to peri-implantites (Bollen et al. 1996). Although those extreme pH values (3 and 9) are not necessarily present in

real clinical situations, parameters chosen in this in vitro study attempted to reflect clinically relevant parameters as much as possible.

Results of this study indicated that the survival rate of dental implants may be affected by the different pHs of saliva. However, there is no systematic study in the literature illustrating the effect of corrosion products on the failure rate of dental implants. Perhaps it is hard to state that the failure of implant is due to corrosion alone, since the etiology of implant failure is multifactorial and required to be investigated with interdisciplinary approach.

Limitations and future scope

In the present study, only electrochemical evaluations were performed and masticatory loading was not considered. In addition, only smooth Ti surfaces were used. Further studies linking the corrosion mechanisms with tribology actions such as wear (under mastication cycles) to represent the clinical oral environment are warranted. Also, these studies should be extended to evaluate different surfaces of Ti dental implants. The applicability of employing suitable surface treatment/deposition techniques to enhance the overall performance of dental implants is essential in future research. The EIS tests were conducted at a potential of E_{corr} (corrosion potential) vs SCE in each testing conditions to simulate clinical oral environment. Such tests at other anodic potentials (e.g., E_{pass} – passivation potential) might provide further understanding of passive film growth.

Furthermore, EDS was used in this study to quantify the elemental components of the Ti surface. Although EDS is a good technique used in electrochemistry to characterize the passive film, it has some limitations in showing surface conditions. Further studies are warranted using X-ray photoelectron spectroscopy (XPS) to evaluate in more details the oxide layer on the Ti surface. In addition, a longer exposure of Ti to saliva is needed.

Acknowledgment

The authors would like to express their gratitude to CAPES (BEX: 4129/09-1) and FAPESP (2007/58926-5) for the scholarship provided to the first author (*Barão, V.A.R*). Authors would also like to thank Prof R Urban and Ms. D Hall for the assistance in SEM images.

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Tabelas (Tables)

Table 1. Nominal composition of cp-Ti and Ti-6Al-4V alloy

Ti type	Composition (in wt%)							
	Ti	**Al**	**V**	**C**	**Fe**	**O_2**	**N_2**	**H_2**
cp-Ti	99.7	-	-	0.006	0.12	0.16	0.004	0.0019
Ti-6Al-4V	89.62	6.1	4.0	0.004	0.16	0.106	0.008	0.0022

Table 2. Mean and standard deviation of surface roughness (Ra and RMS) (in nm) of cp-Ti and Ti-6Al-4V alloy as a function of corrosion process in different pH of saliva

Ti type	Roughness parameter	Corrosion process			
		Control (non corroded)	Corroded (pH 3)	Corroded (pH 6.5)	Corroded (pH 9)
cp-Ti	Ra	12.443±3.797[a]	15.649±4.839[a]	13.588±4.208[a]	13.890±4.269[a]
	RMS	15.980±3.944[a]	20.549±6.737[a]	17.114±1.715[a]	17.801±6.305[a]
Ti-6Al-4V	Ra	5.022±1.552[a]	9.278±2.444[b]	8.286±2.410[b]	5.804±2.248[a]
	RMS	6.773±1.858[a]	12.094±2.417[b]	11.397±2.411[b]	8.829±4.558[ab]

Notes: Means followed by different letters in the same row represent statistically significant difference (P<0.05, Tukey's test).

Figuras (Figures)

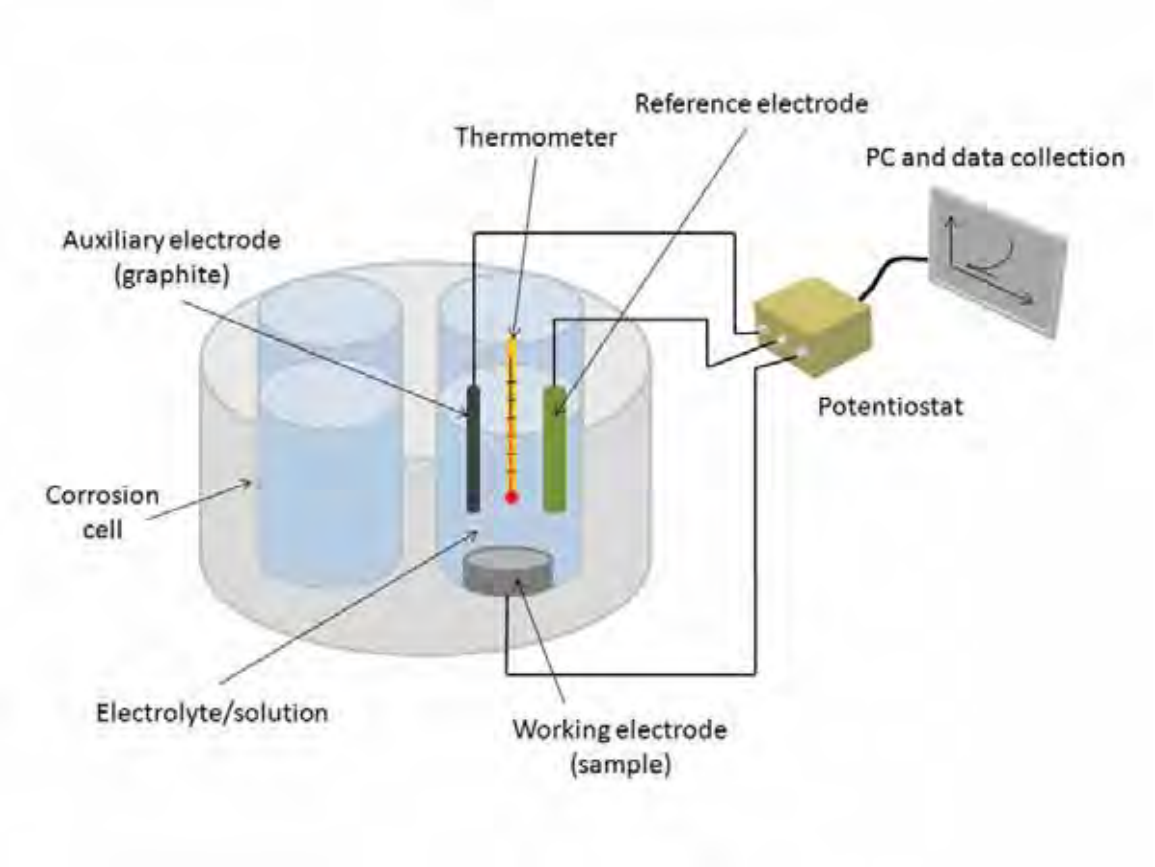

Figure 1. Schematic electrochemical set-up (a standard 3-electrode cell).

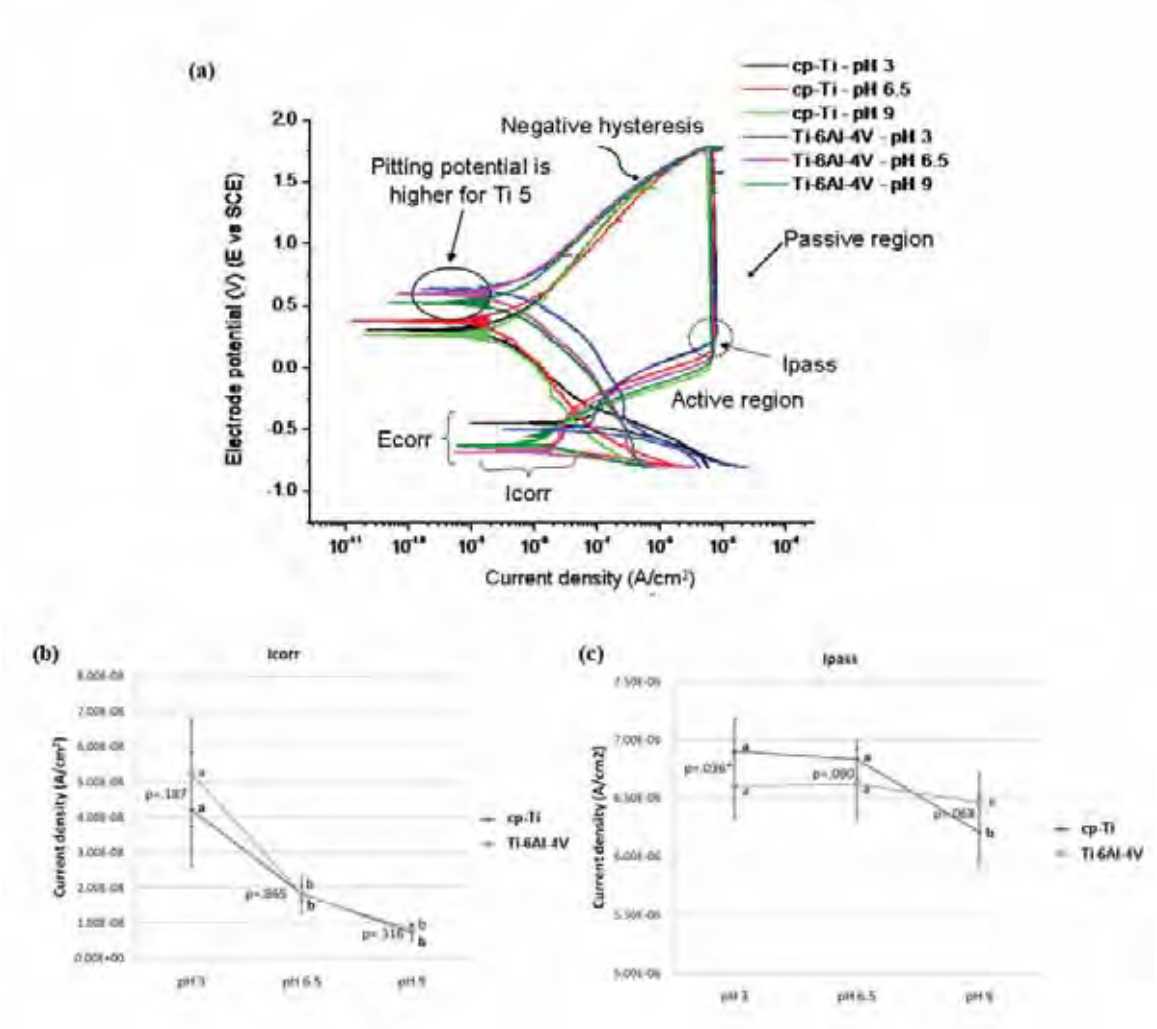

Figure 2. (a) Representative cyclic polarization curves for cp-Ti and Ti-6Al-4V alloy in artificial saliva with different pH values. Evolution of **(b)** corrosion current density (I_{corr}) and **(c)** passivation current density (I_{pass}) for cp-Ti and Ti-6Al-4V alloy in artificial saliva with different pH values. For each variable, small letters compare different pH values at the same Ti type (bold letter for cp-Ti and non-bold letter for Ti-6Al-4V alloy). Different letters indicate significant differences among the groups (α=0.05). *P* value compares cp-Ti with Ti-6Al-4V alloy in each pH (p<.05 indicates statistical significant difference between cp-Ti and Ti-6Al-4V alloy).

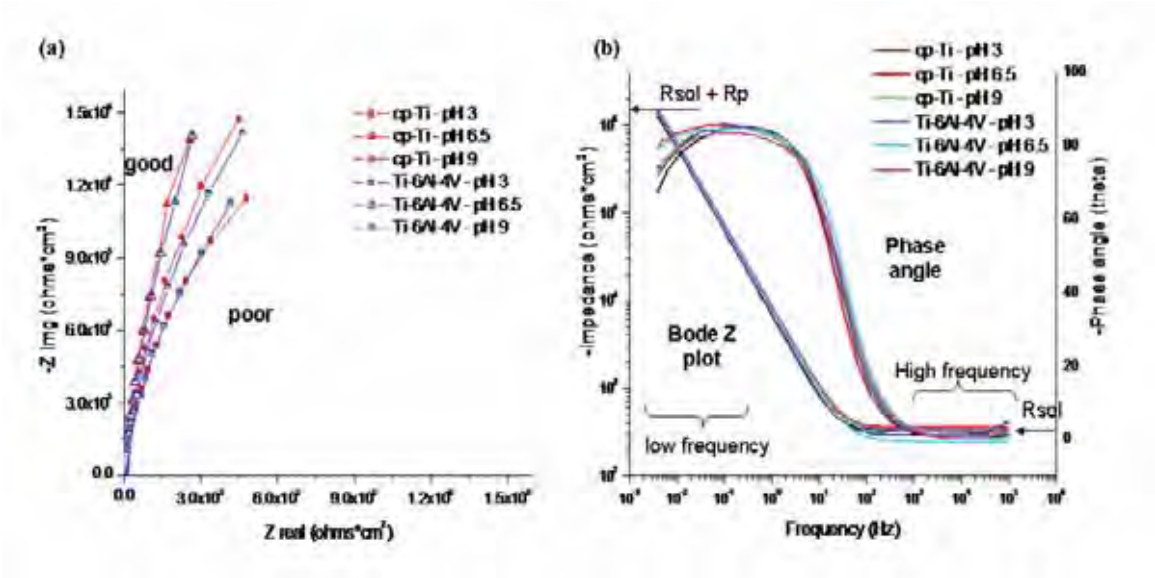

Figure 3. Representative **(a)** Nyquist plots and **(b)** Bode plots from EIS recorded for cp-Ti and Ti-6Al-4V alloy in artificial saliva with different pH values.

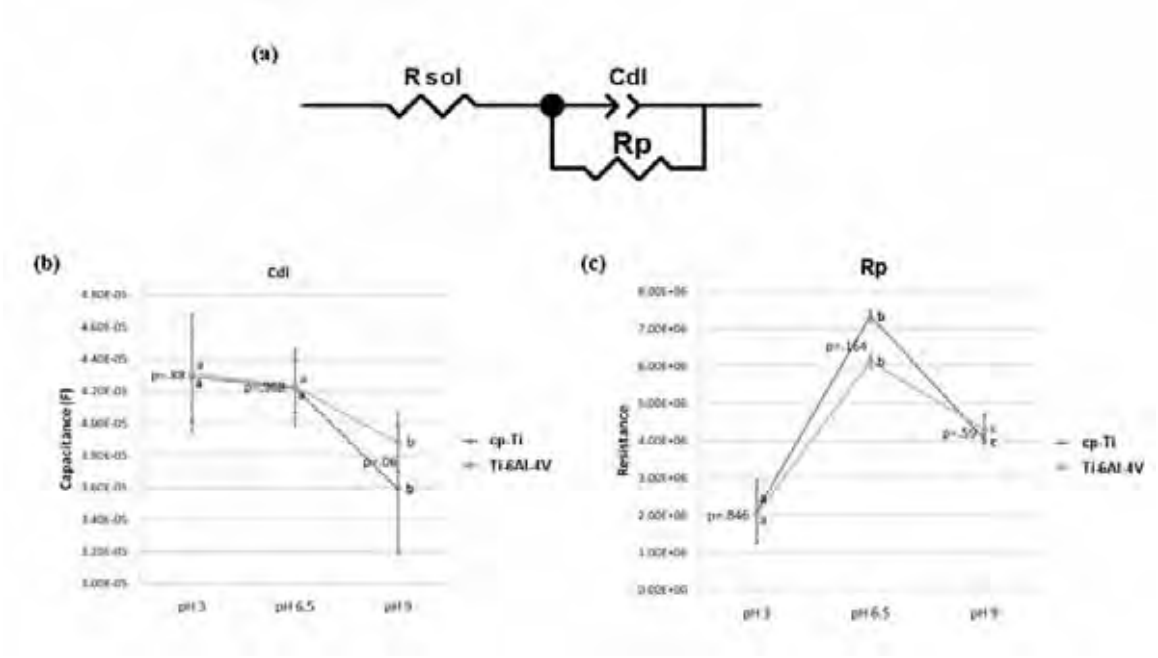

Figure 4. (a) Equivalent circuit **(**Randle's circuit) depicting the corrosion model**.** Evolution of **(b)** capacitance of double layer (C_{dl}) and **(c)** polarization resistance (R_p) for cp-Ti and Ti-6Al-4V alloy in artificial saliva with different pH values. For each variable, small letters compare different pH values at the same Ti type (bold letter for cp-Ti and non-bold letter for Ti-6Al-4V alloy). Different letters indicate significant difference among the groups (α=0.05). *P* value compares cp-Ti with Ti-6Al-4V alloy in each pH (p<.05 indicates statistical significant difference between cp-Ti and Ti-6Al-4V alloy).

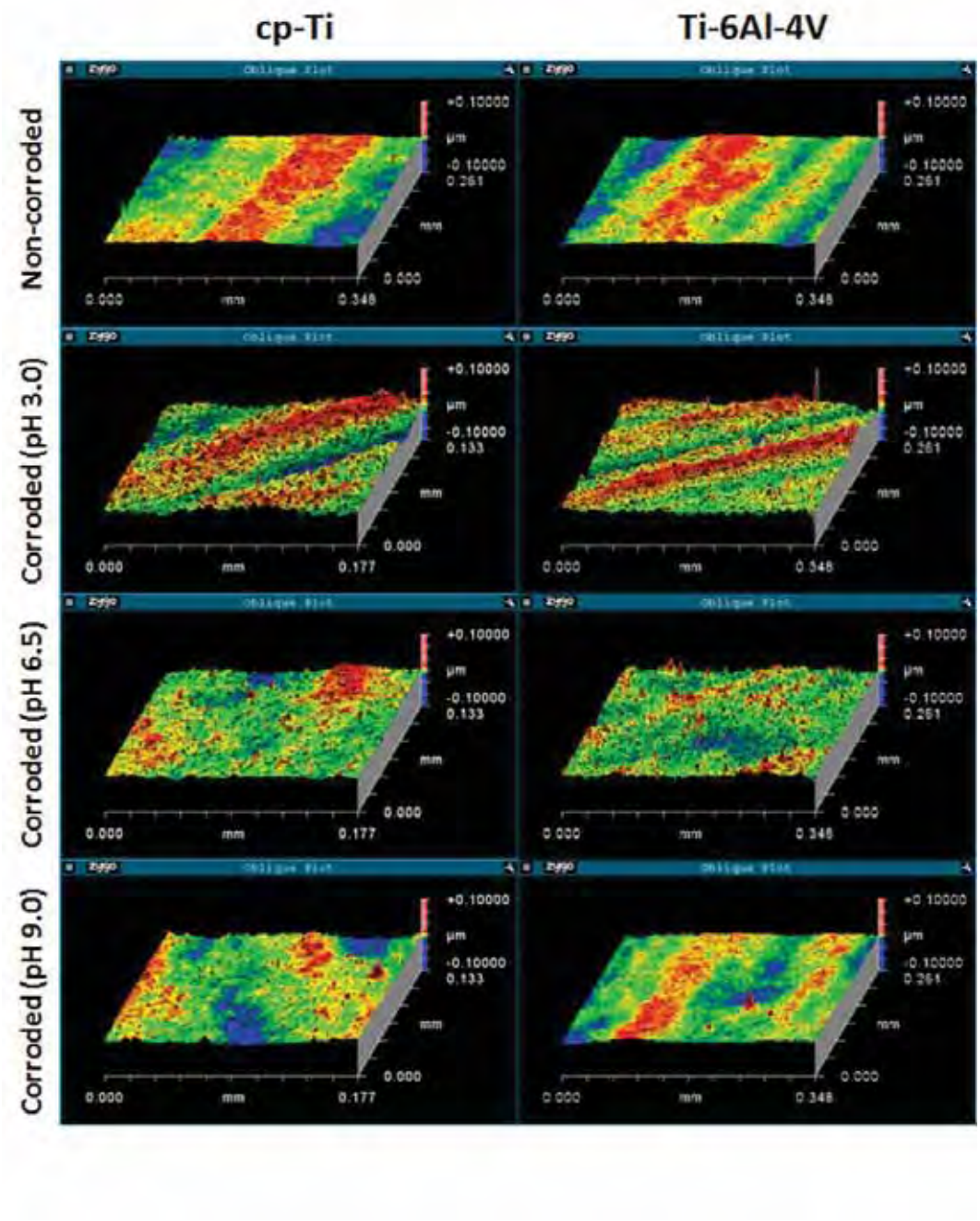

Figure 5. White interferometry microscopy 3D images of cp-Ti and Ti-6Al-4V alloy before and after corrosion. *X* and *Y* scales are in mm and *Z* scale is in µm. The *Z* scale covers 0.2 µm in amplitude. Colors represent the peaks (from ~0 to +0.1 µm of amplitude in the *Z* axis) (red and yellow) and valleys (from ~0 to -0.1 µm of amplitude in the *Z* axis) (green and blue) on the titanium surface topography.

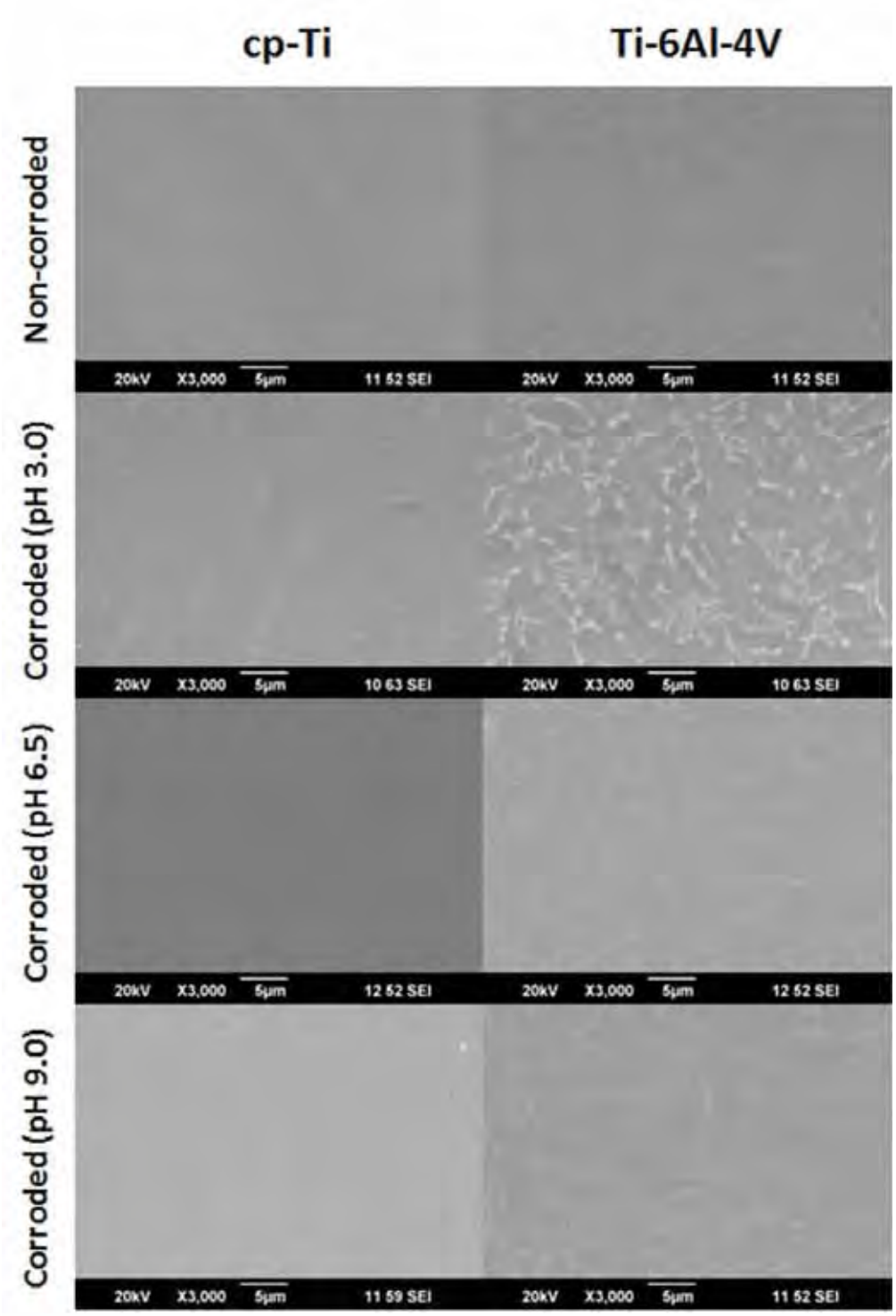

Figure 6. Scanning electron microscope x 3,000 of cp-Ti and Ti-6Al-4V alloy before and after corrosion. Bar = 5 µm.

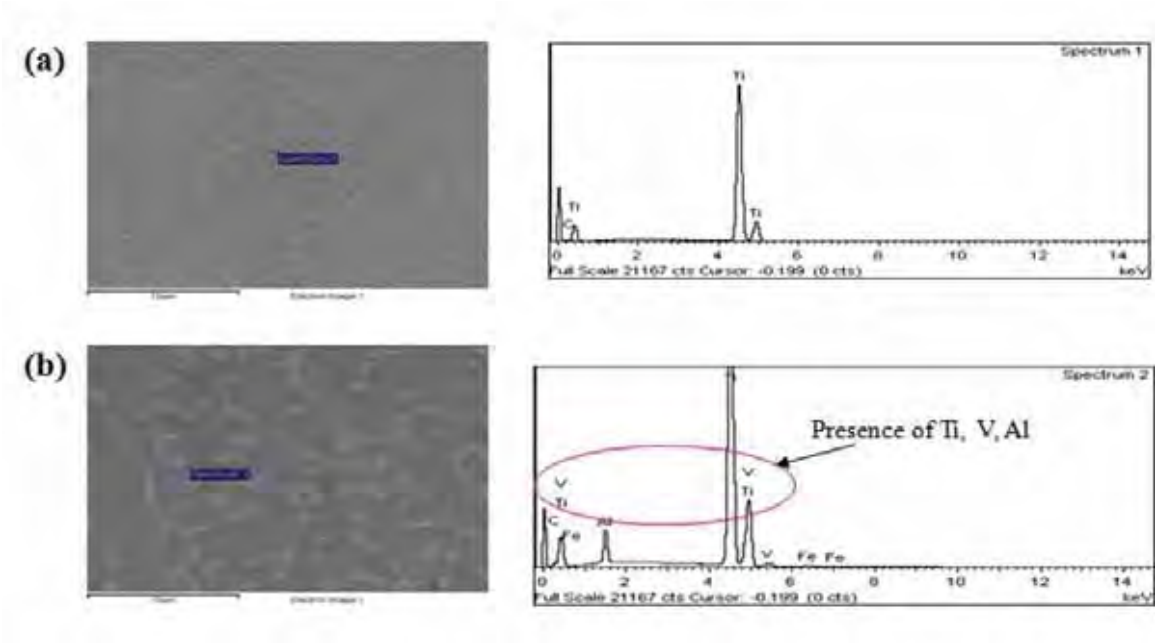

Figure 7. (a) Representative EDS spectra of the cp-Ti after corrosion exposure (at pH 3). **(b)** Representative EDS spectra of the Ti-6Al-4V alloy after corrosion exposure (at pH 3).

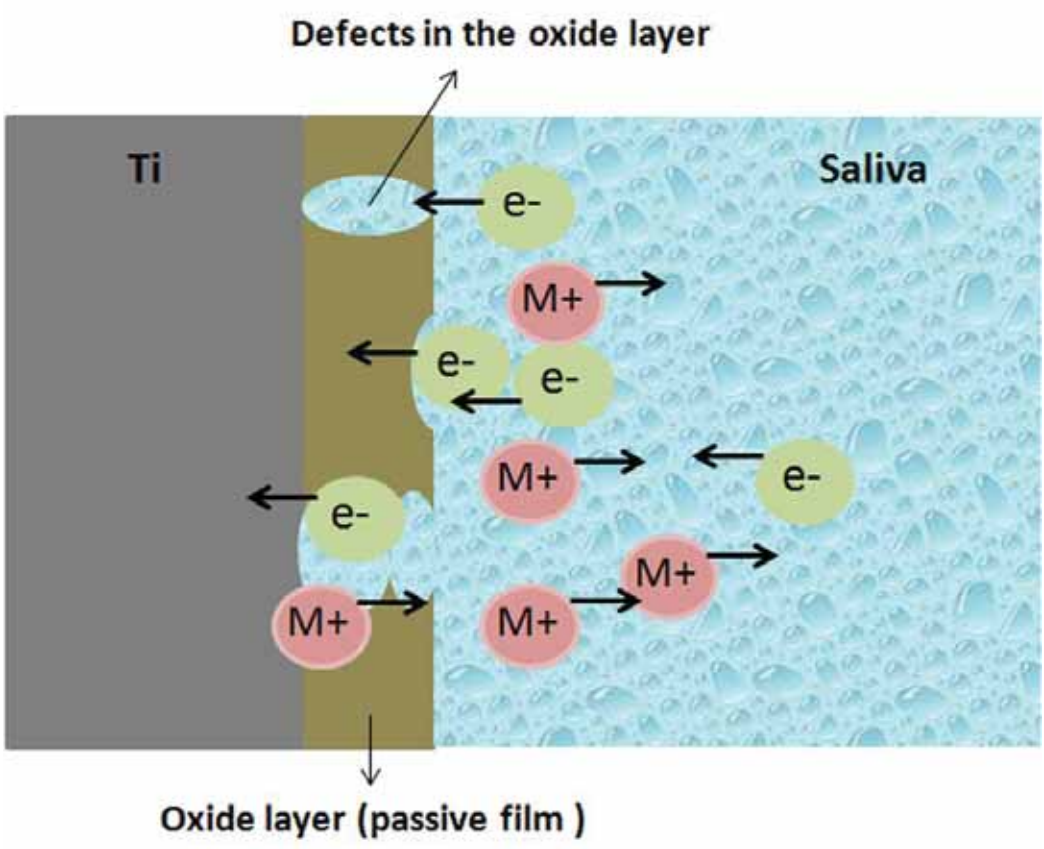

Figure 8. Schematic diagram of the electrochemical reaction layer formed on the Ti surface. Electrons (e-) comes from saliva and metal ions (M+) from Ti.

CAPÍTULO 2 *

Influência da corrosão na Afinidade ao Lipopolissacarídeo para Dois Diferentes Materiais de Titânio

Influence of Corrosion on Lipopolysaccharide Affinity for two Different Titanium Materials

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* Artigo a ser enviado para o *Journal of Materials Science: Materials in Medicine*, e suas normas de publicação encontram-se disponíveis no anexo C.

Influência da corrosão na Afinidade ao Lipopolissacarídeo para Dois Diferentes Materiais de Titânio

3.1 Resumo

Modificações na superfície do titânio devido ao ataque eletroquímico na cavidade oral pode ser um importante fator na afinidade ao lipopolissacarídeo e consequentemente poderia induzir peri-implantite. Nós hipotetizamos que a corrosão (em ambiente oral simulado – pHs 3; 6,5 e 9) aumenta a afinidade ao lipopolissacarídeo para o titânio comercialmente puro (cp-Ti) e a liga Ti-6Al-4V. Discos de titânio foram anodicamente polarizados em um sistema padrão de três eletrodos. Os espécimes foram tratados com lipopolissacarídeo (1,5; 15 e 150 μg/ml) em água livre de lipopolissacarídeo durante 24 horas para avaliar a aderência de lipopolissacarídeo. Os discos foram transferidos a cada 24 horas para solução fresca de água livre de lipopolissacarídeo até completar 72 horas, para investigar a liberação de lipopolissacarídeo. A saliva ácida aumentou a taxa de corrosão do cp-Ti e da liga Ti-6Al-4V e promoveu maior aderência de lipopolissacarídeo na superfície dos discos de titânio ($P<0,05$). A liga Ti-6Al-4V exibiu maior afinidade ao lipopolissacarídeo ($P<0,05$). A liberação do lipopolissacarídeo foi maior no intervalo de 24 horas e reduziu com o passar do tempo. Clinicamente, a corrosão do titânio e sua afinidade ao lipoplissacarídeo pode influenciar na inflamação peri-implantar e no prognóstico do implante.

Influence of Corrosion on Lipopolysaccharide Affinity for two Different Titanium Materials

3.2 Abstract

Titanium surface modifications due to electrochemical attack in the oral cavity may be an important factor for lipopolysaccharide affinity and thereby could induce periimplantitis. We hypothesized that corrosion (in simulated oral environment - pHs 3, 6.5 and 9) increases the lipopolysaccharide affinity for commercially-pure titanium (cp-Ti) and Ti-6Al-4V. Titanium discs were anodically polarized in a standard 3-electrode setting. Specimens were treated with lipopolysaccharide (1.5, 15 and 150 μg/ml) in lipopolysaccharide-free water for 24 hours to evaluated lipopolysaccharide adherence. Discs were then transferred every 24 hours to fresh lipopolysaccharide-free water, up to 72 hours, to investigate lipopolysaccharide elution. Acidic saliva increased the corrosion rate of cp-Ti and Ti-6Al-4V, and promoted greater lipopolysaccharide adherence to titanium surfaces (P<.05). Ti-6Al-4V exhibited greater lipopolysaccharide affinity (P<.05). Lipopolysaccharide elution was greatest at 24-hour interval and reduced over time. Clinically, corrosion of titanium and its surface affinity for lipopolysaccharide could influence periimplant inflammation and implant prognosis.

3.3 Introdução (Introduction)

The oral environment induces a continuous degradation of dental materials [1-2]. Acidic saliva, resulting from infections or food, contributes to the corrosion of dental implants [3-4]. The corrosion products may affect the biocompatibility and function of dental implants by inducing inflammatory reactions that provoke the release of inflammatory mediators from macrophages, leading to bone resorption and possibly contribute to implant failure [3, 5].

Commercially-pure titanium (cp-Ti) and Ti-6Al-4V alloy have been extensively used to fabricate dental implants due to its corrosion resistance, optimal mechanical properties and favorable biocompatibility [6-8]. Although corrosion resistance of titanium relies on the presence of stable and dense oxides formed on the metal surface [7, 9], titanium (Ti) is not inert to corrosion attack when exposed to acid, fluoride and saliva in oral cavity [10-11]. The normal pH in oral cavity varies from 6 to 8 [12]. In case of infections and consumption of some vegetables/grains/milk, the pH of saliva can become acidic or alkaline [1, 12].

Periodontopathic bacteria, including gram-negative bacteria, are found in the periimplant environment and produces lipopolysaccharide (LPS), a membrane constituent of gram-negative bacteria [13]. LPS is a highly bioactive molecule [13] with marked effects on macrophages, lymphocytes, fibroblasts, and osteoblasts [14]. The LPS concentration in the crevicular fluid range from 0.8

to 9.6 µg/ml in a condition of healthy gingival sulcus and gingivitis, respectively [15]. A greater proportion of gram-negative bacteria and LPS in periimplant crevicular fluid are found with chronic inflammation [16-17]. Furthermore, LPS indirectly contribute to inflammation due to its affinity for several restorative materials [18-20].

In vitro studies have investigated the LPS affinity for casting and metal-ceramic alloys [19-22], methacrylate resins [23-24], Ti material [18] and dental ceramic [25]. The affinity was influenced by the type of LPS and material, surface properties and topography, and Ph [18-25]. To our best knowledge, the effect of corrosion on LPS affinity for Ti material is an unexplored issue. The surface modifications of Ti materials due to electrochemical attack may be an important factor for LPS affinity.

We assessed the influence of corrosion process (in simulated oral environment at pHs 3, 6.5 and 9 of artificial saliva) on *Escherichia coli* LPS affinity for cp-Ti and Ti-6Al-4V alloy. We hypothesized that 1. Corrosion increases the LPS affinity for both Ti types tested and 2. There is no difference in the LPS affinity rate for both Ti types.

3.4 Materiais e métodos (Materials and methods)

Specimen preparation

Seventy-two specimens, 15-mm in diameter and 2-mm in thickness, were fabricated from cp-Ti and Ti-6Al-4V alloy (Mac-Master Carr, Elmhurst, IL, USA). Table 1 shows the nominal chemical composition of cp-Ti and Ti-6Al-4V alloy. Both surfaces of the discs were polished with silicon carbide papers (#320, #400, #600, #800) (Carbimet 2, Buehler, Lake Bluff, IL, USA), and polishing cloth (TextMet Polishing Cloth, Buehler) with diamond paste (MetaDi 9-micron, Buehler). Colloidal silica (MasterMed, Buehler) was used as a final polishing procedure, in order to obtain a mirror-finished surface of the Ti discs (Roughness average (Ra) = 0.012 ± 0.004 μm and 0.005 ± 0.002 μm for cp-Ti and Ti-6Al-4V alloy, respectively). Specimens were ultrasonically cleaned with deionized water and 70% propanol, and finally air dried with hot air of 250°C [26].

Electrochemical test

A custom-made electrochemical cell was used to conduct the test (Fig. 1a). The measurements were performed in a standard 3-electrode setting following the American Society for Testing for Materials guidelines (G61 and G31-72). A saturated calomel electrode was used as the reference electrode, graphite rod as the auxiliary electrode and the exposed surface of the Ti as the working electrode (the exposed area was 1.77 cm^2) [26]. A potentiostat (G300, Gamry Inc., Warminster, PA, USA), linked to a computer for data acquisition, was

used to perform the corrosion measurements. Artificial saliva (pHs 3, 6.5 and 9) was used as an electrolyte solution [26].

For each pH condition, 9 specimens of each Ti types were corroded individually on both sides. The composition of artificial saliva [27] was KCl (0.4 g/L), NaCl (0.4 g/L), CaCl2·2H2O (0.906 g/L), NaH2PO4·2H2O (0.690 g/L), Na2S·9H2O (0.005 g/L), and urea (1 g/L). Different pH values were achieved by adding lactic acid (acidic) or NaOH (basic) in an appropriate amount. A total volume of 10 ml of electrolyte was used for each corrosion experiment. After mounting the sample into the cell, approximately 4 hours were allowed to achieve electrochemical stabilization of the system. The temperature of the test solution (artificial saliva) was maintained at 37 ± 1°C to mimic the oral environment.

Initially, open circuit potential was monitored over 3600 seconds to evaluate the free potential. Then, specimens were corroded by inducing different potentials in a forward scan from -0.8 V to +1.8 V (2 mV/sec scan rate). Electrochemical parameters were obtained from the potentiodynamic polarization curves by Tafel's method to investigate the Ti corrosion rate: corrosion current density (Icorr), and passivation current density (Ipass).

Surface roughness measurement

In order to understand the mechanical changes on the Ti surface produced by the corrosion and to determine the possible correlation between surface roughness and LPS affinity, one parameter of surface roughness (Ra) was

investigated pre- (baseline) and post-corrosion using a white light interferometry microscope (Zygo New View 6300, Zygo Corporation, Middlefield, CT). Five measurements were performed on each side of individual Ti discs.

LPS affinity test

From the 36 discs for each Ti type, 9 were not corroded (control), while the others were corroded in saliva with different pHs (n=9) as previously described. LPS affinity protocol followed a previous method [18]. Corroded and non-corroded discs were rendered LPS free by washing them twice in 20 ml of 1:1 chloroform/methanol, followed with three subsequent washes in 20 ml of chloroform. Discs were then rinsed once in 20 ml of LPS-free water (95289 Water, Sigma-Aldrich, St Louis, MO, USA) before LPS treatment. Instruments and glassware were baked at 250°C, over 30 minutes for LPS decontamination.

Commercially available LPS from *E. coli* (055:B5, Sigma-Aldrich) were used for LPS affinity test. Nine corroded discs in each artificial saliva pH, and nine non-corroded discs from each Ti type were placed individually into 2.0 ml of LPS-free water, containing either 1.5 μg/ml (low concentration), 15 μg/ml (medium concentration) or 150 μg/ml (high concentration) of *E. coli* LPS (n=3). In the absence of clinical studies that investigated the concentration of LPS on the gingival sulcus under periimplantites, herein the concentrations of LPS were based on previous in vitro studies [19, 26].

With three specimens per group, the observed power to detect a medium size effect (0.5 according to Cohen's effect size statistics) for this study was

0.854. One additional control disc from each Ti type was placed into 2.0 ml of LPS-free water to ensure that no exogenous contamination occurs during the experiment. Specimens were incubated at 37°C for 24 hours. They were removed from the LPS solutions and rinsed by quickly dipping twice in LPS-free water to remove unattached LPS from the disc surface. Then the discs were placed into 2.0 ml of LPS-free water and incubated to evaluate elution (Fig. 1b). LPS concentrations in the post-treatment solutions were determined with a *Limulus* amoebocyte lysate (LAL) chromomeric endpoint assay (HIT302 LAL assay, Hycult biotech, Cell Science, Canton, MA, USA) after measurement of absorbance at 405 nm of each medium in a liquid scintillation spectrometer (Synergy 2, BioTek, Winooski, VT, USA).

After the 24-hour elution/incubation period, the discs were removed again, quickly rinsed, and placed into 2.0 ml of LPS-free water. The discs were then incubated and subsequently transferred to fresh medium for 24-, 48-, 72-hour elution intervals (Fig. 1b). Thereafter, LPS concentration in the 24-, 48-, 72-hour eluates for each disc was calculated after measurement of eluate through the LAL assay. A total of 50 µl of each medium was evaluated. In order to ensure that the LPS adhered to the test tubes would mix with the medium and to obtain homogeneity of the medium, each tube was vortexed for 10 seconds.

LPS adherence was calculated by subtracting the total LPS concentration in the post-treatment solution from the pre-treatment concentration, measured in endotoxin unit (EU). LPS elution was determined by evaluating the LPS concentration of each eluate in EU/ml.

Statistical analysis

Electrochemical (Icorr and Ipass) and surface roughness data were analyzed by using one-way ANOVA for each factor (pH level or Ti type). Tukey's HSD test was used as a post-hoc technique.

Initial LPS adherence and elution data were evaluated with one-way ANOVA to determine the effects of corrosion and LPS concentration on the adherence to each Ti type. Tukey's HSD test was used as a post-hoc technique when applicable. Independent T-test was used to compare the LPS adherence to cp-Ti and Ti-6Al-4V alloy as a function of corrosion and LPS concentration. One-way repeated measured ANOVA and Pairwise comparisons were used to investigate the differences in elution among groups over time. A significance level of 0.05 was used for all tests (Statistical Package for the Social Sciences, version 17.0; SPSS Inc, Chicago, IL).

3.5 Resultados (Results)

Electrochemical and surface roughness data

Icorr and Ipass values are presented in Table 2. As the values of Icorr and Ipass increased, the corrosion rate was greater. One-way ANOVA showed that Icorr value of both Ti types was affected by saliva pH (P<.001). While saliva pH significantly influenced the Ipass value of cp-Ti (P<.001, ANOVA), no significant effect was observed for Ti-6Al-4V alloy (P=.408, ANOVA). For both Ti types, the values of Icorr significantly decreased with the increased pH (P<.001, Tukey's HSD test), indicating that at a low pH, the corrosion rate of Ti is higher. The Ipass value of cp-Ti generally decreased, at near neutral and basic pHs (P<.001, Tukey's HSD test), correlating with the Icorr results. Regardless of pH value, no significant difference of corrosion rate was observed between Ti types (P>.05, ANOVA), excepted for Ipass value at pH 3 (P=.036, ANOVA).

The means and standard deviations of Ra values of pre- and post-corrosion for both Ti types are displayed in Fig. 2. Corrosion process did not affect the surface roughness of cp-Ti (P=.207, ANOVA), whereas a significant effect was observed for Ti-6Al-4V alloy (P<.001, ANOVA), in which corroded discs at pH 3 and 6.5 exhibited higher surface roughness value (P<.05, Tukey's HSD test). Cp-Ti exhibited greater surface roughness value when compared to Ti-6Al-4V alloy pre- and post-corrosion (P<.05, T-test).

LPS affinity data

No detectable LPS associated with the control discs were observed throughout the experiment. This indicates that no exogenous LPS contamination occurred.

The means and standard deviations of initial LPS adherence are presented in Fig. 3a-c. Corrosion process significantly affected the LPS adherence to cp-Ti at high LPS concentration (P=.015, one-way ANOVA) and to Ti-6Al-4V alloy at medium LPS concentration (P<0.001, one-way ANOVA). LPS adherence was significantly higher for cp-Ti and Ti-6Al-4V alloy at acidic pH (pH 3) (P<.05, Tukey's HSD test).

Higher LPS treatment concentrations resulted in greater LPS adherence to the Ti-6Al-4V alloy, and it was statistically significant in corroded discs at pH 3 (P<.001, ANOVA) and at pH 6.5 (P=.004, ANOVA). Regardless of LPS concentration and corrosion protocol, Ti-6Al-4V alloy exhibited higher LPS adherence than cp-Ti (P<.05, T-test), excepted for non-corroded and corroded (pH 9) discs at low LPS concentration (P=.314 and P=.073, respectively, T-test) and at high LPS concentration (P=.544 and P=.050, respectively, T-test).

Corrosion process did not affect the LPS elution from both Ti types at all intervals (P>.05, one-way ANOVA), excepted for cp-Ti at 48-hour elution period in high LPS concentration, where the non-corroded discs exhibited higher LPS elution, compared to the corroded discs (P<.05, Tukey's HSD test). Groups treated with higher LPS concentration exhibited higher LPS elution levels at all

intervals of evaluation. Those data were statistically significant at 48 and 72 hours of elution for cp-Ti (P<.05, Tukey's HSD test) (Fig. 4a-f).

One-way repeated measured ANOVA showed significant difference among the elution intervals for Ti types at all LPS concentrations and corrosion process (P<.05). At 24-hour elution, all groups presented higher LPS elution when compared to 48- and 72-hour elution (P<.05, Pairwise test). At each of the three elution times, cp-Ti released significantly greater LPS than Ti-6Al-4V alloy (P<.05, T-test) (Fig. 4a-f). After 72 hours of elution, more than 99.9% of initially adhering LPS remained on the surface of both Ti types.

3.6 Discussão (Discussion)

The research hypothesis of LPS affinity increases significantly for cp-Ti and Ti-6Al-4V alloy after corrosion process and that no difference in LPS affinity exists between cp-Ti and Ti-6Al-4V was partially accepted. Corrosion increased the LPS adherence to cp-Ti at high LPS concentration (P=.015) and to Ti-6Al-4V at medium LPS concentration (P<.001). LPS elution from Ti was not affected by corrosion (P>.05). Furthermore, in most of situations, greater LPS adherence was observed to Ti-6Al-4V alloy (P<.05).

When corrosion promoted more LPS adherence to Ti, an acidic pH was involved in the corrosion protocol. Ti is an electrochemically active material. When it is exposed to air, an oxide layer is formed on its surface, conferring its protection against corrosion [9]. However, the presence of defects on the oxide layer of Ti surface can affect its passivity [6]. Previous studies [8, 28] have reported that acidic solution degrades the protective oxide film and induces greater corrosion, which is in agreement with the electrochemical data from this study. It could be speculated that the defective oxide layer promotes greater LPS adherence, due to changes of Ti surface energy or chemical modification of Ti surface. LPS exhibit a low surface energy and are attracted to comparatively higher surface energies [19, 21].

The higher surface roughness observed on the specimens corroding at pH 3 may have an influence on the greater LPS adherence. The surface roughness of implant components could affect the LPS affinity and consequently the LPS

concentration in periimplant crevicular fluid [18]. In addition, roughness is an important property of adhesion and colonization of bacteria [29], therefore, a greater LPS accumulation may be expected on the Ti surface after corrosion, which could induce periimplantitis [30].

The interaction between LPS and Ti differed, based upon LPS concentrations and Ti type. Higher LPS adherence to Ti-6Al-4V alloy was observed in high LPS concentration. Knoernschild et al. [19] described greater LPS adherence to gold and Ni-Cr-Be alloy related to higher LPS treatment concentration. However, the LPS adherence to cp-Ti was lower in milieu with high LPS concentration when compared to solution with medium LPS concentration. It is likely that the cp-Ti surface became LPS-saturated at higher LPS concentration. In the presence of surface saturation, the free LPS in solution interact with the LPS that had been adhered to the cp-Ti surface. Therefore, the LPS intermolecular interactions may reduce the quantity of available LPS that adhered to Ti [19].

Although cp-Ti exhibited higher surface roughness, a greater LPS adherence to Ti-6Al-4V alloy was observed. The difference in oxide film composition may be the driving force toward this observation. Since Ti-6Al-4V alloy has aluminum and vanadium in its composition, aluminum and vanadium oxides are found on the surface of Ti-6Al-4V alloy. Selective interactions between LPS and those specific surface oxides [20] might have occurred, which coincide with our results. The difference in surface energy of both Ti types may be another factor influencing LPS adherence [18]. Surface energy is related to

surface hardness [19]. The surface hardness of Ti-6Al-4V alloy is twice as high than that of cp-Ti [31], and a greater LPS adherence occurred on the former. On the other hand, Nelson et al showed a similar LPS adherence to cp-Ti and Ti-6Al-4V alloy, possibly due to the difference in LPS concentration and the low sensitivity of the method used to detect LPS [18].

Corrosion process did not influence the LPS elution level for both Ti types. Since the specimens were exposed to the same environment (LPS-free water) during the elution time, it is believed that the oxide film formed on the corroded discs is similar to that from non-corroded discs. As expected, specimens treated with high LPS concentration displayed a greater LPS elution levels at all intervals. Those specimens may be LPS-saturated during LPS treatment; therefore, higher quantity of LPS is available to release from Ti surface to the medium. With the higher initial LPS adherence to the Ti-6Al-4V alloy, a trend toward lower levels of LPS elution was observed. Therefore, it can be implied that Ti-6Al-4V alloy presented higher LPS affinity, compared to cp-Ti. However, the degree of such affinity to produce a biologic response remains unknown.

LPS release was higher at 24 hours of elution, and generally stabilized after 48 hours. Others also have reported the greatest LPS elution at 24-hour elution and a reduction in LPS release over time [18, 21, 24]. Even after 72-hour elution time, approximately 99.9% of initially adhering LPS remained on both Ti types surface. This result may indicate that *E. coli* LPS have high attraction to cp-Ti and Ti-6Al-4V alloy surfaces, and the balance between adherence and elution

resulted in low levels of LPS release compared to the amount adhering to the Ti surface [21].

In summary, the corrosion and LPS affinity tests herein revealed that acidic artificial saliva increased the corrosion rate of cp-Ti and Ti-6Al-4V alloy, which promoted greater amount of LPS adherence to Ti surface. In this point of view, situations that acidify the pH of saliva (e.g. infections, certain foods, mouthwash products, smoking, chronic/systemic diseases or medication) may adversely affect the implant surface, which in turn affect the periimplant tissue response that may lead to implant failure.

Several areas of corrosion and bacterial response may have significant impact on the performance and durability of dental implants. Hence, future research addressing the LPS affinity for different types of Ti materials with different chemical surface treatment may be of interest. Also, for more practical implications, studies can be extended to investigate such LPS affinity phenomena under the presence of oral bacteria. LPS from *E. coli* bacterium, which is not a common microorganism of the gingival sulcus, was used in this study. However, the LPS from several gram-negative bacteria present similar basic molecular structure [21]. Furthermore, the LAL assay of this study uses *E. coli* as its standard. Therefore, the LPS used herein had a similar reactivity with the lysate. Further studies are warranted to evaluate the affinity of different LPS types for Ti materials.

3.7 Conclusão (Conclusion)

Within the limitations of this study, the following conclusions were drawn:

- Acidic artificial saliva increased the corrosion rate of cp-Ti and Ti-6Al-4V alloy, and promoted greater LPS adherence to titanium surfaces. LPS elution levels were not affected by corrosion.
- Ti-6Al-4V alloy exhibited greater LPS affinity when compared to cp-Ti, by increasing the levels of LPS adherence and reducing LPS elution.
- LPS elution levels were the highest at 24-hour interval and reduced over time for both titanium types. However, even after 72 hours of elution, greater than 99.9% of initially adhering LPS remained on the surface of both titanium types.

Acknowledgment

The authors would like to express gratitude to the Coordination of Improvement of Personnel Higher Education (CAPES) (BEX 4129/09-1) and the State of Sao Paulo Research Foundation (FAPESP) (2007/58926-5) for the scholarships provided to V.A. Barao.

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Tabelas (Tables)

Table 1. Nominal composition of cp-Ti and Ti-6Al-4V alloy

Ti type	Composition (in wt%)							
	Ti	**Al**	**V**	**C**	**Fe**	**O_2**	**N_2**	**H_2**
cp-Ti	99.7	-	-	0.006	0.12	0.16	0.004	0.0019
Ti-6Al-4V	89.62	6.1	4.0	0.004	0.16	0.106	0.008	0.0022

Table 2. Means and (standard deviation) of corrosion current density (I_{corr}) and passivation current density (I_{pass}), for cp-Ti and Ti-6Al-4V alloy in artificial saliva with different pH values (n=9)

Corrosion parameter	**pH of artificial saliva**		
I_{corr} (A/cm^2)	**3.0**	**6.5**	**9.0**
cp-Ti	4.22E-8 (1.62E-8) a	1.84E-8 (2.13E-9) b	7.28E-9 (2.29E-9) b
Ti-6Al-4V	5.24E-8 (1.52E-8) a	1.81E-8 (5.4E-9) b	8.17E-9 (1.17 E-9) b
I_{pass} (A/cm^2)			
cp-Ti	6.90E-6 (2.78E-7) a,*	6.83E-6 (1.61E-7) a	6.21E-6 (2.90E-7) b
Ti-6Al-4V	6.60E-6 (2.81E-7) a	6.62E-6 (3.01E-7) a	6.46E-6 (2.53E-7) a

Notes:

Means followed by different lowercase letters in rows are statistically different within each titanium type ($p<.05$, Tukey's HSD test).

(*) represents significant difference in I_{pass} value between cp-Ti and Ti-6Al-4V ($p=.036$, one-way ANOVA).

Figuras (Figures)

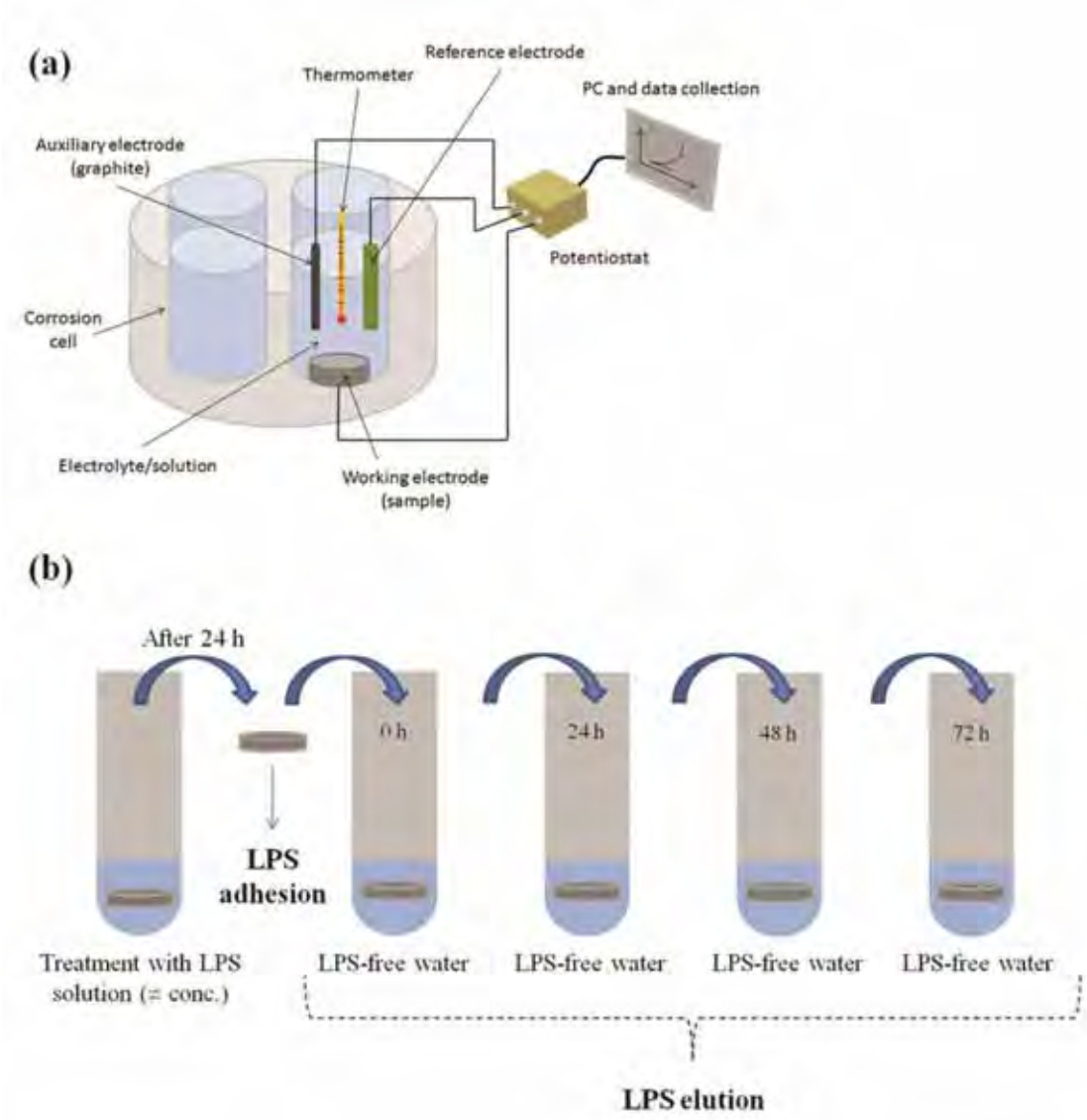

Figure 1. Electrochemical and LPS affinity tests. **(a)** Schematic electrochemical set-up (a standard 3-electrode cell). **(b)** LPS affinity protocol.

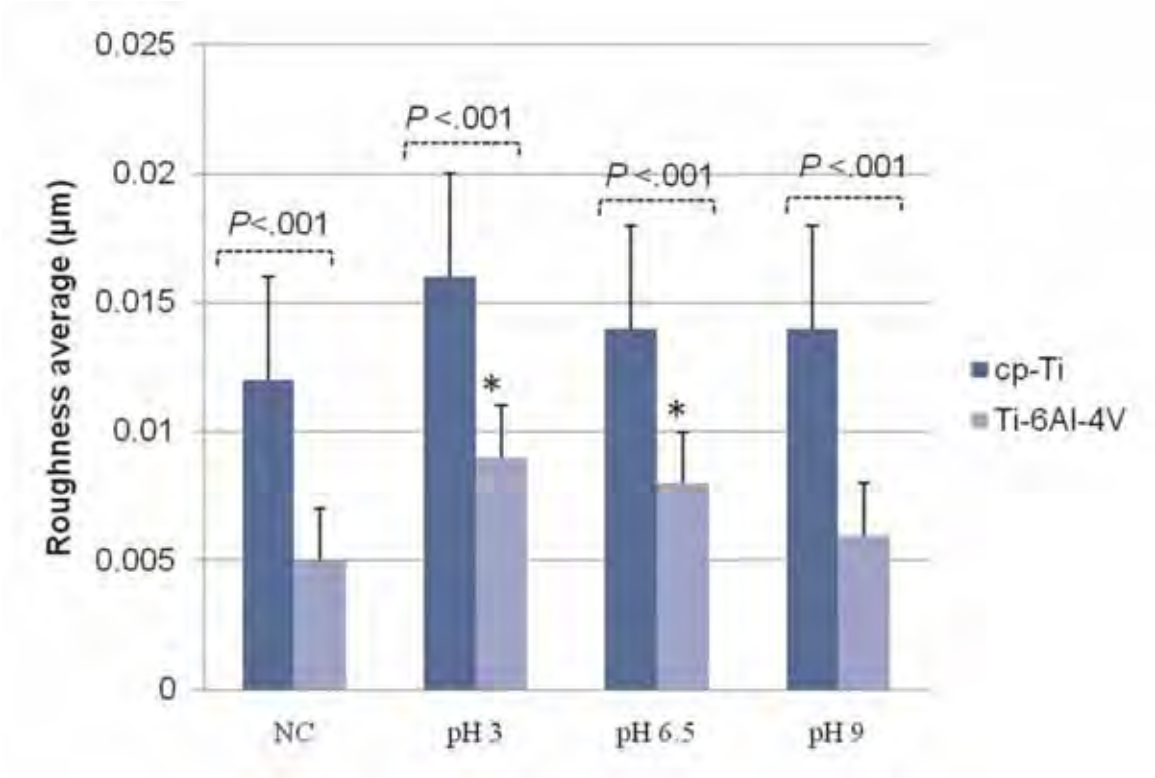

Figure 2. Mean and standard deviation of surface roughness (Ra) of cp-Ti and Ti-6Al-4V alloy pre (non corroded-NC) and post corrosion process (pH 3, 6.5 and 9) (n=9). For each titanium type, (*) expresses significant difference among the non-corrosion and corrosion groups (P<.05, Tukey's HSD test). P value compares cp-Ti with Ti-6Al-4V alloy in each situation (P<.05 indicates statistical significant difference between cp-Ti and Ti-6Al-4V alloy according to ANOVA).

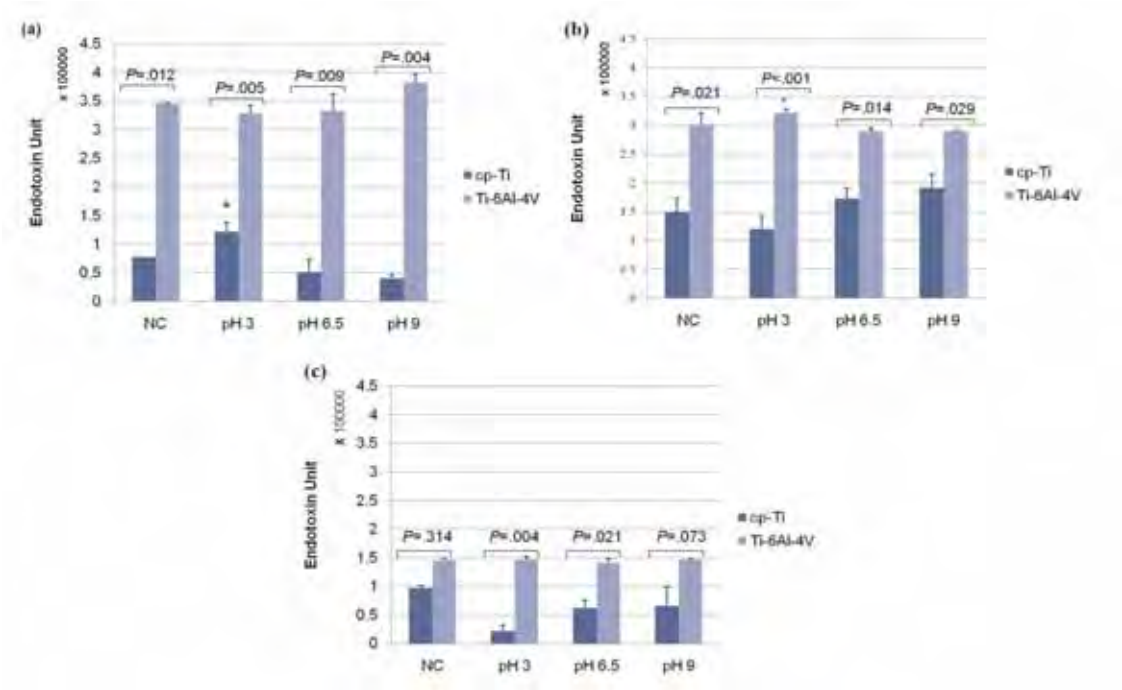

Figure 3. LPS adherence results. **(a-c)** Mean and standard deviation of *E. Coli* LPS initial adherence (Endotoxin unit) to cp-Ti and Ti-6Al-4V alloy. **(a)** At high LPS concentration (150 µg/ml). **(b)** At medium LPS concentration (15 µg/ml). **(c)** At low LPS concentration (1.5 µg/ml). For each titanium type, (*) expresses significant difference among the non-corrosion and corrosion groups ($P<.05$, Tukey's HSD test). *P* value compares cp-Ti with Ti-6Al-4V alloy in each situation ($P<.05$ indicates statistical significant difference between cp-Ti and Ti-6Al-4V alloy according to T-test).

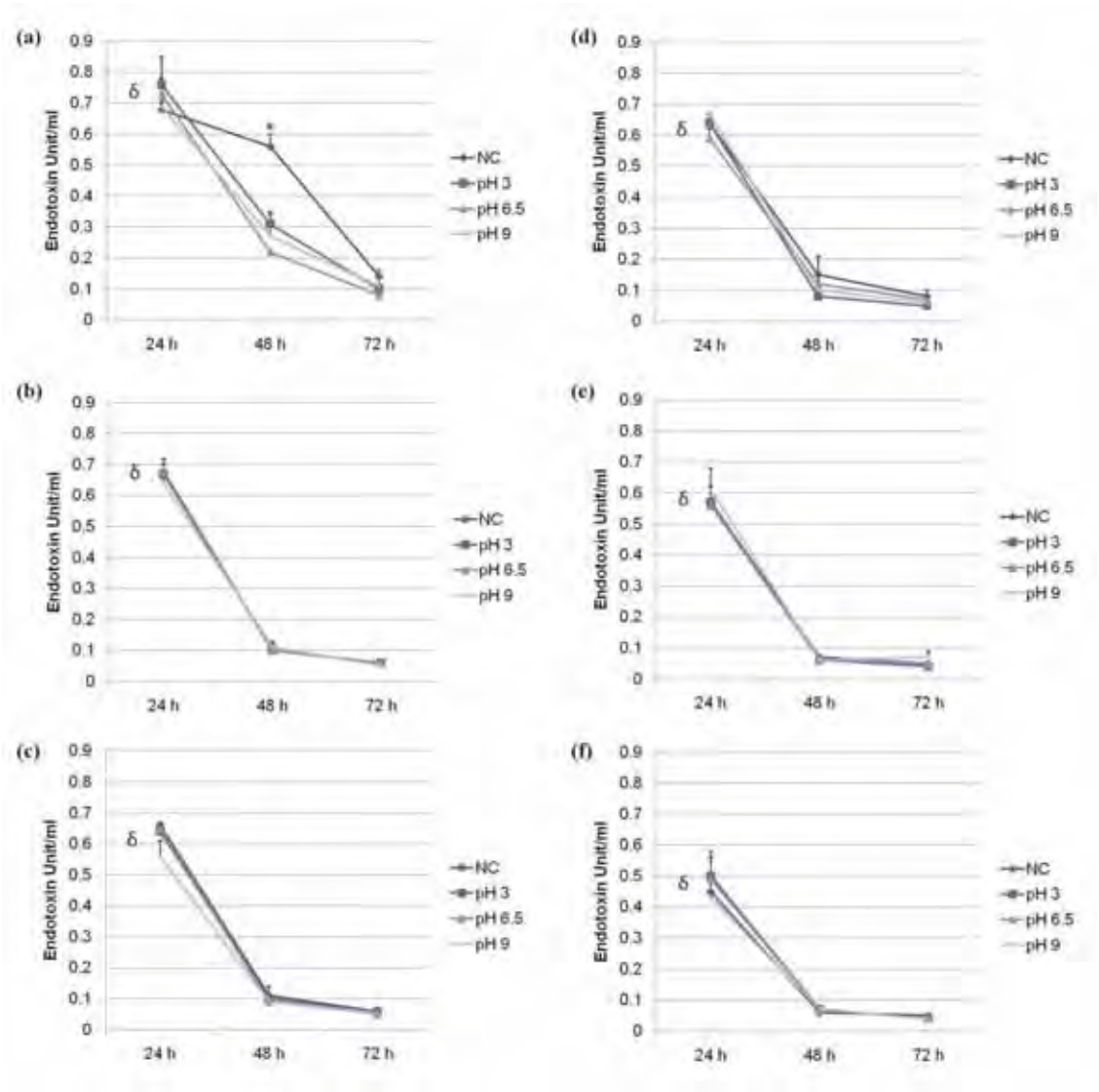

Figure 4. LPS elution results. **(a-f)** Mean and standard deviation of *E. Coli* LPS elution (Endotoxin unit/ml) from non-corroded (NC) and corroded titanium discs at pH 3, 6.5 and 9. **(a)** Cp-Ti following a high LPS concentration treatment (150 µg/ml). **(b)** Cp-Ti following a medium LPS concentration treatment (15 µg/ml). **(c)** Cp-Ti following a low LPS concentration treatment (1.5 µg/ml). **(d)** Ti-6Al-4V alloy following a high LPS concentration treatment (150 µg/ml). **(e)** Ti-6Al-4V alloy following a medium LPS concentration treatment (15 µg/ml). **(f)** Ti-6Al-4V alloy following a low LPS concentration treatment (1.5 µg/ml). (*) expresses significant

difference among the non-corrosion and corrosion groups (*P*<.05, Tukey's HSD test) at 48 hours of elution. (δ) denotes statistically significance difference of 24 hours of elution when compared to 48 and 72 hours of elution intervals (*P*<.05, Pairwise comparison test).

CAPÍTULO 3 *

Qual é o Papel do Lipopolissacarídeo no Comportamento Tribocorrosivo do Titânio?

What is the Role of Lipopolysaccharide on the Tribocorrosive Behavior of Titanium?

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* Artigo a ser enviado para o *Journal of the Mechanical Behavior of Biomedical Materials*, e suas normas de publicação encontram-se disponíveis no anexo D.

Qual é o Papel do Lipopolissacarídeo no Comportamento Tribocorrosivo do Titânio?

4.1 Resumo

Na cavidade bucal, os implantes dentários de titânio são expostos a um complexo processo de degradação o qual é predominantemente influenciado pela intermitente ação mecânica (mastigação), continua exposição a várias soluções químicas (saliva e alimentos) e formação microbiológica (biofilme). Diversos estudos têm investigado a corrosão química do titânio; entretanto, poucos estudos têm reportado sobre os efeitos da interação química, mecânica e microbiológica a qual mimetiza o ambiente oral. Diversos estudos têm investigado a corrosão química e a resistência mecânica do titânio; entretanto, poucos têm reportado sobre o efeito combinado da ação química, mecânica e microbiológica a qual simula o ambiente oral. Uma nova área de pesquisa muldisciplinar, "*tribocorrosão*" (estudo combinado de desgaste e corrosão), foi usada para avaliar tal efeito. A natureza tribocorrosiva do titânio em saliva artificial (pH 6,5) com lipopolissacarídeo (LPS) foi investigada. Vinte e quatro discos de titânio (12 mm de diâmetro e 7 mm de espessura) foram divididos em 8 grupos (n=3) em função do material (titânio comercialmente puro (cpTi) e a liga titânio-aluminio- vanádio (TiAlV) e a concentração de LPS (0, 0,15, 15 and 150 µg/ml). A duração do deslizamento (2000 ciclos), freqüência (1,2 Hz) e carga (20 N – pressão Herteziana estimada de 372 MPa) foram usados para mimetizar o processo mastigatório. A espectroscopia de impedância eletroquímica foi

conduzida antes e após o teste de tribocorrosão para entender as mudanças na cinética de corrosão. As superfícies desgastadas foram avaliadas por meio da interferometria de luz branca e microscopia eletrônica de varredura. A perda total de peso e os valores de rugosidade foram calculados. Os dados foram analizados por meio da ANOVA, e pelos testes de Tukey e T pariado (α=0,05). O LPS afetou o comportamento tribocorrosivo de ambos os tipos de titânio. O LPS estatisticamente acelerou a troca iônica entre o titânio e a saliva, e reduziu a resistência da superfície do titânio a corrosão ($p<0,05$). O deslizamento reduziu a proteção da superfície do titânio. No geral, a liga TiAlV exibiu melhor comportamento corrosivo, mas ambos os tipos de titânio mostraram perda de peso similares ($p>0,05$). O LPS sigificantemente aumentou a perda de peso do cpTi ($p=0,041$), e sua rugosidade ($p<0,001$). O LPS afetou negativamente o comportamento tribocorrosivo do titânio, a que poderia contribuir com a falha dos implantes dentários.

What is the Role of Lipopolysaccharide on the Tribocorrosive Behavior of Titanium?

4.2 Abstract

In an oral environment, titanium dental implants are exposed to a complex degradation process which is predominantly influenced by the intermittent mechanical events (mastication), continuous exposure to varying chemical solutions (saliva and food) and formation of microbiological (biofilm). Several studies have investigated the chemical corrosion and mechanical resistance of titanium; however, very few attempted to report on the effects of combined chemical, mechanical and microbiological interactions, which simulates the oral environment. A new multi-disciplinary research area, "*tribocorrosion*" (a combined study of wear and corrosion), was used to address such issues. The tribocorrosive nature of titanium in artificial saliva (pH 6.5) with lipopolysaccharide (LPS) was investigated. Twenty-four titanium discs (12-mm diameter, 7-mm thickness), were divided into 8 groups (n=3) as a function of material (commercially-pure titanium (cpTi) and titanium-aluminum-vanadium (TiAlV) alloy) and LPS concentrations (0, 0.15, 15 and 150μg/ml). Sliding duration (2000 cycles), frequency (1.2Hz) and load (20 N – estimated Hertzian pressure of 372 MPa) parameters mimicked the mastication process. Electrochemical impedance spectroscopy was conducted before and after tribocorrosion to comprehend the changes in corrosion kinetics. Worn surfaces were examined using white-light-interferometry and scanning electron microscopy. Total weight

loss and roughness values were calculated. Data were analyzed using ANOVA, Tukey's and paired-t tests (α=.05). LPS affected the tribocorrosive behavior of both titanium types. LPS statistically accelerated the ion exchange between titanium and saliva, and reduced the resistance of the titanium surface against corrosion ($p<.05$). Sliding events decreased the protectiveness of titanium surface. In general, TiAlV exhibited better corrosion behavior, but both titanium types showed similar in total weight loss ($p>.05$). LPS significantly increased the cpTi weight loss ($p=.041$), and its roughness ($p<.001$). LPS negatively affected the corrosion/wear behavior of titanium, which may contribute to the failure of dental implants.

4.3 Introdução (Introduction)

Dental implants are widely used and they are considered as an ideal solution to substitute missing teeth (Heuer et al., 2007). Commercially pure titanium (cpTi) is preferred in dentistry (Mabilleau et al., 2006), whereas titanium alloys, such as titanium-aluminum-vanadium (TiAlV), are favored in orthopedics because of their superior mechanical strength. In general, titanium (Ti) has favorable mechanical properties, excellent resistance to corrosion and acceptable biocompatibility (Cortada et al., 2000; de Assis et al., 2006; Vieira et al., 2006). The spontaneous ability of Ti to form a stable and dense oxide layer (TiO_2) is responsible for its corrosion resistance and the reason for wide acceptance, particularly in biomedical application (Huang, 2003).

Although Ti materials exhibit superior corrosion resistance, they are not inert to corrosive attack under specified conditions, such as pH variation and presence of fluorides etc. (Chaturvedi, 2009). Further, removal of Ti oxide layer (totally or partially, thickness varies from 10-20 nm) under mechanical or chemical incidents possibly activate corrosion mechanisms on the exposed Ti surface as observed with many other metal alloys (Chaturvedi, 2009; Henry, 1999). When inserted in oral cavity, dental implants are exposed to several adverse mechanical, chemical and microbiological events, leading into complex degradation processes (Nakagawa et al., 2002). During mastication, which is a unique mechanical action, implants are subjected to both axial and oblique forces which create micromovements at several interfaces, i.e.

implant/prosthetic crown, implant/abutment, abutment/prosthetic crown and implant/bone interfaces (Vieira et al., 2006). In such situations fretting-corrosion, a surface damage mode caused by low-amplitude oscillatory sliding motion between two contacting surfaces (Dalmiglio et al., 2008), can induce wear and corrosion. Particles created from wear (surface damage) of implants promote the release of inflammatory mediators from various cells (Azzi and Szpunar, 2007) which contribute to bone resorption (Nikolopoulou, 2006), and the encapsulation of the implant by fibrous tissue (Jones, 2001) with subsequent failure of the implant (Nikolopoulou, 2006; Sinnett-Jones et al., 2005).

In addition, dental implants are exposed to an aggressive environment including agents such as bacterial biofilm and saliva (Correa et al., 2009). The thermal, ionic, microbiologic, and enzymatic properties of this environment are ideal to induce the biodegradation of metals (Fathi et al., 2003). Lipopolysaccharide (LPS), a membrane constituent of gram-negative bacteria, is a highly bioactive molecule (Simon et al., 1969) and has a marked effects on cells like macrophages, lymphocytes, fibroblasts, and osteoblasts (Wilson, 1995). LPS is presented in the gingival crevicular fluid (Casarin et al., 2010; Simon et al., 1969), mainly on the surfaces of periodontally diseased teeth (Wilson, 1995), contributing to inflammation, osteoclasis, inhibition of cell growth, and delayed healing (Knoernschild et al., 1996). In the peri-implant region, LPS influences peri-implant inflammation and implant prognosis (Nelson et al., 1997).

Several studies have attempted to evaluate the chemical corrosion of Ti (Huang, 2003; Joska and Fojt, 2010; Joska et al., 2010; Mabilleau et al., 2006;

Mareci et al., 2009; Messer et al., 2010; Messer et al., 2009; Nakagawa et al., 1999; Nakagawa et al., 2002; Popa et al., 2008; Schiff et al., 2002; Souza et al., 2009); however, very few have reported on the effect of combined chemical, mechanical and microbiological interactions, which resemble the oral environment. In this investigation, a multi-disciplinary research area, entitled "*tribocorrosion*", is employed to address this issue in a comprehensive manner.

The research area 'tribocorrosion' has been defined as "a degradation phenomenon of material science (wear, cracking, corrosion, etc.) subjected to the combined action of mechanical loading (friction, abrasion, erosion, etc.) and corrosion attack caused by the environment (chemical and/or electrochemical interaction)" (Mathew et al., 2009). The combination of such processes can result in various forms of deterioration, including surface wear and fatigue. Moreover, when the tribocorrosion occurs in a biological environment, such as in oral system, the presence of biological species creates further complexity in understanding the materials degradation pathways and dominant driving mechanisms generated by the interplay of wear and corrosion (Landolt et al., 2001).

Previous study (Souza et al., 2010b) investigated the tribocorrosion behavior of Ti in the presence of mixed oral biofilms. Although biofilms induced low friction on Ti during tribocorrosion test, its corrosion resistance was reduced probably due to the reduction of pH caused by the presence of micro-organisms and their secreted products. The reduction of friction could affect the mechanical

integrity and stability (elastic deformation, rupture and irreversible shear) of internal connections of dental implants (Souza et al., 2010a).

However, to the authors' best knowledge, the contribution of LPS on the corrosion/wear of Ti material has not been evaluated yet. In this paper, the role of LPS on cpTi and TiAlV in a simulated oral environment has been investigated. The synergistic effect of electrochemical corrosion and wear under mechanical sliding motion has been quantitatively evaluated. It was hypothesized that LPS induces high wear/corrosion irrespective of the type of Ti.

4.4 Materiais e métodos (Materials and methods)

Surface preparation

In the present study, cpTi and TiAlV alloy discs, 12-mm diameter and 7-mm thickness, were milled from titanium rods (Mac-Master Carr, Elmhurst, IL, USA). The nominal chemical composition, density and molecular weight of cpTi and TiAlV alloy are shown in Table 1. A total of 24 discs were used. The samples were divided into 8 groups (n=3) as a function of Ti type (cpTi and TiAlV alloy) and *Escherichia coli* LPS (055:B5, Sigma-Aldrich, USA) concentration (0 - control, 0.15, 15 and 150 μg/ml). Ti discs were wet-ground with 320 to 800 grit silicone carbide paper (Carbimet 2, Buehler, Lake Bluff, IL, USA), and further polished with polishing cloth (TextMet Polishing Cloth, Buehler), diamond paste (MetaDi 9-micron, Buehler) and lubricant (MetaDi Fluid, Buehler). The surface was then mirror finished with chemomet polishing cloth (Chemomet I, Buheler) and colloidal silica polishing suspension (MasterMed, Buehler) (final average roughness (R_a) of 8.68±1.53 nm and 10.63±1.44 nm for cpTi and TiAlV alloy, respectively). The discs were ultrasonically cleaned (FS 20, Fisher Scientific, Pittsburg, PA, USA) in deionized water and 70% propanol, and finally dried with hot air of 250^{o}C. Samples were randomly allotted in each group. Artificial saliva was prepared according to Liu et al. (Liu et al., 2007) and its composition was KCl (0.4 g/L), NaCl (0.4 g/L), $CaCl_2$·2H2O (0.906 g/L), NaH_2PO_4·$2H_2O$ (0.690 g/L), Na2S·$9H_2O$ (0.005 g/L), and urea (1 g/L).

Corrosion/wear test

Tribocorrosion tests can be conducted under different electrochemical conditions, such as free potential (potential is monitored), potentiostatic (current is monitored) and potentiodynamic (potential is varied). In this study, potentiostatic test was chosen as it facilitates the estimation of the corrosion loss during the tribocorrosion exposure. This will assist in understanding the influence of LPS and mechanical stress on the tribocorrosion behavior of Ti and the synergistic interactions between wear and corrosion. For this reason, it is necessary to choose a potential value based on the polarization curves of Ti. These are found through basic electrochemical tests as follow.

Basic electrochemical test

A basic electrochemical test was conducted in a custom made acrylic electrochemical cell (Figure 1a) with a standard 3-electrode setting according to the American Society for Testing for Materials (ASTM) guidelines (G61 and G31-72). A saturated calomel electrode (SCE) was used as the reference electrode, graphite rod as the auxiliary electrode and the exposed surface of the Ti as the working electrode (the exposed area was 1.77 cm^2). A potentiostat (G300, Gamry Inc., Warminster, PA, USA), linked to a computer for data acquisition, was used to perform the corrosion measurements. A total volume of 150 ml of electrolyte was used for each corrosion experiment. In this trial saliva with no LPS (control) was used as an electrolyte. Similar polarization curves of Ti were observed in environment with and without LPS (Barao et al., 2011). After mounting the

sample into the cell, approximately 4 hours were allowed to achieve electrochemical stabilization of the system. The temperature of the test solution was maintained at 37 ± 1°C to mimic the oral environment.

Initially, open circuit potential (OCP) was monitored during a period of 3600 seconds to evaluate the potential and to stabilize the system. Further, the samples were cyclically polarized from -0.8 V to 1.8 V at a scan rate of 2 mV/sec. Figure 2 illustrated the cyclic polarization curves of cpTi and TiAlV alloy in artificial saliva at pH 6.5 (normal oral saliva pH). Both Ti types exhibited similar potentiodynamic curves, and a potential value of -0.3 V vs. SCE close to the free corrosion potential (E_{oc}) within the anodic region of the curve was chosen to be used during tribocorrosion experiment. It is important to clarify that the selected potential corresponds to an anodic region when doing polarization curves beginning in a far cathodic potential of -0.8 V, when the oxide film is partially reduced.

Tribocorrosion test

The tribocorrosion apparatus used for the experiments is shown in the Figure 1b. The tribological system consisted of an alumina ceramic ball (28 mm in diameter) rubbing against the sample (pin with flat surface) in an electrolyte chamber. The standard 3-electrode model as shown on the basic electrochemical test was employed. The exposed area of the sample to the electrolyte was 1.13 cm^2. A total of 150ml of artificial saliva at pH 6.5 was used in each test. To evaluate the effect of LPS concentration on the tribocorrosion behavior of Ti,

different LPS concentrations were added to the artificial saliva to prepare 0.15 µg/ml, 15 µg/ml and 150 µg/ml of LPS. In the oral crevicular fluid the LPS concentration varied from 0.8 to 9.6 µg/ml in case of healthy gingival sulcus and inflammation, respectively (Tzamouranis et al., 1979). Herein, higher LPS concentration was used owing to potentiate the possible effect of LPS on the corrosion/wear behavior of Ti, and also, it was based in a previous study (Barao et al., 2011). Tests were performed at a specific anodic potentiostatic condition (-0.3 V vs. SCE) chosen based on the potentiodynamic curves established during basic electrochemical test.

A standard protocol was employed for the tribocorrosion test (Figure 1c). Initially, samples were electrochemically cleaned at cathodic potential of -0.9 V vs. SCE). This is done in an effort to achieve identical starting conditions. OCP was monitored during a period of 600 seconds to evaluate the potential and to stabilize the system (with a newly formed TiO_2 film). Then, an electrochemical impedance spectroscopy (EIS) test was conducted to investigate the properties of the oxide film formed on the Ti surface (corrosion kinetics). The EIS measurements were performed in the frequency range from 100 KHz to 5 mHz, with AC sine wave amplitude of 10 mV at its corrosion potential. The measurement of potential and current were used to determine the real (Z') and imaginary (Z") components of the impedance, which were plotted with the Nyquist plot, or the total impedance (|Z|) and phase angle. EIS results were used to model the corrosion kinetics and to understand the properties of the oxide film formed on the Ti surface. Data were adjusted using the Zview2 software

(Scribner Associates Inc., Southern Pines, NC, USA) by adopting two well-known equivalent circuits (Bozzini et al., 2008; Messer et al., 2009; Moisel et al., 2008; Zhang et al., 2011) as further discussed. Double layer capacitance (C_{dl}) and polarization resistance (R_p) were determined. Impedance experimental data were fitted with chi-square error <0.001 (Vidal and Munoz, 2008).

During the tribocorrosion test, evolution of current was monitored through a potentiostatic test, according to the protocol presented in Figure 1c. The sliding duration (2000 cycles), frequency (1.2 Hz), load (20 N, Hertzian pressure estimation of 372 MPa), and oscillatory amplitude (±5°) parameters were selected to simulate the oral environment and mastication process. The coefficient of friction was measured throughout the sliding period. After sliding, EIS measurements were performed to investigate the change in the surface chemistry/condition after the tribocorrosion exposure. Finally, open circuit potential was monitored as a part of final stabilization of the test. After the test, samples were cleaned with deionized water as per the standard protocol.

Surface characterization

A white light interferometry microscope (Zygo New View 6300, Zygo Corporation, Middlefield, CT, USA) was used to capture three-dimensional (3-D) of the untouched and worn/corroded Ti surface discs. In order to understand the changes on the damaged surface under the sliding motion and the presence of LPS, surface characterization were conducted before (baseline) and after tribocorrosion exposure with Zygo microscope. Surface roughness was

characterized through a white light interferometry microscope (Zygo New View 6300, Zygo Corporation). The average roughness R_a was plotted. Two areas were chosen for investigation: inside the wear scar and the area adjacent to the wear scar. Five measurements were performed in each area for every sample. A scanning electron microscope (SEM) (Jeol JSM-6490 LV, Oxford Instruments, Oxford, UK) was also used to characterize the surfaces.

Total wear-corrosion weight loss

The weight loss during tribocorrosion was estimated and the synergistic interaction between wear and corrosion can be studied by using the following method according to Stack and Abdulrahman (Stack and Abdulrahman, 2010): The total weight loss (K_{wc}) can be expressed as:

$$K_{wc} = K_w + K_c \quad (1)$$

where, K_w is the total weight loss due to (Mechanical) wear and K_c is the total weight loss due to corrosion. The K_w and K_c variables can be divided as follow:

$$K_w = K_{wo} + \Delta K_w \quad (2)$$

and

$$K_c = K_{co} + \Delta K_c \quad (3)$$

Where, K_{wo} is the weight loss in the absence of corrosion, K_{co} is the corrosion weight loss in the absence of wear, ΔK_w is the effect of corrosion on the weight loss due to wear and ΔK_c is the effect of wear on the corrosion rate.

Total weight loss of the Ti samples (K_{wc}) was calculated by profilometric analysis of the wear scar as described above. The weight loss due to corrosion (K_c) was estimated using Faraday's Law (Mathew et al., 2010):

$$Kc = \frac{M \times i \times t}{n \times F} \quad (4)$$

and

$$i \times t = Q \quad (5)$$

where 'M' is the atomic mass of the material or equivalent weight in g/mol, 'i' is the total current, 't' is the total exposure time, 'n' is the number of electrons involved in the corrosion process which can be 2^+, 3^+ and 4^+ (for the simplest in this study we assumed n=2), 'F' is Faraday's constant (96500 C/mol^{-1}), and 'Q' is the charge passed through the working electrode (in coulombs) .

To calculate the weight loss in the absence of corrosion (K_{wo}), a specific tribocorrosion experiment was conducted in a cathodic protection potential (-0.9 V vs. SCE, the selection of this potential was based on the potentiodynamic curve), in which corrosion is negligible and material loss is only due to the tribological interaction between the two sliding bodies (Mischler, 2008).

Then the effect of corrosion on the weight loss due to wear, ΔK_w is estimated by

$$\Delta K_w = K_w - K_{wo} \quad (6)$$

The corrosion rate in the absence of wear (K_{co}) was calculated by estimating the current under potentiostatic condition (without sliding) and based on Faradays equation (Equation 4). In addition, the effect of wear on the corrosion rate ΔK_c is calculated by

$\Delta K_c = K_c - K_{co}$ (7)

Mechanistic and Synergistic study

In order to understand the involved wear and corrosion mechanisms, a criteria was established by Stack et al. (Stack and Abdulrahman, 2010) based on the ratio of weight loss due to corrosion (K_c) and weight loss due to wear (K_w). The mechanistic transitions were classified as follows:

$K_c/K_w < 0.1$ Wear (8)

$0.1 \leq K_c/K_w < 1$ Wear-corrosion (9)

$1 \leq K_c/K_w < 10$ Corrosion-wear (10)

$K_c/K_w \geq 10$ Corrosion (11)

Based on such wear/corrosion regimes and to elaborate the synergistic interactions, another ratio $\Delta K_w/\Delta K_c$ (enhancement of wear due to corrosion to enhancement of corrosion due to wear) was estimated and classified into three groups (additive, synergistic or antagonistic (Stack and Abdulrahman, 2010) as presented below:

$\Delta K_w/\Delta K_c < 0.1$ Additive (12)

$1 > \Delta K_w/\Delta K_c \geq 0.1$ Additive-synergistic (13)

$\Delta K_w/\Delta K_c > 1$ Synergistic (14)

Generally, an additive behavior is defined when the interplay of corrosion and wear reduces the total material weight loss (beneficial effect). On the other hand, synergistic behavior is defined when corrosion enhances the wear; hence the total weight loss (Stack and Abdulrahman, 2010).

Statistical analysis

Electrochemical parameters such as C_{dl} and R_p were statistically analyzed using one-way ANOVA for analyzing each factor separately (Ti type and LPS concentration) either before or after sliding. Total weight loss, and roughness data were also analyzed by one-way ANOVA. Tukey HSD test was chosen as the multiple-comparison technique when necessary. T- test was used to compare cpTi and TiAlV. Paired-T test was used to compare the electrochemical data before and after sliding for each Ti type. A significant level of 0.05 was used for all tests (Statistical Package for the Social Sciences, version 17.0; SPSS Inc, Chicago, IL, USA).

4.5 Resultados e Discussão (Results and Discussion)

Evolution of current and friction coefficient

Figure 3 illustrates the evolution of current and friction coefficient during sliding period for cpTi and TiAlV alloy. It is clearly noticeable that when sliding started, the current suddenly increased and during the whole period of sliding, it shows a consistent and noticeable fluctuations. This indicates a destruction of passive oxide film on the Ti surface under sliding action (Fernandes et al., 2006), which results in the exposure of bare surface to the solution and reformation of oxide films, shifting from passive to active regime (Mischler, 2008). TiO_2 films in their native form present poor tribological properties and fractured under wear regimes (i.e. fretting and sliding) (Fernandes et al., 2006). Constant depassivation and repassivation processes on the worn surface during sliding were observed (Mischler et al., 1998). The current converged to the original value when sliding is stopped since a new and stable protective film was formed (passivation) on the Ti surface, covering the electrochemically active area of exposed specimen. Apparently, it indicates that the presence of LPS did not change the evolution of current for both Ti types.

The friction coefficient exhibited a constant value in the range of (0.3-0.4) during the sliding test. The slight fluctuations may be attributed to build-up and accumulation of third-body particles in the sample/ball contact region (Barril et al., 2004; Vieira et al., 2006). In addition, LPS groups showed similar coefficient of friction when compared with the control.

Electrochemical impedance spectroscopy (EIS) measurements

The results from the EIS before and after sliding are studied through Nyquist (impedance real vs. impedance imaginary, Figure 4) and Bode plots (bode phase plot and bode impedance plot, Figure 5). Nyquist plots demonstrate the electrochemical resistance of Ti surface. It is clearly observed that the presence of LPS reduced the semicircular diameter of capacitance loop, indicating poor corrosion resistance for both cpTi and TiAlV alloy. After sliding, the diameter of capacitance loop suffered a higher decrease (Figure 4), indicating some modification of the characteristics of the passive film formed on the Ti surface (Fernandes et al., 2006).

Bode impedance plots provide the variation of impedance as a function of frequency of the electrochemical double layer (with or without the presence of passive film) formed at the interface of Ti and solution during corrosion process (corrosion kinetics). Bode phase plot, just one time constant was observed for all groups, except for cpTi after sliding which presented two time constants (Figure 5). One time constant indicates the presence of compact and homogeneous passive film while two time constants indicate the formation of a porous film on the Ti surface (Souza et al., 2009). At high frequency, the phase angle and impedance exhibited low values for LPS groups (mainly at 150 μg/ml of LPS) both before and after sliding, corroborating with the Nyquist results.

EIS model

Two equivalent circuits were employed to estimate the formation and characteristics of the oxide film on the Ti surface as a function of LPS presence, sliding period and Ti type. In Randle's equivalent circuit (Figure 6a) the oxide layer consists of a compact layer with a constant phase element (CPE - C_{dl}) and a polarization resistance (R_p). In the second circuit (Figure 6b) we assumed that the oxide layer is formed by two layers: the porous outer layer represented by the components $C_{dl\ out}$ (capacity of the metal/film/electrolyte interface) and $R_{p\ out}$ (outer layer polarization resistance), and the inner (barrier) layer, for lower frequencies, represented by the components $C_{dl\ in}$ and $R_{p\ in}$ (inner layer polarization resistance). R_{sol} represents the solution resistance for both circuits. The impedance of a CPE is defined by $Z_{CPE} = 1/((jw)^n C)$, where $j = \sqrt{(-1)}$ and $w = 2\pi f$, and the exponent 'n' of the CPE is related to non-equilibrium current distribution due to surface roughness (Ariza and Rocha, 2005; Hsu et al., 2004). The parameter 'C' is a constant, representing true capacitance of the oxide barrier layer. All results of EIS were modeled by Randle's circuit except for cpTi after tribocorrosion test that was represented by the second circuit.

The presence of LPS influenced significantly on the corrosion kinetics and resistance of both Ti types (C_{dl} cpTi $p=.018$ before sliding, $p=.431$ after sliding; C_{dl} TiAlV $p=.127$ before sliding, $p=.026$ after sliding; R_p cpTi $p<0.001$ before sliding, $p=.058$ after sliding; R_p TiAlV $p=.003$ before sliding, $p=.028$ after sliding). Figure 6c-d shows the evolution of R_p and C_{dl} and the post-hoc comparisons (Tukey HSD test, T test and Paired-T test) for both Ti types, before and after sliding. C_{dl}

increased with the increase of LPS concentration. These values were statistically significant for cpTi before sliding, and for TiAlV alloy after sliding. C_{dl} of cpTi was statistically higher when compared to TiAlV alloy after sliding. For both Ti types R_p was statistically reduced in presence of LPS. TiAlV alloy exhibited higher R_p in comparison to cpTi regardless of the presence or absence of LPS. As observed by others, the TiO_2 formed on the surface of TiAlV alloys is an efficient barrier to corrosion, and increases charge transfer resistance at the corrosion interface (Hsu et al., 2004); therefore these samples exhibited high polarization resistance (Fernandes et al., 2006). In addition the lower corrosion resistance of cpTi after sliding compared to TiAlV may be due to the presence of a porous layer in the previous case. The electrolyte can be penetrated through such pores in the passive film to the underlying surface of cpTi (Joska et al., 2010).

Most of the studies of corrosion resistance of Ti are conducted in saline or artificial saliva. Little is known about corrosion kinetics and variation in the mechanisms induced by released-cell products such as LPS. Mabilleau et al. (Mabilleau et al., 2006) found that LPS-treated cells increased corrosion of Ti in saliva as evidenced by the increased Ti surface roughness. Messer et al. (Messer et al., 2010) showed an increase of corrosion rate of machined implants when exposed to simulated inflammatory (THP1 cells and LPS) and hyperglycemic (dextrose) conditions.

LPS are composed by lipid and polysaccharide joined by a covalent bond. According to Messer et al. (Messer et al., 2009) the presence of dextrose may carry away metal ions from alloy surface, encouraging further corrosion.

Therefore, the polysaccharide part of the LPS molecule may also induced high metal release from the Ti to the media which may explain the reduction of the corrosion inhibiting characteristics of the passive oxide film formed on Ti surface. In addition, the passive film can be formed with same defects in environment containing LPS, explaining the C_{dl} and R_p results in the present study.

After the wear test C_{dl} increased and R_p decreased for both Ti types for all groups (Figure 6). Some authors (Lucas and Lemons, 1992) stated that under static conditions the oxide layer on the Ti surface is stable and protective, but under sliding and loading the film stability is compromised. This may explain the findings of the current study. Kamotori et al. (Komotori et al., 2007) observed a reduction of corrosion resistance of TiAlV alloy under the simultaneous action of corrosion and wear in Ringer's solution. Authors found that the reduction is intensified by increasing the scratching rate and applied potential.

It is important to highlight that both the C_{dl} and R_p values may be affected by changing of the specimens dimensions promoted by the wear scar (worn surface) itself and by the higher roughness after tribocorrosion test. By estimating those topographical changes, an increase of 0.08 to 0.12% of the original area of the specimens is observed. Due to the small area changes (from unworn area to worn area) such effects are neglected for C_{dl} and R_p calculation.

Surface characterization

Figure 7 illustrates the white light interferometry images of cpTi and TiAlV alloy before and after tribocorrosion (around the wear scar) as a function of LPS

concentration. An increase in surface damage is observed after electrochemical attack. After tribocorrosion test a wear scar with well defined boundary was observed on the Ti surface for all groups (Figure 8a). The surface profile of a representative wear scar is showed (Figure 8b).

Figure 9 shows the surface roughness (R_a) of the samples before and after tribocorrosion as a function of different LPS concentration. LPS did not affect the R_a values of Ti around the wear scar area ($p>.05$; ANOVA); however, a tendency of increase in surface roughness was observed when the samples were exposed to saliva containing LPS. The roughness inside the wear scar was affected by LPS for both Ti types ($p<.0001$; ANOVA): the higher the LPS concentration, the higher the surface roughness ($p<.05$; Tukey HSD test). Both Ti types exhibited similar surface roughness values ($p\geq.068$; T-test) except for 15 μg/ml LPS groups at peripheral area ($p=.027$; T-test) and for 150 μg/ml LPS groups inside the wear scar ($p=.040$; T-test).

It is known that corrosion process can increase the roughness of Ti (Correa et al., 2009; Mabilleau et al., 2006) based on the corrosion mechanisms. Our results showed that LPS accelerate the wear/corrosion of cpTi and TiAlV alloy, which may explain the greater surface roughness of LPS groups. Surface roughness is an important property of adhesion and colonization of bacteria (Morgan and Wilson, 2001); therefore, a greater biofilm accumulation might be expected on the Ti surface after wear/corrosion in presence of LPS, which could lead to peri-implantitis (Bollen et al., 1996).

SEM images of the wear scars of both Ti types are presented in Figure 10. The worn surface is characterized by wear marks aligned in the direction of sliding. Under the tribocorrosion test conditions, two areas can be observed in the wear track. Boundary wear scar area exhibited some smearing as a result of wear debris spread during sliding test which removes the protective passive film and actively corrode the Ti surface. The area inside of the wear scar is noticeable with severe material damage and presence of wear debris (third body). Particles are observed in the central part of the wear scar probably due to the plastic surface deformation. Those particles are likely to delaminate and detach from surface, inducing oscillations of the friction coefficient (Vieira et al., 2006). Such effects also cause the transitions in the wear mechanisms from two body to three body. Delamination and cracking were observed mainly in the 150 µg/ml LPS group. The backscattering images of the 150 µg/ml LPS groups clearly show cracking (arrows) of the Ti surface.

Wear-corrosion weight loss distribution

As explained in the section 2.4 the total weight loss could be separated in to individual contributions from wear and corrosion and listed in the Table 2. The variation of weight loss as a function of LPS concentration is shown in Figure 11. The individual contributions of corrosion (K_c) and wear (K_w) to the weight loss are presented. The combined effect of corrosion and wear (K_{wc}) is also illustrated in the Figure 11. In general, LPS significantly influenced the total weight loss due to corrosion (K_c p=.316 for cpTi and p=.029 for TiAlV alloy; ANOVA), the total weight

loss due to wear (K_w p=.300 for cpTi and p=.014 for TiAlV alloy; ANOVA) and the total weight loss due to wear and corrosion (K_{wc} p=.05 for cpTi and p=.130 for TiAlV alloy; ANOVA). For both Ti types, LPS increased the total weight loss (Figure 11). In LPS groups the passive oxide film is less protective (as observed in C_{dl} and R_p results) which may induce higher release of metals from Ti, and, consequently, high weight loss. Fernandes et al. (Fernandes et al., 2006) observed correlation between degradation of the passive film with high weight loss of plasma nitride and plasma nitride + oxidized TiAlV alloy after wear. It is also believed that in the presence of LPS the passive film is harder to reform, leading to high total weight loss. No significant difference in the total weight loss (K_c, K_w and K_{wc}) was observed between cpTi and TiAlV alloy (p>.05, T-test).

Mechanistic and synergistic interaction between wear and corrosion

Identification of wear/corrosion regime transitions is made by using the ratio of weight loss due to corrosion and weight loss due to wear (K_c/K_w ratio). K_c/K_w values ranging from 0.11 to 0.16 were obtained (Table 2). According to these results a wear dominated tribocorrosion mechanisms is the driving degradation process. (Stack and Abdulrahman, 2010). Those regimes of wear-corrosion were defined as additive, synergistic or antagonistic based on the $\Delta K_w/\Delta K_c$ ratio values (Stack and Abdulrahman, 2010). All groups exhibited a synergistic effect in which corrosion and wear are enhancing each other (detrimental effect). However, for the TiAlV alloy at 150 µg/ml LPS concentration, additive effect was observed in which corrosion possibly reduced

the wear (Table 2). This later result can be explained by the decrease of total weight loss of TiAlV alloy at 150 μg/ml LPS concentration (Figure 11).

These results indicate the complexity of the wear and/or corrosion mechanisms, during tribocorrosion process. In general, material deterioration results from two mechanisms: mechanical wear and wear-accelerated corrosion (Mischler, 2008). Mechanical wear (i.e. sliding) is related to the metal detached from the material surface. During sliding, the oxide film on the Ti surface was removed and the new surface of Ti was exposed and more likely to be corroded than the TiO_2 surface. Therefore, the wear accelerates corrosion by removing the Ti protection until another passive film is formed (Mischler, 2008). In the present study, the wear-accelerated corrosion mechanism was more pronounced in the presence of LPS. The new Ti surface after sliding suffered from increased attack by the LPS, which induced a high corrosion rate and consequently more weight loss.

A simple schematic diagram has been developed to demonstrate the influence of LPS on the wear/corrosion mechanism of Ti (Figure 12). Initially, Ti surface was protected by the oxide film. Before sliding, the polysaccharide part of LPS may attacks the oxide film, inducing some defects. Part of the Ti surface was exposed, and an exchange of ions between Ti (metal ions - M+) and saliva (electrons - e^-) occurred. During the sliding, the rest of passive film was removed. Some debris was observed and the new surface of Ti was attacked again by the LPS, which increased the total wear weight loss. A new passive film was formed when sliding was stopped. But according to our results, this film is less protective

than the native film (as observed by the C_{dl} and R_p results). In addition, the influence of LPS on the new formed film may be based on an adsorption tendency of LPS on Ti. However, all these speculations are not yet proved.

Clinical relevance, limitations and future scope

When Ti dental implants are inserted in oral cavity, a stable passive film is formed. However, due to several unfavorable conditions this passive film can break down and corrosion may take place. The present study showed that the damage due to sliding wear, performed at 1.2 Hz resembling the mastication frequency, reduced the corrosion resistance of cpTi and TiAlV alloy. In addition, the presence of components of membrane of gram negative bacteria, such as LPS, accelerated the wear/corrosion process of Ti. Therefore, patients with oral infections (i.e. periodontitis) may be more likely to have their dental implants corroded. Also, the surface roughness of Ti was increased by the presence of LPS, which may induce greater bacterial biofilm accumulation and consequently peri-implantitis, and further implant therapy failure. Previous study showed that bacteria increased the wear/corrosion of Ti (Souza et al., 2010b). Therefore, this whole process will be transformed in a “vicious circle” in which LPS induces corrosion, corrosion increases surface roughness, surface roughness promote more bacteria adherence, bacteria induces corrosion and so on. For this reason it is very important that patients with oral Ti implants are assisted/instructed to practice a good oral hygiene.

The employed tribocorrosion set-up did not completely resemble the actual adverse factors/conditions that implants are subjected in oral cavity. The simulated force was uni-directional and constant in magnitude. Implants are exposed to vertical, horizontal and oblique forces, and high-intensity forces created by parafunctional habits (i.e. bruxism). Furthermore, the motion amplitude was larger than what is expected *in-vivo* and uni-directional rather than multi-directional. Smaller, multi-directional amplitudes may generate a fretting-type tribological contact conditions. In oral cavity implants are inserted into the bone, which may assist to distribute the stress generated during loading. However, in this study Ti was firmly held. The microbiological environment created in this study was based on LPS only (i.e. bacteria were missing). Further investigations with actual bacteria enriched with LPS are necessary. Specially focused investigations are required to understand the effect of corrosion products on the cell proliferation and viability, inflammatory reaction, and bone level.

4.6 Conclusão (Conclusion)

The tribocorrosion behavior of commercially pure titanium (cp-Ti) and titanium-aluminum-vanadium (TiAlV) alloy was investigated in the presence of artificial saliva as a function of lipopolysaccharide presence. Based on the outcomes of the present *in-vitro* study the following conclusions can be drawn:

- Lipopolysaccharide affected negatively the wear/corrosion behavior of cpTi and TiAlV alloy by increasing the capacitance and reducing the resistance of the titanium oxide film.
- Both titanium types exhibited inferior corrosion behavior after sliding as observed in the capacitance and resistance of double layer data and Nyquist plots.
- The total wear-corrosion weight loss was high when lipopolysaccharide was added regardless of titanium type. Wear-corrosion was the dominant mechanistic model.
- The presence of lipopolysaccharide increased the roughness of the worn surface of both titanium types.
- Clinically, the results of this study showed that patients with oral infections (presence of LPS) may be more likely to have their dental implants corroded and with greater bacterial biofilm accumulation which could negatively affect the implant prognosis.

Acknowledgements

Authors would like to express gratitude to CAPES (BEX: 4129/09-1) and FAPESP (2007/ 58926-5) for the scholarships provided to *Barão, V.A.R*. Authors would like to thank Prof R Urban and Ms. D Hall (Department of Orthopedic Surgery, Rush University Medical Center) for the assistance in SEM images.

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Tabelas (Tables)

Table 1. Nominal composition, density and molecular weight of cpTi and TiAlV alloy

Ti type	Composition (wt%)								Density	Molecular
	Ti	Al	V	C	Fe	O_2	N_2	H_2	(cm^3)	weight (u)
cpTi	99.7	-	-	0.006	0.12	0.16	0.004	0.0019	4.50	47.85
TiAlV	89.62	6.1	4.0	0.004	0.16	0.106	0.008	0.0022	4.44	46.72

Table 2. Weight loss distribution as a function of LPS concentration for cpTi and TiAlV alloy in artificial saliva

Ti type	K_{wc} (μg)	K_c (μg)	K_w (μg)	K_c/K_w	K_{wo} (μg)	ΔK_w (μg)	K_{co} (μg)	ΔK_c (μg)	$\Delta K_w/\Delta K_c$
cpTi									
Control	96.31±15.84	13.21±1.25	83.09±1.71	0.16±0.05	14.17	68.92±17.09	3.60E-3±3.43E-3	13.21±0.88	5.27±1.3
0.15 μg/ml	111.27±9.95	15.68±1.25	95.58±8.71	0.16±0.01	12.69	82.90±8.71	1.21E-2±3.76E-3	15.67±0.87	5.29±0.1
15 μg/ml	125.98±24.91	15.64± 2.16	110.33±27.06	0.15±0.06	17.54	92.78±27.06	3.32E-3±1.42E-2	15.64±1.52	6.1±1.8
150 μg/ml	152.33±64.10	16.69±4.16	135.35±59.98	0.12±0.02	11.88	146.07±59.98	7.48E-3±7.49E-3	16.97±4.16	7.0±1.7
TiAlV									
Control	106.01±2.23	12.95±2.49	93.05±2.48	0.14±0.01	36.84	56.21±40.56	-4.84E-3±7.06E-3	12.96±18.37	4.3± 0.2
0.15 μg/ml	114.99±10.40	14.96±1.28	100.00±10.40	0.15±0.02	6.09	93.93±38.84	7.25E-3±1.34E-2	14.95±0.01	6.3± 0.5
15 μg/ml	153.95±8.78	15.07±0.24	138.87±8.64	0.11±0.01	0.013	4.53±54.03	1.91E-3±9.51E-3	15.07±0.24	0.3±0.6
150 μg/ml	129.81±2.58	16.05±1.94	113.76±24.62	0.15±0.03	0.015	-41.28± 70.35	-7.36E-3±1.25E-2	16.05±1.95	-2.7±1.6

K_{wc}: total weight loss due to wear and corrosion (tribocorrosion)

K_c: total weight loss due to corrosion

K_w: total weight loss due to (mechanical) wear

K_{wo}: weight loss in the absence of corrosion

ΔK_w: effect of corrosion on the weight loss due to wear

K_{co}: corrosion weight loss in the absence of wear

ΔK_c: effect of mechanical wear on the corrosion rate.

Figuras (Figures)

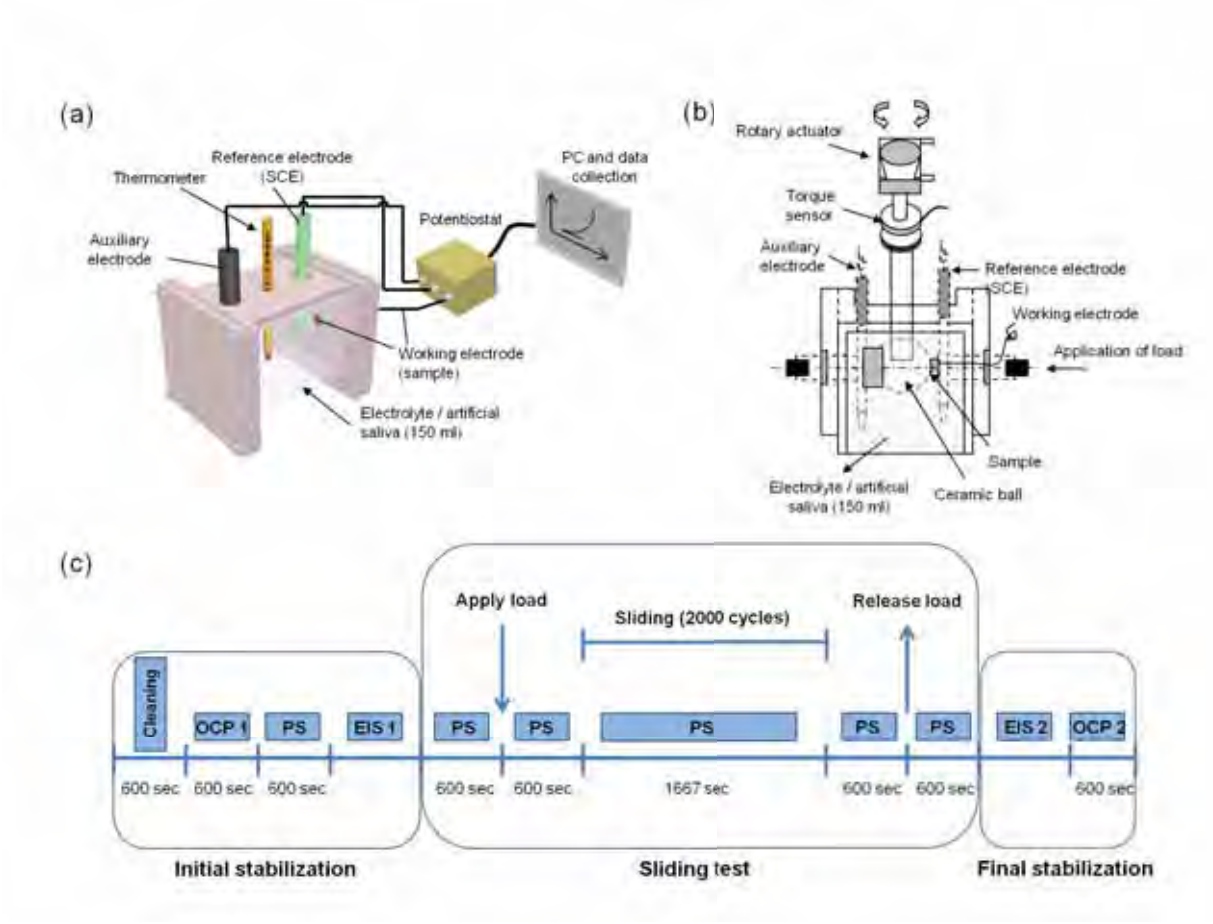

Figure 1. (a) Schematic electrochemical set-up (a standard 3-electrode cell) used during basic corrosion test. **(b)** Schematic tribocorrosion set-up. **(c)** Standard protocol used during tribocorrosion test (OCP – open circuit potential; PS – potentiostatic test; EIS – electrochemical impedance spectroscopy).

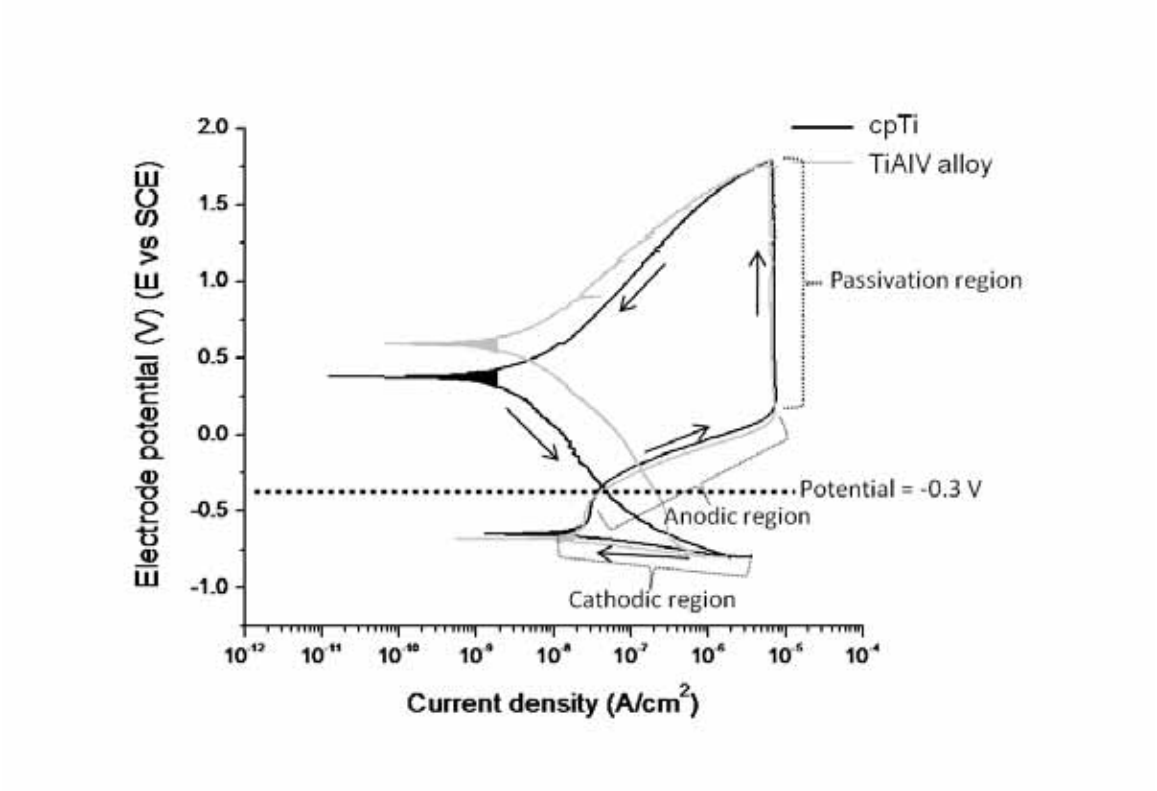

Figure 2. Representative cyclic polarization curves for cpTi and TiAlV alloy in artificial saliva at pH 6.5.

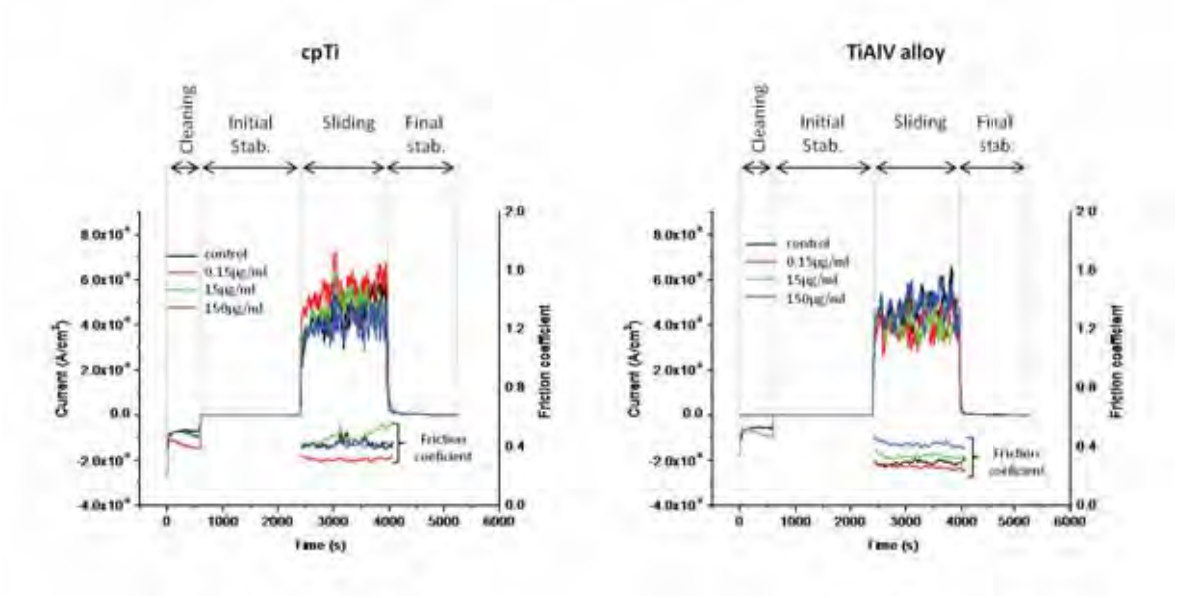

Figure 3. Representative curves of evolution of current density during the wear/corrosion test and the evolution of friction coefficient during sliding period for cpTi and TiAlV alloy in artificial saliva as a function of different LPS concentration.

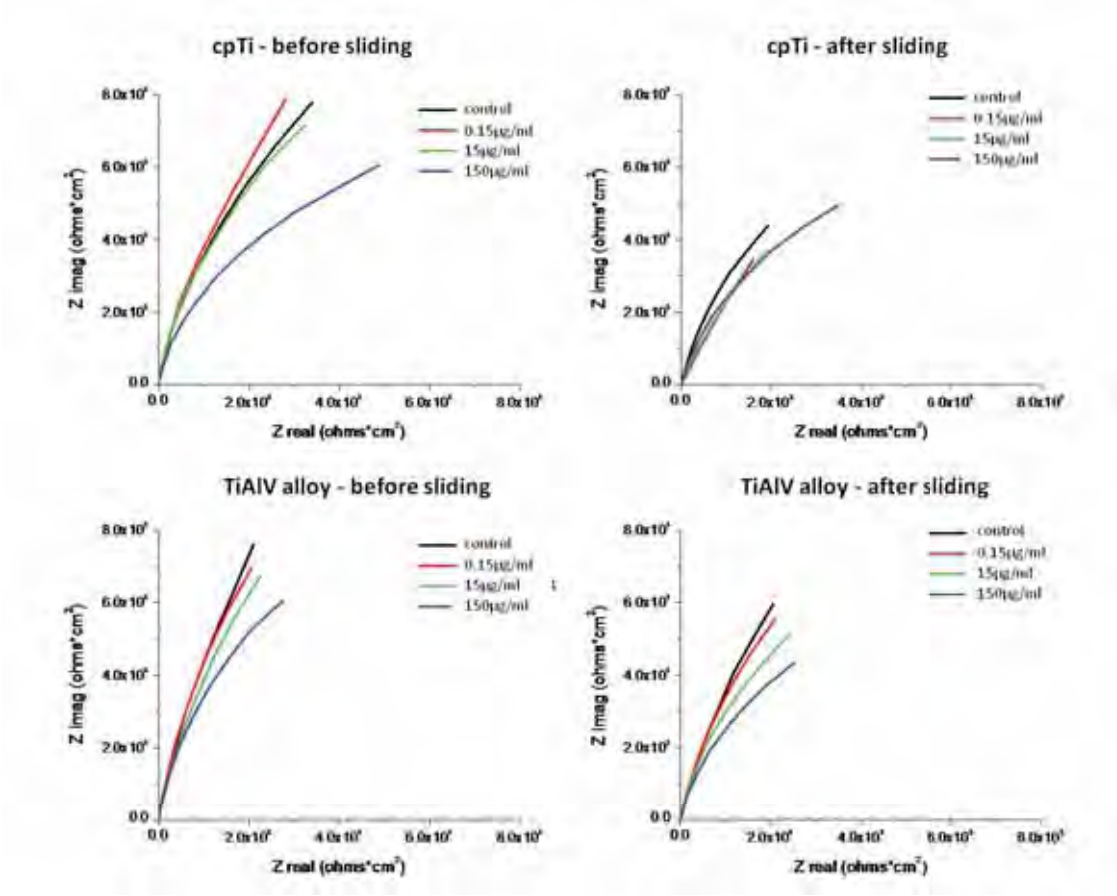

Figure 4. Representative Nyquist plots from EIS recorded for cpTi and TiAlV alloy in artificial saliva as a function of different LPS concentration, before and after sliding.

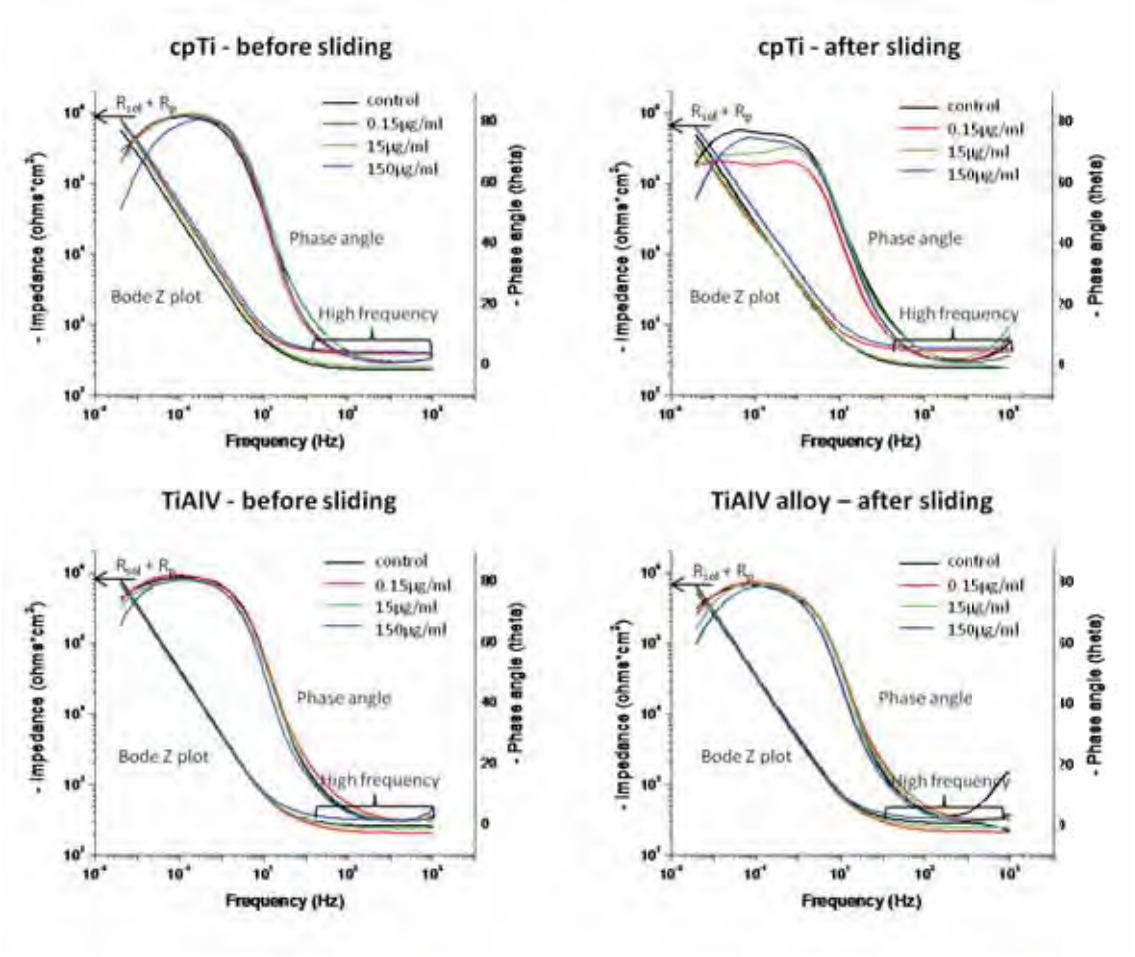

Figure 5. Representative Bode plots from EIS recorded for cpTi and TiAlV alloy in artificial saliva as a function of different LPS concentration, before and after sliding. (R_{sol} - resistance of solution and R_p - polarization resistance).

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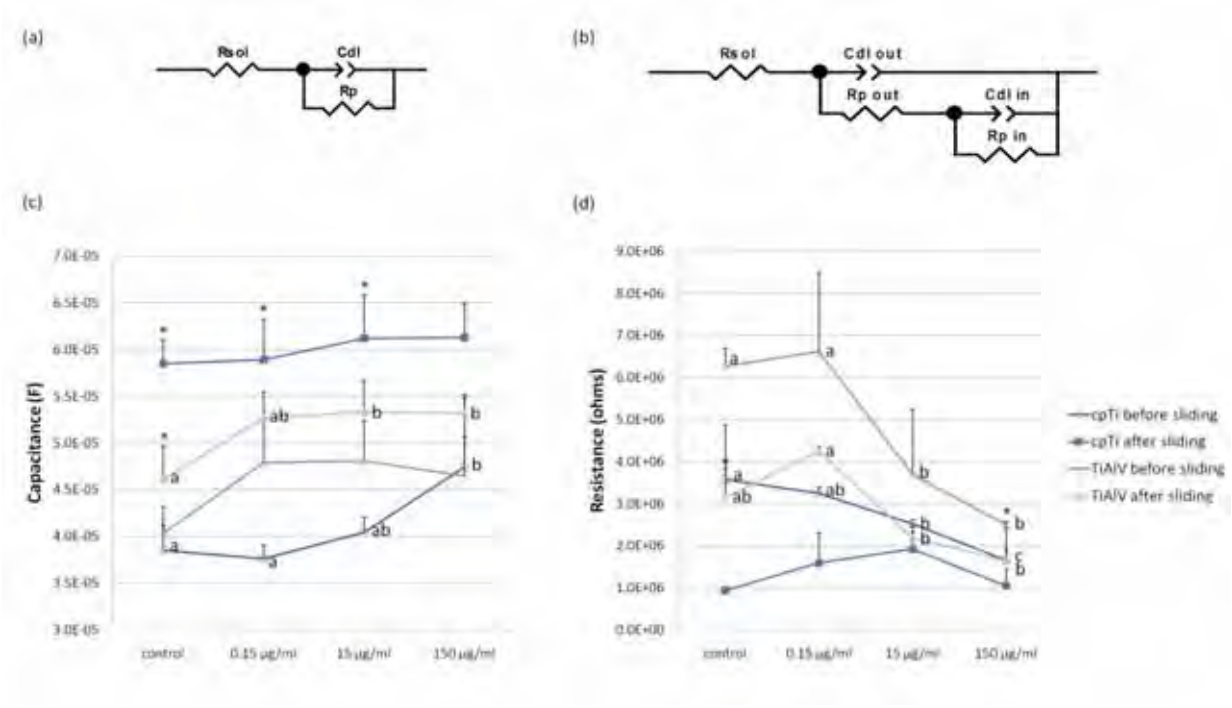

Figure 6. (a-b) Equivalent circuit models selected for impedance spectra analysis. Evolution of **(c)** capacitance of double layer (C_{dl}), and **(d)** polarization resistance (R_p) for cpTi and TiAlV alloy in artificial saliva as a function of different LPS concentration. Lower case letters were used to compare the different LPS concentrations at the same Ti type and sliding period. Different letters indicate significant difference among the groups (α=0.05). For each variable, (*) express significant difference between sliding periods at the same Ti type and LPS concentration (α=0.05).

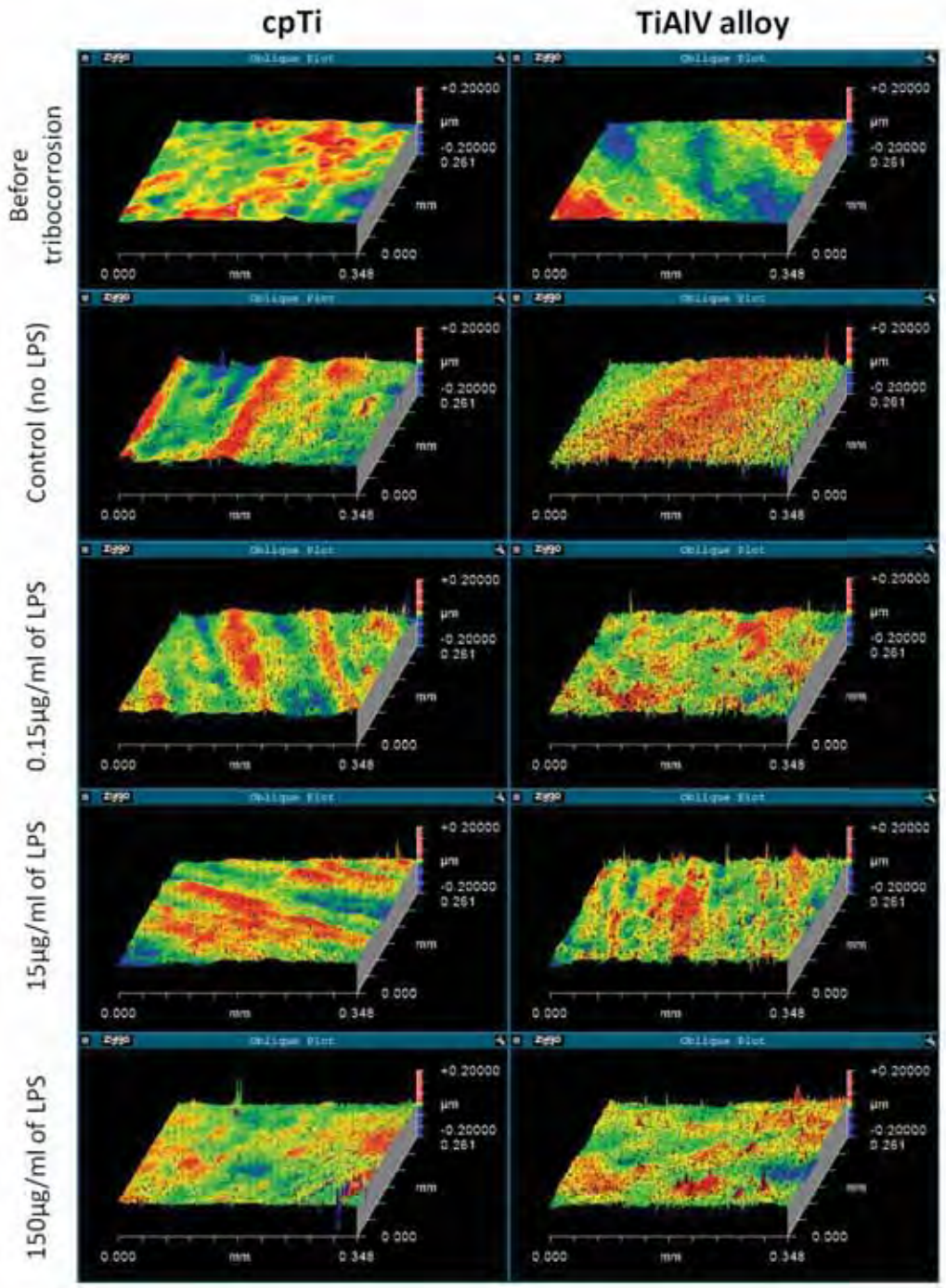

Figure 7. White interferometry microscopy 3D images of cpTi and TiAlV alloy before and after tribocorrosion (around the wear scar) as a function of LPS concentration. *X* and *Y* scales are in mm, and *Z* scale is in µm. The *Z* scale covers 0.4 µm in amplitude.

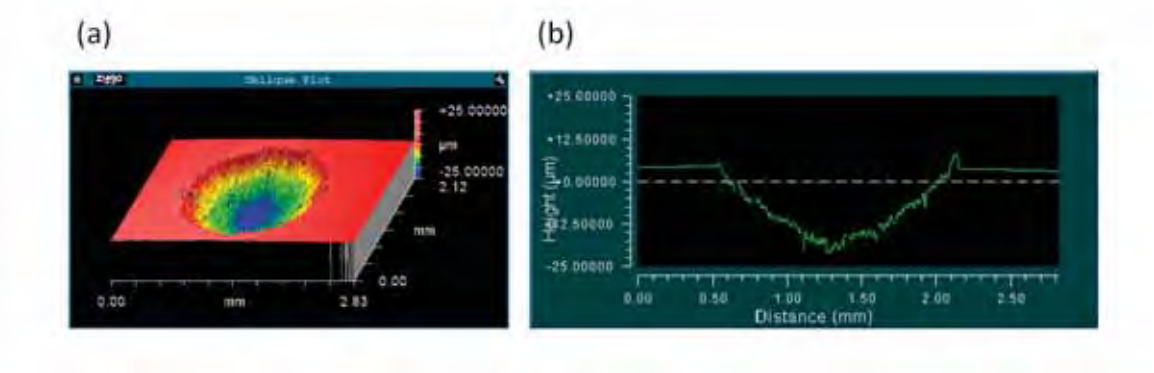

Figure 8. White interferometry microscopy 3D images and surface profile of a representative wear scar of Ti. *X* and *Y* scales are in mm, and *Z* scale is in µm. The *Z* scale covers 50 µm in amplitude.

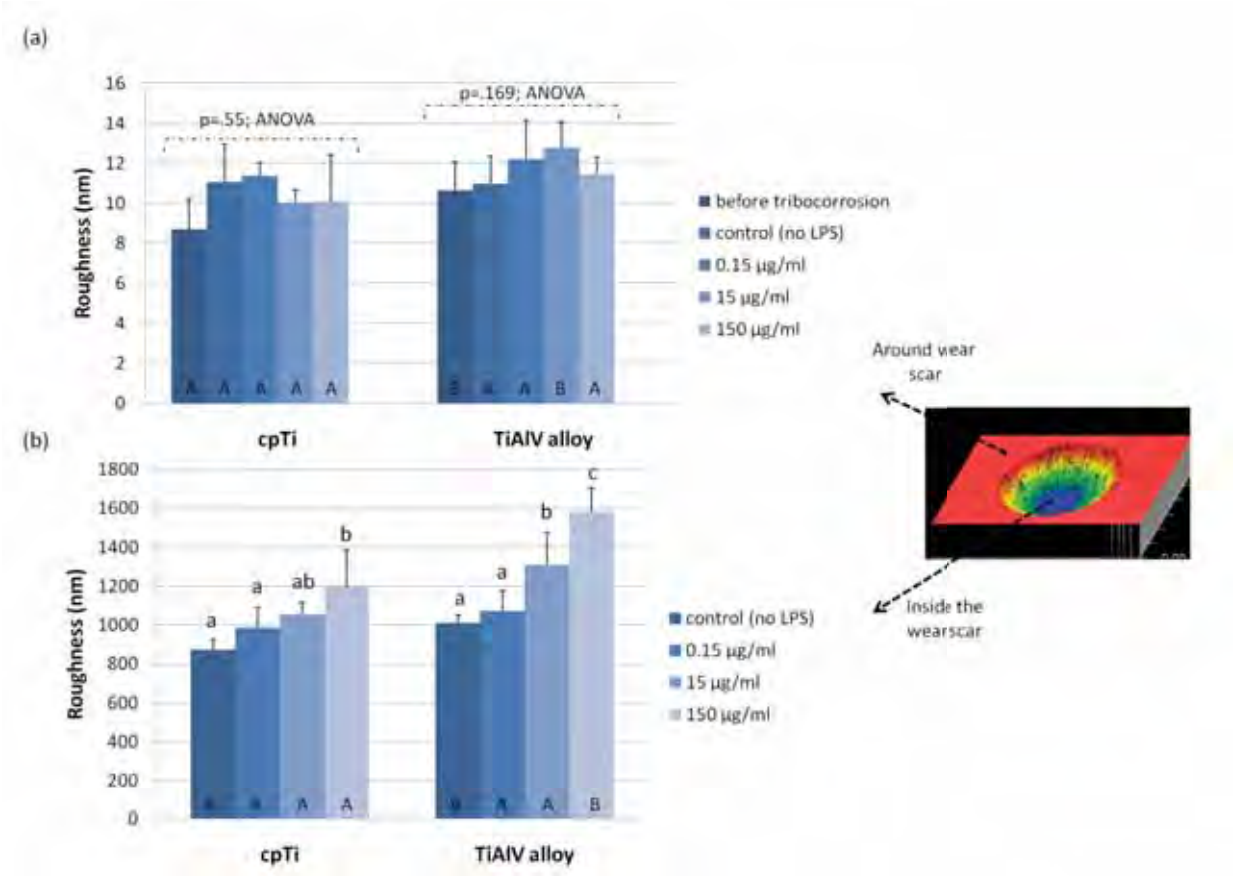

Figure 9. Roughness average (Ra) of cpTi and TiAlV alloy **(a)** around the wear scar; **(b)** inside the wear scar. In the same Ti type, means with different lower case letters are significant different ($p<.05$). In the same LPS concentration, different upper case letter shows significant different between cpTi and TiAlV alloy ($p<.05$).

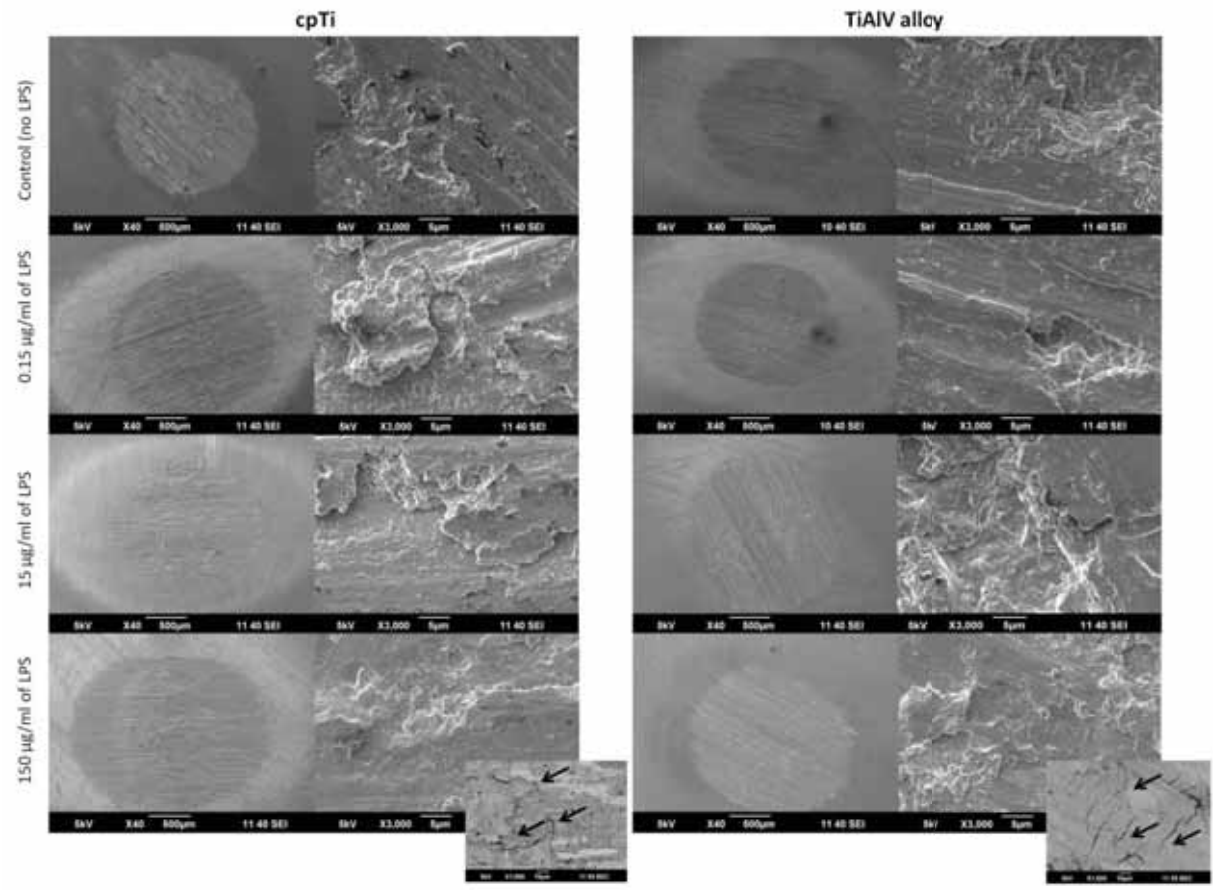

Figure 10. SEM photographs of corroded wear surface of cpTi and TiAlV alloy in artificial saliva as a function of different LPS concentration. Arrows indicate the cracking on the titanium surfaces through backscattering images technique.

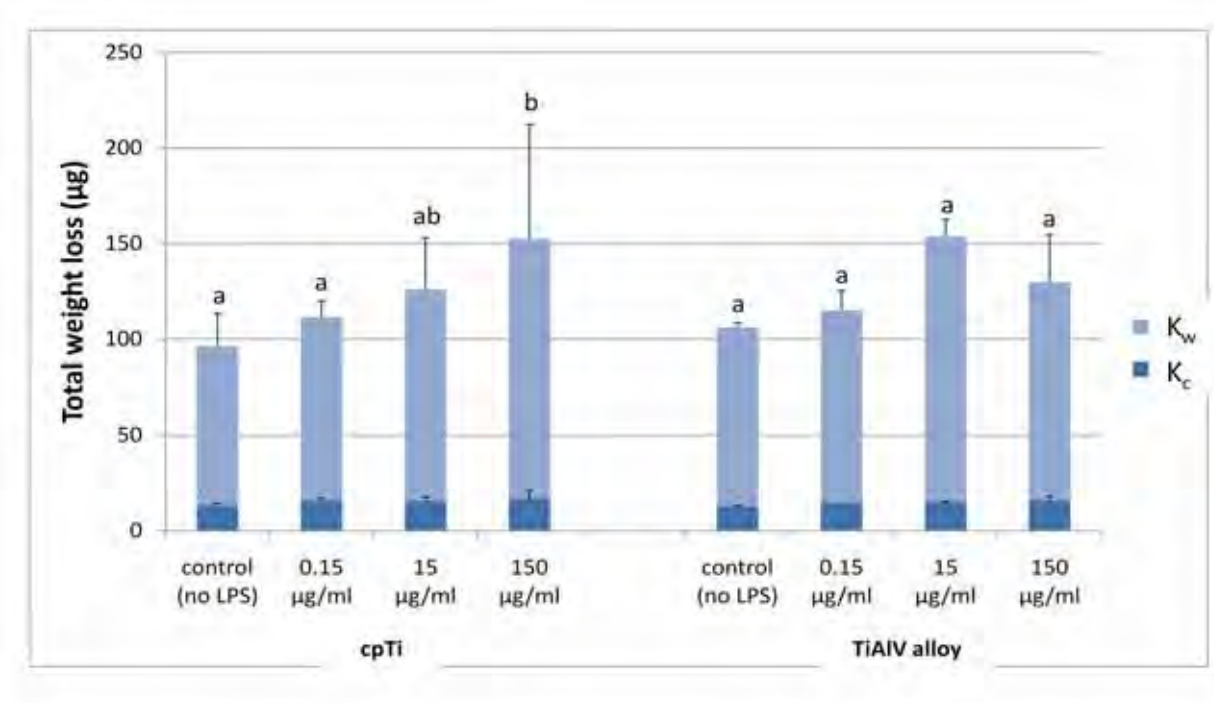

Figure 11. Variation of weight loss of cpTi and TiAlV alloy as a function of different LPS concentration. The individual contribution of corrosion (K_c) and wear (K_w) to the weight loss are expressed. The combined effect of corrosion and wear (K_{wc}) is illustrated by the whole bar. In the same Ti type, means with different lower case letters are significant different ($p<.05$) regarding K_{wc} values.

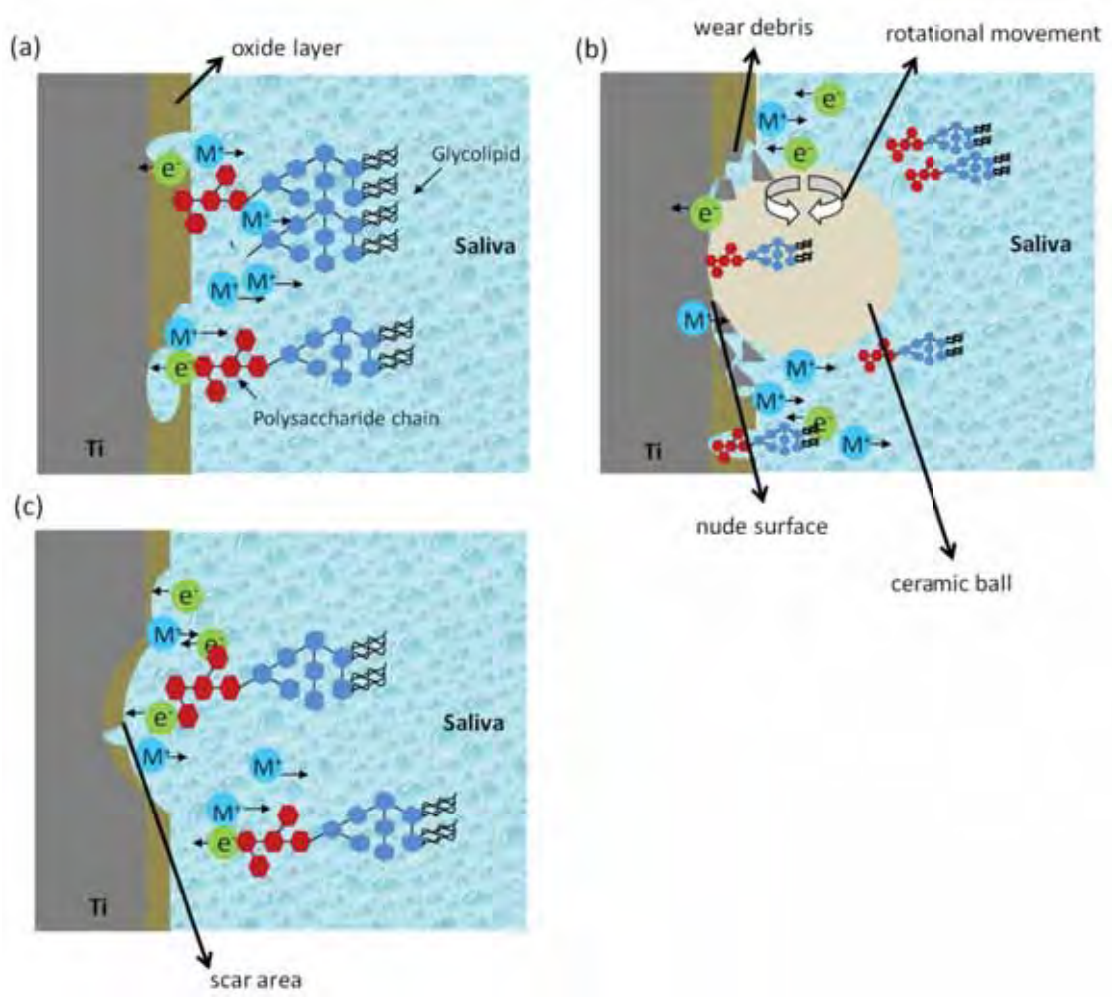

Figure 12. Schematic diagram of the tribological contact zone which exhibit the influence of LPS on the wear/corrosion mechanism of Ti. **(a)** befor e sliding; **(b)** during sliding; **(c)** after sliding. (e^- - electrons and M^+ - metal ions).

CAPÍTULO 4 *

Comparação de Diferentes Designs de Overdentures Implanto-Retidas e Prótese Total Fixa Implanto-Suportada na Distribuição de Tensões em Mandíbula Edêntula – Análise pelo Método de Elementos Finitos Tridimensional Baseado em TC

Comparison of Different Designs of Implant-Retained Overdentures and Fixed Full-Arch Implant-Supported Prosthesis on Stress Distribution in Edentulous Mandible – A CT-based Three-Dimensional Finite Element Analysis

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* Artigo a ser enviado para o *The International Journal of Oral & Maxillofacial Implants*, e suas normas de publicação encontram-se disponíveis no anexo E.

Comparação de Diferentes Designs de Overdentures Implanto-Retidas e Prótese Total Fixa Implanto-Suportada na Distribuição de Tensões em Mandíbula Edêntula – Análise pelo Método de Elementos Finitos Tridimensional Baseado em TC

5.1 Resumo

Objetivo: Comparar o efeito de diferentes designs de overdentures implanto-retidas e prótese total fixa implanto-suportada na distribuição de tensões em mandíbula edêntula pelo método de elementos finitos tridimensional (MEF-3D) baseado em tomografia computadorizada (TC).

Materiais e métodos: Quatro modelos tridimensionais de elementos finitos de uma mandíbula edêntula humana com mucosa e quatro implantes posicionados na região interforame foram construídos e reabilitados com diferentes designs de próteses. No grupo OR a mandíbula foi restaurada com overdenture retida por quatro implantes não esplitados com sistema de retenção do tipo O'ring; nos grupos BC-C e BC as mandíbulas foram restauradas com overdentures retidas por quatro implantes esplintados com sistema de retenção barra-clipe associado ou não a dois cantilevers distais, respectivamente; no grupo FD a mandíbula foi restaurada com prótese total fixa suportada por quatro implantes. Os modelos foram suportados pelos músculos da mastigação e pelas articulações temporomandibulares. Uma força oblíqua de 100 N (30 graus) foi aplicada na região de primeiro molar inferior esquerdo em cada prótese na direção

vestíbulo-lingual. Análises qualitativa e quantitativa das tensões de von Mises (σ_{vM}), máxima (σ_{max}) (tração) e mínima (σ_{min}) (compressão) tensões principais (em MPa) foram obtidas.

Resultados: O grupo BC-C exibiu os maiores valores de tensões (σ_{vM} = 398,8; σ_{max} = 580,5 e σ_{min} = -455,2) enquanto que o grupo FD mostrou as menores tensões (σ_{vM} = 128,9; σ_{max} = 185,9 e σ_{min} = -172,1) nos implantes/componetes protéticos. No grupo das overdentures, o uso de implantes não esplintados (grupo OR) reduziu as tensões nos implantes/componentes protéticos (59,4% para σ_{vM}, 66,2% para σ_{max} e 57,7% para σ_{min} versus grupo BC-C) e tecidos de suporte (redução máxima de tensão de 72% e 79,5% para σ_{max}, e de 15,7% e 85,7% para σ_{min} no oso cortical e medular, respectivamente). O osso cortical exibiu maior concentração de tensões que o osso medular em todos os grupos.

Conclusão: O uso de prótese total fixa implanto-suportada e prótese total removível retida por implantes não esplinatados para reabilitar mandíbula edêntula reduziu na tensões no tecido ósseo peri-implantar, mucosa e implantes/componentes protéticos.

Comparison of Different Designs of Implant-Retained Overdentures and Fixed Full-Arch Implant-Supported Prosthesis on Stress Distribution in Edentulous Mandible – A CT-based Three-Dimensional Finite Element Analysis

5.2 Abstract

Purpose: To compare the effect of different designs of implant-retained overdentures and fixed full-arch implant-supported prosthesis on stress distribution in edentulous mandible by using a three-dimensional finite element analysis (3D-FEA) based on a computerized tomography (CT).

Materials and methods: Four 3D FE models of an edentulous human mandible with mucosa and four implants placed in the interforamina area were constructed and restored with different designs of dentures. In the OR group, the mandible was restored with an overdenture retained by four unsplinted implants with O'ring attachment; in the BC-C and BC groups, the mandibles were restored with overdentures retained by four splinted implants with bar-clip anchor associated or not with two distally placed cantilevers, respectively; in the FD group, the mandible was restored with a fixed full-arch four-implant-supported prosthesis. The models were supported by the masticatory muscles and temporomandibular joints. A 100-N oblique load (30 degrees) was applied on the left first molar of each denture in a buccolingual direction. Qualitative and quantitative analysis based on the von Mises stress (σ_{vM}), the maximum (σ_{max})

(tensile) and minimum (σ_{min}) (compressive) principal stresses (in MPa) were obtained.

Results: BC-C group exhibited the highest stress values (σ_{vM} = 398.8, σ_{max} = 580.5 and σ_{min} = -455.2) while FD group showed the lowest one (σ_{vM} = 128.9, σ_{max} = 185.9 and σ_{min} = -172.1) in the implant/prosthetic components. Within overdenture groups, the use of unsplinted implants (OR group) reduced the stress level in the implant/prosthetic components (59.4% for σ_{vM}, 66.2% for σ_{max} and 57.7% for σ_{min} versus BC-C group) and supporting tissues (maximum stress reduction of 72% and 79.5% for σ_{max}, and 15.7% and 85.7% for σ_{min} on the cortical bone and the trabecular bone, respectively). Cortical bone exhibited greater stress concentration than the trabecular bone for all groups.

Conclusion: The use of fixed implant dentures and removable dentures retained by unsplinted implants to rehabilitate completely edentulous mandible reduced the stresses in the periimplant bone tissue, mucosa and implant/prosthetic components.

5.3 Introdução (Introduction)

The oral rehabilitation of edentulous patients has been improved by the use of dental implants (1-5). Two successfully implant treatment concepts are clinically available such as fixed and removable prosthesis (6, 7). The use of either two or four implants to retain mandibular overdentures has been indicated and similar clinical and radiographic outcomes were found (8-11). In situations that require increased retention (e.g. high muscle attachment, prominent mylohyoid ridges and extreme gaggers) and in presence of short or narrow implants, the use of more than two implants is necessary to retain mandibular overdentures (12, 13). In order to support mandibular fixed full-arch implant prosthesis, four to six implants are placed in the foramina area (14, 15).

Several factors play a role on the decision between fixed and removable implant dentures as inter-foraminal space, inter-jaw relationship, oral hygiene, speech, cost and patient's preference (16). Overdentures are indicated when patients are not satisfied with the stability and retention of the conventional removable denture but no complain about pain and discomfort of the mucosa should exist (14, 17). In case of reduced salivary flow, overdentures may generate some discomfort for the patient. Fixed full-arch implant-supported prosthesis is indicated in the presence of enough bone and inter-arch space to receive implants in the anterior region of mandible (14). On the other hand, when there is necessity to replace soft and hard tissues and to support the facial tissue by the buccal denture flange, fixed prosthesis is contraindicated (18, 19).

Additionally, the cost of fixed treatment is higher than that of overdentures; nevertheless, it requires fewer post-operative visits for adjustments (14).

A clinical study showed that periimplant bone maintenance was similar when comparing implant-retained overdentures and fixed full-arch implant-supported prosthesis (20). Also, patient satisfaction (21, 22), prosthetic outcomes, and survival rates of implants did not differ between the two treatment modalities (22). Conversely, a previous study showed that patients treated with implant-retained overdentures were less satisfied and presented lower oral health quality than those who received fixed prosthesis (23).

Implant-retained overdentures are considered a simple, cost-effective, viable, less invasive and successful treatment option for edentulous patients (24-28). However, controversies toward the design of attachment systems for overdentures still exist (28, 29). Several *in vivo* studies (30, 31) and biomechanical studies using finite element analysis (FEA) (27, 30, 32), strain gauge analysis (25, 33) and photoelastic analysis (12, 34) displayed better stress distribution for overdentures retained by unsplinted implants (e.g. O'ring attachment system) while others showed superiority with the use of splinted implants (e.g. bar-clip attachment system) (24, 29, 35-37).

The loading transmission and distribution of masticatory forces influence the success and failure of implant restorations over time (38-40). It is believed that loading distribution pattern in implant-retained overdentures differs from those in implant-supported fixed restorations (25). However, to authors' best

knowledge, no study has investigated the stress pattern of implant-retained overdentures versus fixed full-arch supported prosthesis.

Therefore, the present study aimed to compare the effect of different designs of implant-retained overdentures and fixed full-arch implant-supported prosthesis on stress distribution in edentulous mandible by using a three-dimensional finite element analysis (3D-FEA). The research hypothesis was that mandibular fixed full-arch implant-supported prosthesis would result in less stress level in the implant/prosthetic components and periimplant bone tissue when compared to implant-retained overdentures. Also, it was hypothesized that the different designs of overdenture attachment systems would induce distinguished stress distribution and level in the implant/prosthetic components and periimplant bone tissue.

5.4 Materiais e métodos (Materials and methods)

Four 3D finite element models of a complete edentulous human mandible with mucosa and four implants placed in the interforamina area were constructed and restored with different designs of implant-retained overdentures and fixed full-arch implant-supported prosthesis. In the OR group, edentulous mandible was restored with overdenture retained by four unsplinted implants with O'ring attachment system; in the BC-C and BC groups, edentulous mandibles were restored with overdentures retained by four splinted implants with bar-clip attachment system associated or not with two distally placed cantilevers, respectively. Finally, in the FD group, edentulous mandible was restored with fixed full-arch four-implant-supported prosthesis.

Model Design

The 3D geometry of the edentulous mandible was reconstructed from cone beam computerized tomography (CT) (I-Cat Cone Beam Volumetric Tomography and Panoramic Dental Imaging System, Imaging Sciences International, Hatfield, PA, USA) of a complete edentulous mandible of a 60-year-old man (Fig. 1). The mandibular section profiles were collected at 2mm-increments. The selected subject was informed about the procedure to be instituted and signed an informed consent term in accordance with the recommendations of the Human Research Ethics Committee of Aracatuba Dental School – UNESP, Brazil (Process number: 2008-00939). The patient was

rehabilitated with conventional complete dentures. The existing lower complete denture was duplicated in self-polymerized acrylic resin mixed with barium sulfate in a ratio of 3:1 in order to provide radiopacity of the denture during CT scan. The radiopaque duplicated denture was adjusted and placed into patient's mouth, and a CT scan was conducted (Fig. 1). It was done an effort to provide the accurate relationship between denture and mandible.

The files of the CT assessment were imported into the Simpleware 4.1 software package (Simpleware Ltd, Rennes Drive, Exeter, UK) which allowed the construction of the 3D solid geometries of the edentulous mandible and denture. Based on the actual position of the mandible and denture, the precise geometry of the mucosa was deduced and it was in contact with the inner surface of the denture (41). In the edentulous mandible, both cortical and trabecular bones were delimitated based on the CT data. The thickness of the mucosa and cortical bone were approximately 3.0 mm and 1.5 mm in the interforamina area, respectively.

Four cylindrical titanium implants with 11.5-mm length and 3.75-mm diameter were modeled in CAD software (SolidWorks 2010, Dassault Systèmes SolidWorks Corp., Concord, MA, USA) and virtually inserted into each model. In all models, implants were placed in the center of the mandibular crest at 10 and 20 mm away from the midline on both sides of the mandible (42)(Fig. 2).

The different attachment systems of the overdentures and the superstructure of the fixed full-arch implant-supported prosthesis were created into the same CAD software (SolidWorks 2010). The distal cantilever of the BC-C

group had 3.5 mm in height, 1.8 mm in width and 7 mm in length (43). In the FD group, the superstructure presented 4 mm in height and 6 mm in width (44) and a cantilever with 10 mm in length (42) (Fig. 2).

The implants and prosthetic components were imported into the Simpleware software and merged with the edentulous mandible and denture. For the FD group, the bottom part of the denture was reduced and planned. In the overdenture groups, the space for each attachment system in the inner surface of the denture was provided by a boolean operation (Fig. 2). Finally, models were meshed with parabolic tetrahedral interpolation solid elements into Simpleware software. The mesh refinement was established based on the convergence of analysis (6%) (45). The models had a total number of 319,644 elements and 89,912 nodes in OR group, 318,909 elements and 89,616 nodes in BC group, 269,481 elements and 80,225 nodes in BC-C group, and 244,338 elements and 70,387 nodes in FD group (Fig. 3).

Material properties and interface conditions

Meshed models were imported into the finite element analysis software (Abaqus 6.10-EF1, Dassault Systèmes Simulus Corp., Providence, RI, USA) to investigate the stress distribution. All materials were considered isotropic, homogeneous and linearly elastic. The mechanical properties (elastic modulus and coefficient of Poisson) of the materials are presented in table 1 and were extracted from the existing literature (27, 42, 44, 46-49).

Total bonding between bone and implants was assumed to simulate a complete implant osseointegration so that no motion between the two structures occurs under applied loading (40, 50-52). The same type of contact was provided to the other structures.

Constrains and loading conditions

In the present study, the models were supported by the masticatory muscles and temporomandibular joints. The forces created by the four elevator masticatory muscles (temporal, masseter, medial pterygoid and lateral pterygoid) and their positioning on the mandibular body were based on previous studies (50-52) (Fig. 4). A total of ten nodes of the elements were used to define each muscular area action. Forces directions were established by the cosines α, β and γ, (51) which represents the *x*, *y* and *z* axes of each force, respectively (Table 2). The applied muscular forces were 59.23 N for masseter, 39.60 N for medial pterygoid, 34.44 N for lateral pterygoid and 34.09 N for temporal (50-52).

In order to simulate the mean value of posterior bite force in humans, a 100-N oblique load (30 degrees in relation to the long axis of the implant) was applied on the left first molar of each denture in a buccolingual direction (Fig. 4). Considering that the implant-prosthetic components complex consists of a ductile material (titanium and gold alloys), the von Mises equivalent stress (σ_{vM}) was obtained. On the other hand, as the cortical bone, trabecular bone and mucosa are friable materials (nonductile materials), the maximum (σ_{max}) (tensile) and minimum (σ_{min}) (compressive) principal stresses were also obtained to better

understand the influence of different prosthesis designs on stress distribution in the periimplant bone tissue.

5.5 Resultados (Results)

The stress values (σ_{vM}, σ_{max} and σ_{min}) within implant/prosthetic components and supporting tissues (cortical bone, trabecular bone and mucosa) for all groups are displayed in fig. 5. Regardless of stress analysis criteria, BC-C group exhibited the highest stress values which was located into the implant/prosthetic components (σ_{vM} = 398.8 MPa, σ_{max} = 580.5 MPa and σ_{min} = -455.2 MPa) while FD group showed the lowest one (σ_{vM} = 128.9 MPa, σ_{max} = 185.9 MPa and σ_{min} = -172.1 MPa). Comparing the overdenture groups, the use of unsplinted implants associated to the O'ring attachment system reduced the stress level in the implant/prosthetic components (reduction of 59.4% for σ_{vM}, 66.2% for σ_{max} and 57.7% for σ_{min} versus BC-C group).

It is clearly visible that the highest stress levels located under the applied loading; i.e. in the bar portion between the two left implants for splinted groups and in the distal ball abutment in the left side for O'ring group (Fig 6a-d). In the overdenture groups, the σ_{vM} seems to be distributed to all implants while, in the FD group, the stress was mostly located at the implants and cantilever on the loaded side. On the implants, stress was mainly placed from the neck to the middle third region (Fig. 6a-d).

Concerning the supporting tissues, whereas the BC group displayed the greatest stress levels within cortical bone (σ_{vM} = 97.1 MPa, σ_{max} = 100.7 MPa and σ_{min} = -93.5 MPa), trabecular bone (σ_{vM} = 28.9 MPa, σ_{max} = 24.5 MPa and σ_{min} = -38.6 MPa) and mucosa (σ_{vM} = 1.93 MPa, σ_{max} = 4.15 MPa and σ_{min} = -5.33 MPa),

the FD group showed the lowest stress levels (cortical bone - σ_{vM} = 51.0 MPa, σ_{max} = 60.4 MPa and σ_{min} = -51.9 MPa; trabecular bone - σ_{vM} = 6.43 MPa, σ_{max} = 4.21 MPa and σ_{min} = -4.6 MPa; mucosa - σ_{vM} = 0.8 MPa, σ_{max} = 1.2 MPa and σ_{min} = -0.7 MPa), except for the σ_{max} on the cortical bone in which the O'ring group took place (28.1 MPa). In the overdenture group, the use of unsplinted implants decreased the stress concentration in all supporting tissues (maximum stress reduction of 72% and 79.5% for σ_{max}, and 15.7% and 85.7% for σ_{min} on the cortical and the trabecular bones, respectively) (Fig. 5).

Cortical bone exhibited greater stress concentration than the trabecular bone for all groups (Fig. 5). The maximum stress concentration on bone tissue was observed in the periimplant area (Figs 7 and 8). In the cortical bone, the highest tensile stress (σ_{max}) was observed on the buccal side of the periimplant area while the greatest compressive stress (σ_{min}) was noted on the lingual side (Fig. 7). It is interesting to note that in OR and FD groups the stress on periimplant cortical bone was mainly limited to the loaded side. On the other hand, for the BC and BC-C groups, the stress was distributed to all periimplant cortical bone areas (Fig 7).

5.6 Discussão (Discussion)

The research hypotheses - that mandibular fixed full-arch implant-supported prosthesis would result in less stress level in the implant/prosthetic components and periimplant bone tissue when compared to implant-retained overdentures, and that the different designs of overdenture attachment systems would induce distinguished stress distribution and level in the implant/prosthetic components and periimplant bone tissue – were confirmed by the present 3D FEA study.

Concerning the implant/prosthetic components set, the use of implant-retained overdenture associated with bar-clip attachment system with two distally placed cantilevers (BC-C group) displayed the greatest stress level. Although cantilever extensions of bar attachments have been recommended for mandibular implant-retained overdentures to increase denture stability against non-axial loading (13, 53), previous clinical study (54) showed higher rate of bar fracture associated with distal cantilevers when compared with those without cantilever in 4-implant-retained overdentures. It may be explained by the outcomes of the current study in which the σ_{vM} increased by 29.7% in the bar system associated with distal cantilevers versus bar system without distal cantilevers. Additionally, great load on the distal implants was observed in cantilevered implant-retained maxillary overdentures during *in vivo* masticatory cycles (55). Even though the presence of distal cantilevers, the fixed full-arch implant-supported prosthesis (FD group) exhibited the lowest stress levels on the

superstructure, which might be explained by the size (height and width) and shape of the bar creating a more stable and robust system. Also, the cantilever length was limited to 10 mm and several studies agree with up to 20-mm cantilever extension (44, 56-58). No study has compared the technical complication rates (e.g. bar fracture) between removable and fixed dentures with bar systems; however, the current results presumably indicate less bar fracture rate to the fixed treatment design clinically. Gallucci et al showed that from a total of 45 completely edentulous patients treated with implant-supported fixed mandibular prosthesis, 4.4% of patients (n=2) had fracture of metal framework after 5 months and 3.3 years of delivery due to imperfections at the time of casting/soldering of the framework (4). In relation to the bar design of overdentures, from a total of 31 patients, 6.5% (n=2) exhibited bar fracture and 9.7% (n=3) exhibited other failures (i.e. loosening of the retention screw) after a mean period of 3.5 years (59). Several other factors as insufficient metal thickness, inferior solder joints, excessive cantilever length, inadequate strength of alloys, parafunctional habits of patients, and incorrect framework design affect the rate of metal framework fractures in dental prosthesis retained by implants (60).

Within overdenture groups, the O'ring system reduced the stress level on the implant/prosthetic components. The flexibility and resiliency provided by the O'ring rubber may work as a stress-breaking system explaining the current results (25, 27). This result is supported by clinical outcome in which the survival rate of mandibular overdenture rehabilitation was higher for ball attachment

(98.8%) when compared to dolder bar (97.7%) after 10 years of follow-up (61). On the other hand, the postinsertion maintenance of bar-clip attachment in mandibular implant overdenture was lower than those observed in ball attachment (62, 63).

In relation to the supporting tissues (cortical bone, trabecular bone and mucosa), the BC group promoted the highest stress levels whereas the FD group the lowest one. In the FD group, the denture is completely supported by the implants and no contact with mucosa occurs. Additionally, the presence of a strong framework increases the stability of the whole system which decreases the stress mainly on the periimplant cortical bone. This observation is supported by clinical data in which full-arch implant-supported mandibular restoration exhibited higher cumulative success rate of implants (96.2%) when compared to implant-retained mandibular overdentures (93.7% and 93.9% for ball anchor and dolder bar, respectively) in a 10-year follow-up study (61).

In the overdenture groups, the use of unsplinted implant (OR group) optimized the stress level on the supporting tissues when compared to splinted implants (BC and BC-C groups), which is in agreement with several studies (12, 25, 27, 30-34). It may be owing to the fact that solitary anchors allow the mandible's flexure (27, 32, 64) and that the O'ring rubber used in the current study promoted a stress-breaking effect onto the bone tissue (27). *In vivo* study showed higher implant loss in case of overdenture retained by two splinted implants (6.5%) than unsplinted implants (5.2%). On the other hand, previous clinical study showed that splinting mini-implants with a rigid superstructure

decreased bone stress and induced less marginal bone loss than with single mini-implants to retain mandibular overdentures (37).

It is worth noting that the presence of cantilever extension on the bar-clip system (BC-C group) decreased the stress level on the supporting tissues when compared with the bar-clip design without cantilever (BC group). It may be explained by the fact that higher stress was concentrated in the implant/prosthetic components of the BC-C group away from the surrounding implant crestal bone and that the presence of cantilever reduced the overdenture contact in the denture bearing area, which relieves the supporting tissues. Therefore, distal extensions may provide higher stability against lateral forces and seclude the denture bearing area from loading forces (65). Nevertheless, Sadowsky and Caputo in a photoelastic study advocated that cantilevered bar overdenture exhibited higher stress level to the distal ipsilateral implants than non-cantilevered bar overdenture (43). It is important to highlight that the overdenture cantilever was 7-mm long in the current study while it was 11.5-mm long in Sadowsky and Caputo study (43).

Within cortical bone, peak of stress was observed on the periimplant region. When the implant is loaded, the stress is transferred to its first material contact (i.e. periimplant cortical bone), which explains the clinical marginal bone loss around implants (38). Correlation between high occlusal stress onto the implants and marginal bone loss has been showed (66-70). The higher stress values observed in cortical bone may rely on its higher elastic modulus when compared with trabecular bone (32, 71). Herein, a small amount of remaining

stress was spread to trabecular bone. In all groups, the stresses on the buccal and lingual periimplant cortical bone were greater than those observed on the mesial and distal regions, which is in agreement with previous studies(58, 72).

It is interesting to highlight that the stress distribution in the cortical bone surrounding the implants differed between the removable and fixed groups. In the former, the stress spread to the ipsilateral implants whereas it was concentrated at the loaded side in the fixed group. This may be attributed to the denture bearing area contact of the overdenture group and the greater stability of the fixed group.

The present study has several limitations and some assumptions were made in the models' construction. All materials were homogeneous, isotropic and linearly elastic in nature. However, it is known that the cortical bone is transversely isotropic and heterogeneous (73). Additionally, previous study showed that the use of anisotropic bone in complete mandible increased up to 70% the stress and strain levels on the periimplant bone (74). The interface between the structures was assumed to be glued. No thread was represented in the implants. However, since the present study was comparative in nature, such assumptions do not affect the current outcomes as they were presented in all groups (75). In order to validate such results, further FEA studies considering the anisotropic property of bone and coefficient of friction among the structures (mainly at implant/bone interface) are warranted. Furthermore, animal and clinical studies are necessary to prove such biomechanical outcomes.

5.7 Conclusão (Conclusion)

Based on the outcomes of the present study and within the limitations of the methodology used the following conclusions can be drawn:

1. Cantilevered bar-clip overdenture (BC-C group) displayed the highest von Mises stress, maximum and minimum principal stresses values within implant/prosthetic components whereas the fixed full-arch implant-supported prosthesis (FD group) presented the lowest ones.
2. In the supporting tissues (cortical bone, trabecular bone and mucosa), bar-clip overdenture (BC group) showed the greatest stress values followed by cantilevered bar-clip overdenture (BC-C group), O'ring overdenture (OR group) and fixed full-arch implant-supported prosthesis (FD group).
3. Within overdenture groups, the use of unsplinted implants to retain the overdentures (OR group) reduced the von Mises stress, maximum and minimum principal stresses levels in both implant/prosthetic components and supporting tissues.
4. Within supporting tissues, periimplant cortical bone exhibited the highest stress values for all groups. In the overdenture groups, the stress was transferred to the ipsilateral periimplant cortical bone.

Acknowledgment

The authors would like to express gratitude to the State of Sao Paulo Research Foundation (FAPESP) and for the scholarship (2007/58926-5) and grant support (2010/09857-3) provided.

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Tabelas (Tables)

Table 1. Mechanical properties of the materials used in the study

Material	Structure	Elastic modulus (MPa)	Coefficient of Poisson	Reference
___	Cortical bone	13,700	0.3	Barbier et al. (1998)(46)
___	Trabecular bone	1,370	0.3	Barbier et al. (1998)(46)
___	Mucosa	680	0.45	Barao et al. (2008)(47)
Titanium (Ti-6Al-4V)	Implant	103,400	0.35	Sertgoz and Gunever (1996)(44)
Gold alloy	Fixed full-arch implant-supported prosthesis superstructure and overdenture bar	120,000	0.25	Zarone et al. (2003)(42)
Acrylic resin	Artificial denture teeth and denture base	8,300	0.28	Darbar et al. (1995)(48)
Plastic	Overdenture clip	3,000	0.28	Barao et al. (2009)(27)
Stainless steel	O'ring capsule	19,000	0.31	Barao et al. (2009)(27)
Rubber	O'ring rubber	5	0.45	Chun et al. (2005)(49)

Table 2. Directional cosines of the resultant muscular forces based on Cruz et al (2003) (51)

	Muscles							
	Masseter		Medial pterygoid		Lateral pterygoid		Temporal	
	right	left	right	left	right	left	right	left
Cos (α)	-0.043	0.043	0.587	-0.587	0.714	-0.714	-0.325	0.325
Cos (β)	-0.011	0.011	-0.165	0.165	-0.692	0.692	0.219	-0.219
Cos (γ)	0.999	-0.999	0.792	-0.792	0.106	-0.106	0.920	-0.920

Figuras (Figures)

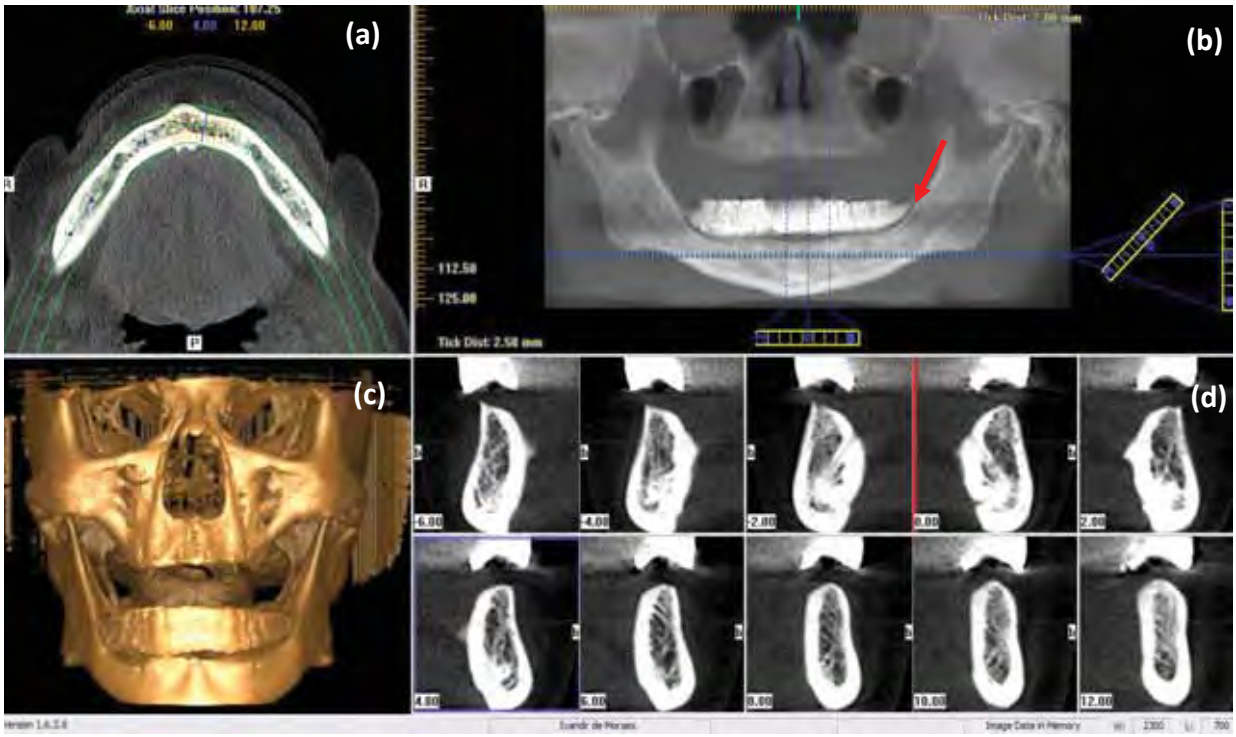

Fig. 1. Mandible CT scan of an edentulous patient with the radiopaque duplicated lower denture in position **(a)** Inferior view of the mandibular arch. **(b)** Panoramic view of the mandible with the lower denture in position (red arrow). **(c)** Instant 3D reconstruction of the CT scan. **(d)** Transverse sections (2-mm thickness) of the mandible.

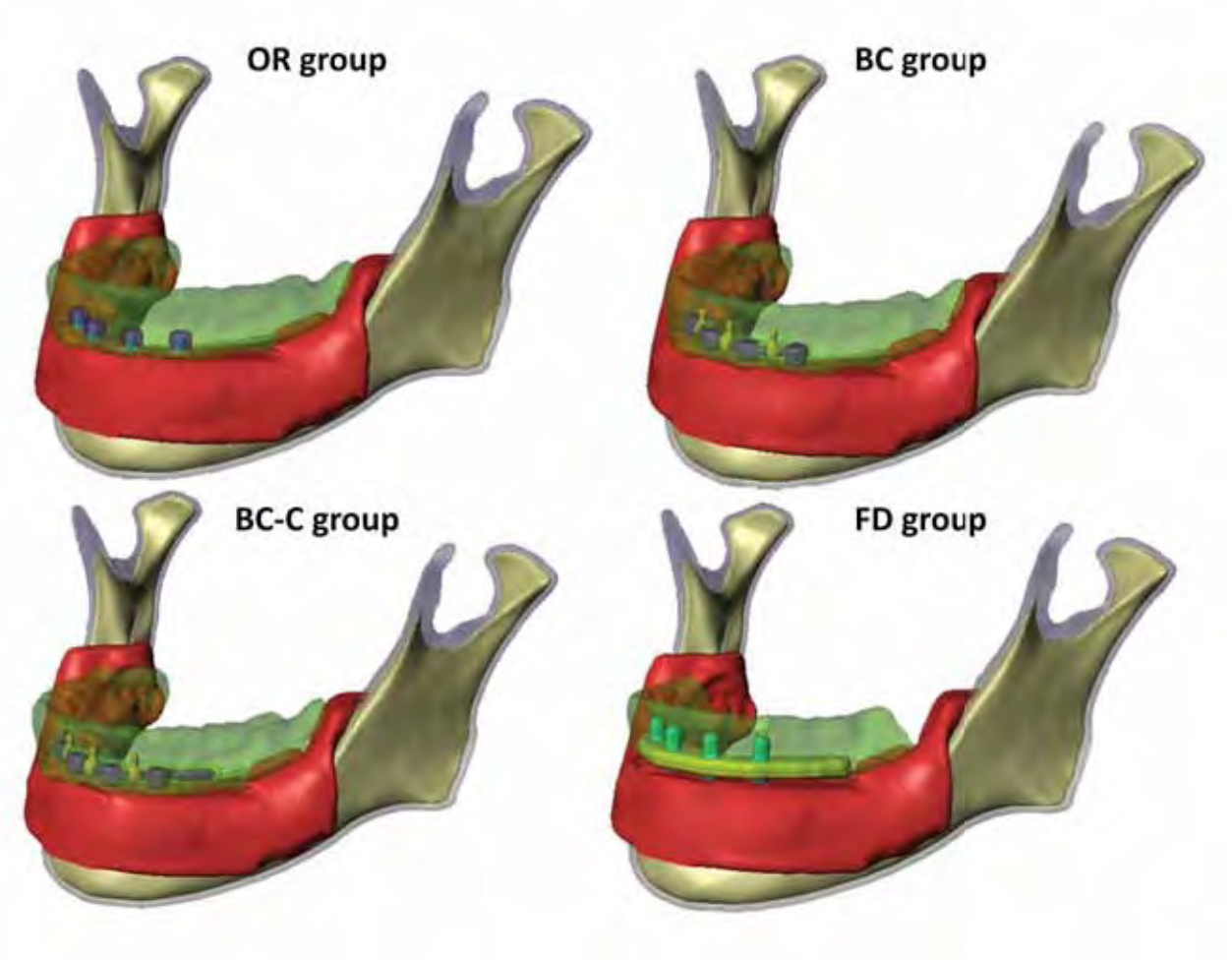

Fig. 2. 3D Complete models of all groups showing the cortical bone, trabecular bone, mucosa, denture, implants and prosthetic components.

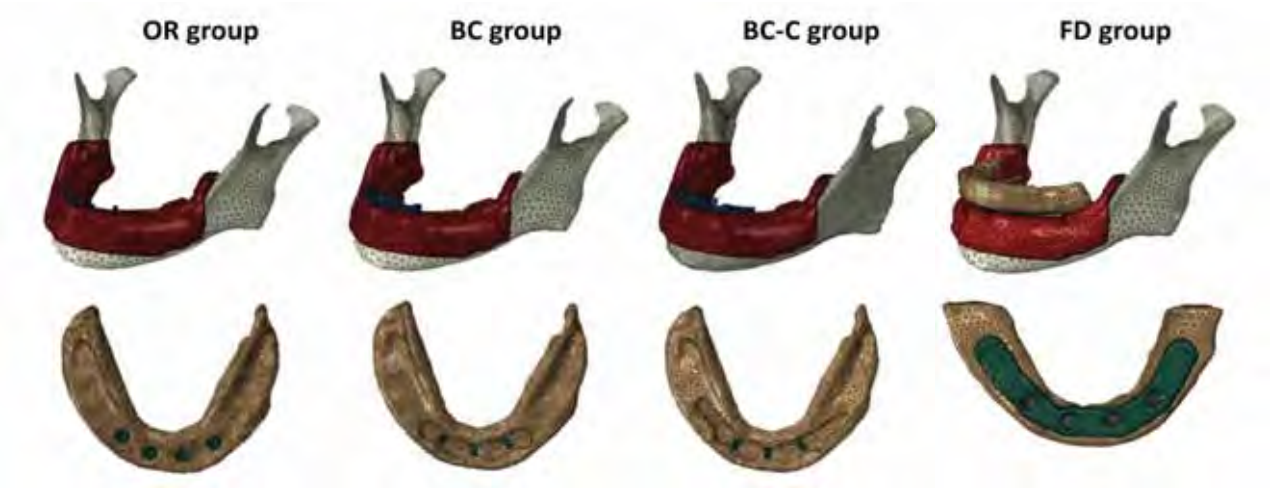

Fig. 3. Global view of meshing in each group

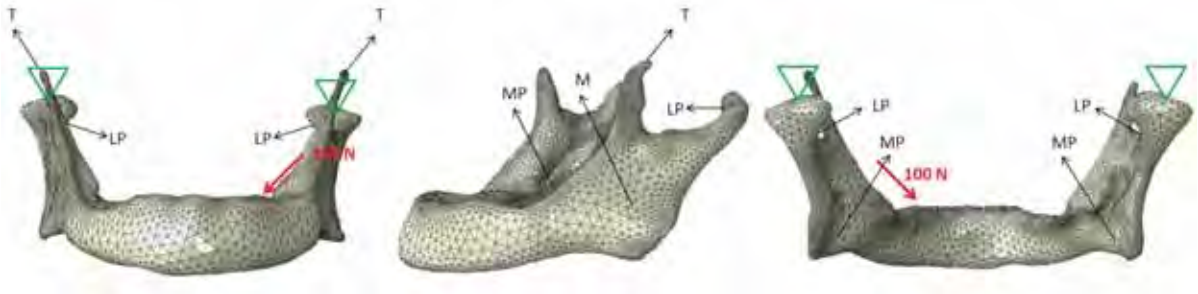

Fig. 4. Boundary conditions of the mandible FE model used in all groups based on Cruz et al (50-52). Black arrows indicate the direction of the applied masticatory muscle forces (T – temporal; LP – Lateral Pterygoid; MP – Medial Pterygoid and M – Masseter). Green triangles show the fixation of the temporomandibular joints. Red arrow shows the applied masticatory force on the left first molar region.

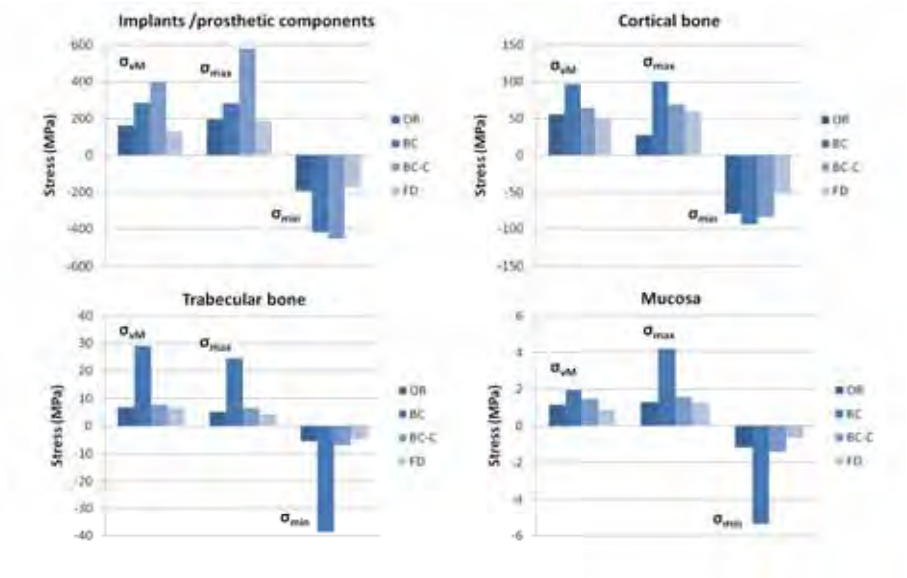

Fig. 5. von Mises stress (σ_{vM}), maximum (σ_{max})and minimum (σ_{min}) principal stress values (in MPa) within implant/prosthetic components, cortical bone, trabecular bone and mucosa for all groups

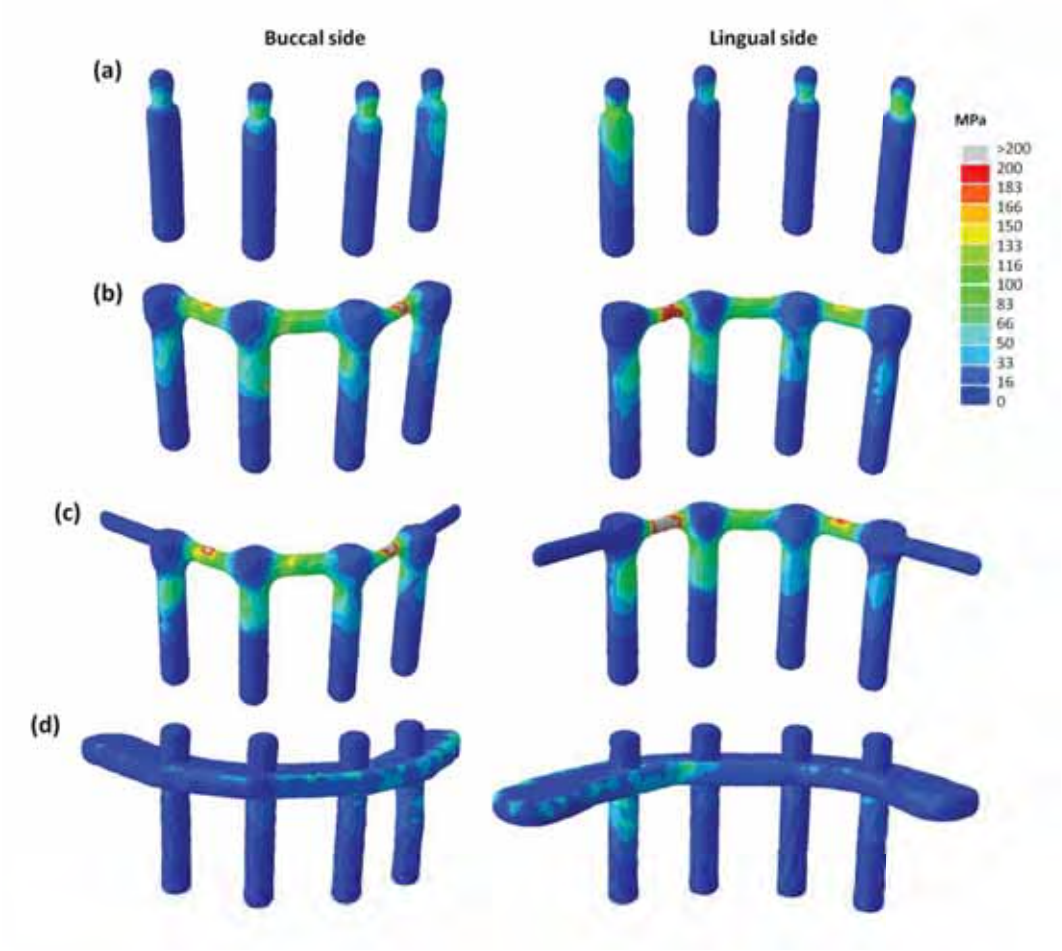

Fig. 6. von Mises stress (σ_{vM}) distribution (in MPa) within implant/prosthetic components for (a) OR, (b) BC, (c) BC-C and (d) FD groups.

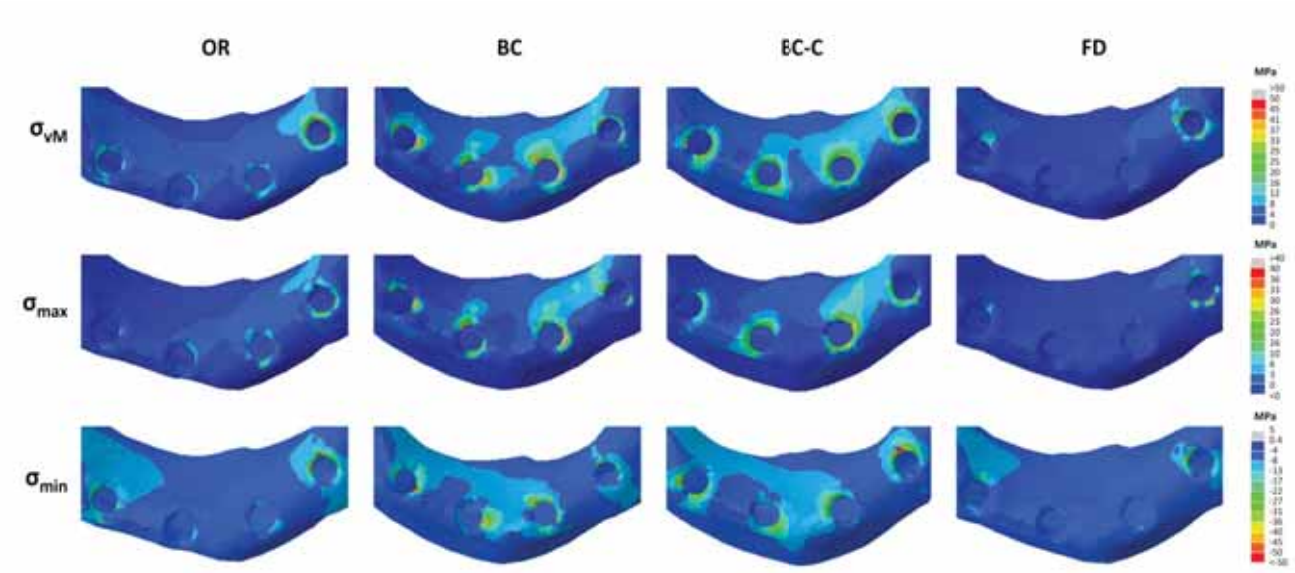

Fig. 7. von Mises stress (σ_{vM}), maximum (σ_{max})and minimum (σ_{min}) principal stress distributions (in MPa) within cortical bone for all groups.

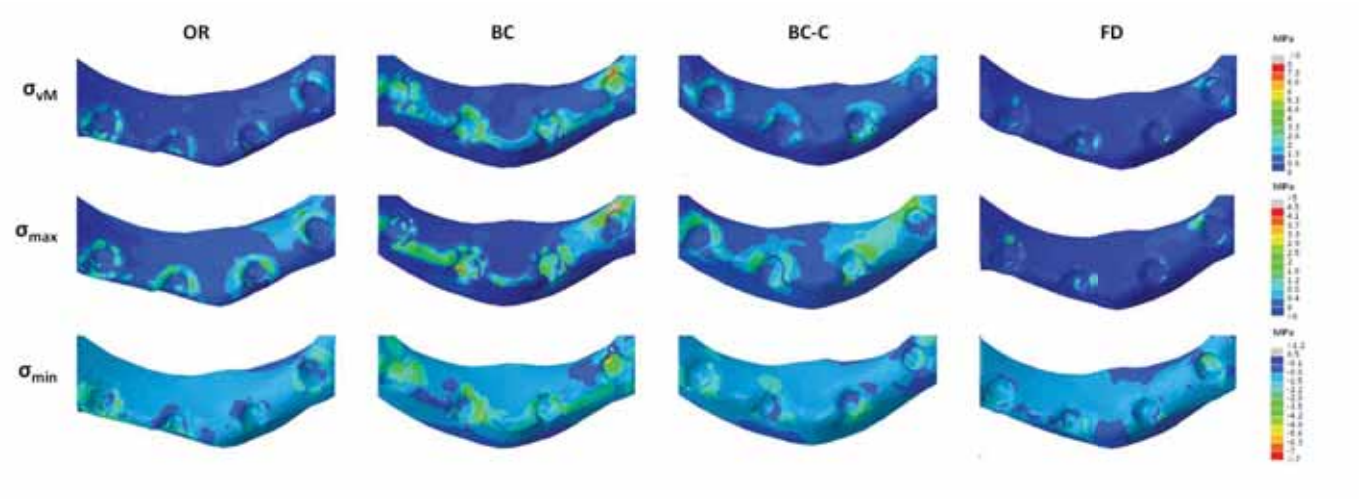

Fig. 8. von Mises stress (σ_{vM}), maximum (σ_{max})and minimum (σ_{min}) principal stress distributions (in MPa) within trabecular bone for all groups

ANEXO

Anexo A. Aprovação do Comitê de Ética em Pesquisa da Faculdade de Odontologia de Araçatuba - UNESP

unesp UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO" Campus de Araçatuba

COMITÊ DE ÉTICA EM PESQUISA –CEP-

OF. 038/2008
CEP
SFCD/bri

Araçatuba, 11 de abril de 2008.

Referência Processo FOA 2008-00939

O Coordenador do Comitê de Ética em Pesquisa desta Unidade, tendo em vista o parecer favorável da relatora que analisou o projeto **"ANÁLISE COMPARATIVA DA DISTRIBUIÇÃO INTERNA DE TENSÕES ENTRE OVERDENTURES IMPLANTO-RETIDAS E PRÓTESES FIXAS IMPLANTO-SUPORTADAS DO TIPO PROTOCOLO, ATRAVÉS DO MÉTODO DE ELEMENTOS FINITOS TRIDIMENSIONAL"** expede o seguinte parecer:

Aprovado:

Informamos a Vossa Senhoria que de acordo com as normas contidas na resolução CNS 215, **deverá ser enviado relatórios parciais em 10/04/2009 e 10/04/2010 e o relatório final em 10/04/2011.**

Prof. Dr. Stefan Fiúza de Carvalho Dekon
Coordenador do CEP

Ilmo. Senhor
Dr. WIRLEY GONÇALVES ASSUNÇÃO
Araçatuba-SP-

Ciente.De acordo.
11/4/2008
Dr.Wirley Gonçalves Assunção

Faculdade de Odontologia e Curso de Medicina Veterinária –
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Anexo B. Normas para publicação do periódico Clinical Oral Implants Research

Clinical Oral Implants Research

Official publication of the European Association for Osseointegration

Edited by:
Niklaus P. Lang

Print ISSN: 0905-7161
Online ISSN: 1600-0501
Frequency: Monthly
Current Volume: 22 / 2011
ISI Journal Citation Reports® Ranking: 2009: Engineering, Biomedical: 13 / 59; Dentistry, Oral Surgery & Medicine: 6 / 64
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3.6. Suspension of Submission Mid-way in the Submission Process
You may suspend a submission at any phase before clicking the 'Submit' button and save it to submit later. The manuscript can

then be located under 'Unsubmitted Manuscripts' and you can click on 'Continue Submission' to continue your submission when you choose to.

3.7. E-mail Confirmation of Submission
After submission you will receive an e-mail to confirm receipt of your manuscript. If you do not receive the confirmation email after 24 hours, please check your e-mail address carefully in the system. If the e-mail address is correct please contact your IT department. The error may be caused by some sort of spam filtering on your e-mail server. Also, the e-mails should be received if the IT department adds our email server (uranus.scholarone.com) to their whitelist.

3.8. Manuscript Status
You can access ScholarOne Manuscripts (formerly known as Manuscript Central) any time to check your 'Author Centre' for the status of your manuscript. The Journal will inform you by e-mail once a decision has been made.

3.9. Submission of Revised Manuscripts
To submit your revised manuscript, locate your manuscript under 'Manuscripts with Decisions' and click on 'Submit a Revision' . Please remember to delete any old files uploaded when you upload your revised manuscript.

4. MANUSCRIPT TYPES ACCEPTED

Original research articles of high scientific merit in the field of material sciences, physiology of wound healing, biology of tissue integration of implants, diagnosis and treatment planning, prevention of pathologic processes jeopardizing the longevity of implants, clinical trials on implant systems, stomatognathic physiology related to oral implants, new developments in therapeutic concepts and prosthetic rehabilitation.

Review articles by experts on new developments in basic sciences related to implant dentistry and clinically applied concepts. Reviews are generally by invitation only and have to be approved by the Editor-in-Chief before submission.

Case reports and case series, but only if they provide or document new fundamental knowledge and if they use language understandable to the clinician.

Novel developments if they provide a technical novelty for any implant system.

Short communications of important research findings in a concise format and for rapid publication.

Treatment rational by experts with evidence-based treatment approach.

Proceedings of international meetings may also be considered for publication at the discretion of the Editor.

5. MANUSCRIPT FORMAT AND STRUCTURE

5.1. Page Charge
Articles exceeding 10 published pages are subject to a charge of USD 160 per additional page. One published page amounts approximately to 5,500 characters (excluding figures and tables).

5.2. Format

Language: The language of publication is English. Authors for whom English is a second language might choose to have their manuscript professionally edited by an English speaking person before submission to make sure the English is of high quality. A list of independent suppliers of editing services can be found at http://authorservices.wiley.com/bauthor/english_language.asp. All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication

Abbreviations, Symbols and Nomenclature: The symbol % is to be used for percent, h for hour, min for minute, and s for second. In vitro, in vivo, in situ and other Latin expressions are to be italicised. Use only standard abbreviations. All units will be metric. Use no roman numerals in the text. In decimals, a decimal point and not a comma will be used. Avoid abbreviations in the title. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement. In cases of doubt, the spelling orthodoxy of Webster's third new international dictionary will be adhered to.

Scientific Names: Proper names of bacteria should be binomial and should be singly underlined on the typescript. The full proper name (e.g., Streptococcus sanguis) must be given upon first mention. The generic name may be abbreviated thereafter with the first letter of the genus (e.g., S. sanguis). If abbreviation of the generic name could cause confusion, the full name should be used. If the vernacular form of a genus name (e.g., streptococci) is used, the first letter of the vernacular name is not capitalised and the name is not underlined. Use of two letters of the genus (e.g., Ps. for Peptostreptococcus) is incorrect, even though it might avoid ambiguity. With regard to drugs, generic names should be used instead of proprietary names. If a proprietary name is used, it must be attached when the term is first used.

5.2. Structure

All manuscripts submitted to *Clinical Oral Implants Research* should include Title Page, Abstract, Main Text and Acknowledgements, Tables, Figures and Figure Legends as appropriate.

Title Page: should contain the title of the article, full name(s) of the authors (no more than 6) and institutional affiliation(s), a running title not exceeding 60 letters and spaces, and the name, telephone and fax numbers, email and complete mailing address of the author responsible for correspondence. The author must list appropriate key words for indexing purposes.

Abstract: should not to exceed 250 words. This should be structured into: objectives, material and methods, results, conclusions, and no other information.

Main Text of Original Research Article should include Introduction, Material and Methods, Results and Discussion.

Introduction: Summarise the rationale and purpose of the study, giving only strictly pertinent references. Do not review existing literature extensively. State clearly the working hypothesis.

Material and Methods: Material and methods should be presented in sufficient detail to allow confirmation of the observations. Published methods should be referenced and discussed only briefly, unless modifications have been made. Indicate the statistical methods used, if applicable.

Results: Present your results in a logical sequence in the text, tables, and illustrations. Do not repeat in the text all data in the tables and illustrations. The important observations should be emphasised.

Discussion: Summarise the findings without repeating in detail the data given in the Results section. Relate your observations to other relevant studies and point out the implications of the findings and their limitations. Cite other relevant studies.

Main Text of Short Communications: Short communications are limited to two printed pages including illustrations and references and need not follow the usual division into material and methods, etc., but should have an abstract.

Acknowledgements: Acknowledge only persons who have made substantive contributions to the study. Authors are responsible for obtaining written permission from everyone acknowledged by name because readers may infer their endorsement of the data and conclusions. Sources of financial support should be acknowledged.

5.3. References
References should quote the last name(s) of the author(s) and the year of publication (Black & Miller 1988). Three or more authors should always be referred to as, for example, (Fox et al. 1977).
A list of references should be given at the end of the paper and should follow the recommendations in Units, symbols and abbreviations: a guide for biological and medical editors and authors (1988), p. 52, London: The Royal Society of Medicine.

a) The arrangement of the references should be alphabetical by author's surname.

b) The order of the items in each reference should be:
(i) for journal references:
name(s) of author(s), year, title of paper, title of journal, volume number, first and last page numbers.
(ii) for book references:
name(s) of author(s), year, title of book, edition, volume, chapter and/ or page number, town of publication, publisher.

c) Author's names should be arranged thus: Daniels, J.A., Kelly, R.A. & Til, T.C.
Note the use of the ampersand and omission of comma before it. Author's names when repeated in the next reference are always spelled out in full.

d) The year of publication should be surrounded by parentheses: (1966).

c) The title of the paper should be included, without quotation marks.

f) The journal title should be written in full, italicised, and followed by volume number in bold type, and page numbers.
Examples:
Tonetti, M. S., Schmid, J., Hämmerle,C. H. & Lang, N. P. (1993) Intraepithelial antigen-presenting cells in the keratinized mucosa around teeth and osseointegrated implants. *Clinical Oral Implants Research* **4**: 177-186.
Poole, B., Ohkuma, S. & Warburton, M. (1978) Some aspects of the intracellular breakdown of erogenous and endogenous proteins. In: Segal, H.S. & Doyle, D.J., eds. Protein turnover and lysosome function, 1st edition, p. 43. New York: Academic Press.

We recommend the use of a tool such as Reference Manager for reference management and formatting. Reference Manager reference styles can be searched for here: www.refman.com/support/rmstyles.asp

5.4. Tables, Figures and Figure Legends

Tables: Tables should be numbered consecutively with Arabic numerals. Type each table on a separate sheet, with titles making them self-explanatory. Due regard should be given to the proportions of the printed page.

Figures: All figures should clarify the text and their number should be kept to a minimum. Details must be large enough to retain

their clarity after reduction in size. Illustrations should preferably fill a single-column width (81 mm) after reduction, although in exceptional cases 120mm (double-column) and 168 mm (full page) widths will be accepted. Micrographs should be designed to be reproduced without reduction, and they should be dressed directly on the micrograph with a linear size scale, arrows, and other designators as needed. Each figure should have a legend

Preparation of Electronic Figures for Publication: Although low quality images are adequate for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit EPS (lineart) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented programmes. Scans (TIFF only) should have a resolution of 300 dpi (halftone) or 600 to 1200 dpi (line drawings) in relation to the reproduction size (see below). EPS files should be saved with fonts embedded (and with a TIFF preview if possible). For scanned images, the scanning resolution (at final image size) should be as follows to ensure good reproduction: lineart: >600 dpi; half-tones (including gel photographs): >300 dpi; figures containing both halftone and line images: >600 dpi.

Further information can be obtained at Wiley-Blackwell's guidelines for figures:
http://authorservices.wiley.com/bauthor/illustration.asp

Check your electronic artwork before submitting it: http://authorservices.wiley.com/bauthor/eachecklist.asp

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6. AFTER ACCEPTANCE

Upon acceptance of a paper for publication, the manuscript will be forwarded to the Production Editor who is responsible for the production of the journal.

6.1 Proof Corrections
The corresponding author will receive an email alert containing a link to a web site. A working email address must therefore be provided for the corresponding author. The proof can be downloaded as a PDF (portable document format) file from this site. Acrobat Reader will be required in order to read this file. This software can be downloaded (free of charge) from the following Web site: www.adobe.com/products/acrobat/readstep2.html . This will enable the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof. Hard copy proofs will be posted if no e-mail address is available; in your absence, please arrange for a colleague to access your e-mail to retrieve the proofs. Proofs must be returned to the Production Editor within three days of receipt.

Excessive changes made by the author in the proofs, excluding typesetting errors, will be charged separately. Other than in exceptional circumstances, all illustrations are retained by the publisher. Please note that the author is responsible for all statements made in his work, including changes made by the copy editor.

Articles should not normally exceed 10 printed pages, including illustrations and references. Additional pages will be charged to the author(s) at the rate of USD 160 per page.

6.2 Early View (Publication Prior to Print)
Clinical Oral Implants Research is covered by Wiley-Blackwell's Early View service. Early View articles are complete full-text articles published online in advance of their publication in a printed issue. Early View articles are complete and final. They have been fully reviewed, revised and edited for publication, and the authors' final corrections have been incorporated. Because they are in final form, no changes can be made after online publication. The nature of Early View articles means that they do not yet have volume, issue or page numbers, so Early View articles cannot be cited in the traditional way. They are therefore given a Digital Object Identifier (DOI), which allows the article to be cited and tracked before it is allocated to an issue. After print publication, the DOI remains valid and can continue to be used to cite and access the article.

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Anexo C. Normas para publicação do periódico *Journal of Materials Science: Materials in Medicine*

Journal of Materials Science: Materials in Medicine

ISSN: 0957-4530 (print version)
ISSN: 1573-4838 (electronic version)

Title Page

The title page should include:

- The name(s) of the author(s)
- A concise and informative title
- The affiliation(s) and address(es) of the author(s)
- The e-mail address, telephone and fax numbers of the corresponding author

Abstract

Please provide an abstract of 100 to 150 words. The abstract should not contain any undefined abbreviations or unspecified references.

TEXT

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
  Note: If you use Word 2007, do not create the equations with the default equation editor but use the Microsoft equation editor or MathType instead.
- Save your file in doc format. Do not submit docx files.

- Word template (zip, 154 kB)

Manuscripts with mathematical content can also be submitted in LaTeX.

- LaTeX macro package (zip, 182 kB)

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.
Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full.

SCIENTIFIC STYLE

Please always use internationally accepted signs and symbols for units, SI units.

REFERENCES

Citation

Reference citations in the text should be identified by numbers in square brackets. Some examples:
1. Negotiation research spans many disciplines [3].
2. This result was later contradicted by Becker and Seligman [5].
3. This effect has been widely studied [1-3, 7].

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.
The entries in the list should be numbered consecutively.

- Journal article

  Smith JJ. The world of science. Am J Sci. 1999;36:234–5.

- Article by DOI

  Slifka MK, Whitton JL. Clinical implications of dysregulated cytokine production. J Mol Med. 2000; doi:10.1007/s001090000086

- Book

  Blenkinsopp A, Paxton P. Symptoms in the pharmacy: a guide to the management of common illness. 3rd ed. Oxford: Blackwell Science; 1998.

- Book chapter

  Wyllie AH, Kerr JFR, Currie AR. Cell death: the significance of apoptosis. In: Bourne GH, Danielli JF, Jeon KW, editors. International review of cytology. London: Academic; 1980. pp. 251–306.

- Online document

  Doe J. Title of subordinate document. In: The dictionary of substances and their effects. Royal Society of Chemistry. 1999. http://www.rsc.org/dose/title of subordinate document. Accessed 15 Jan 1999.

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations, see

- www.issn.org/2-22661-LTWA-online.php

For authors using EndNote, Springer provides an output style that supports the formatting of in-text citations and reference list.

- EndNote style (zip, 3 kB)

TABLES

- All tables are to be numbered using Arabic numerals.
- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.
- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

ARTWORK

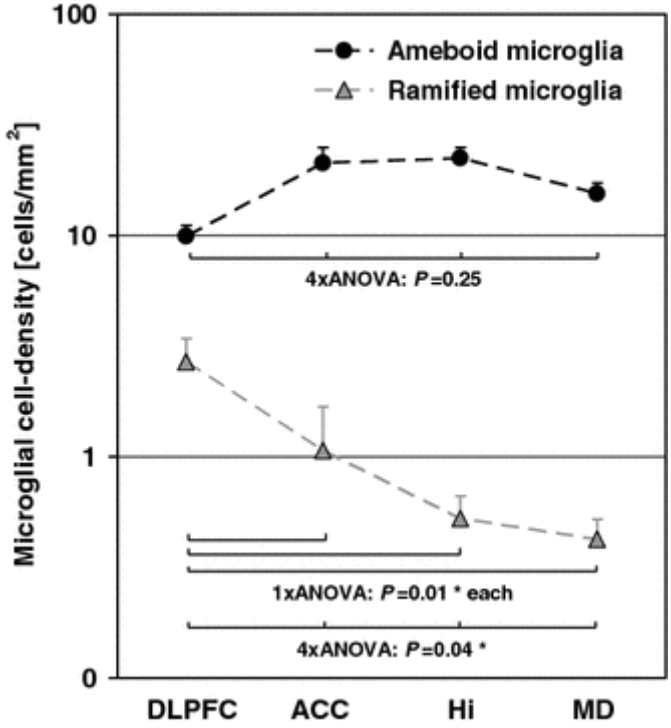
For the best quality final product, it is highly recommended that you submit all of your artwork – photographs, line drawings, etc. – in an electronic format. Your art will then be produced to the highest standards with the greatest accuracy to detail. The published work will directly reflect the quality of the artwork provided.

Electronic Figure Submission

- Supply all figures electronically.
- Indicate what graphics program was used to create the artwork.
- For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MS Office files are also acceptable.

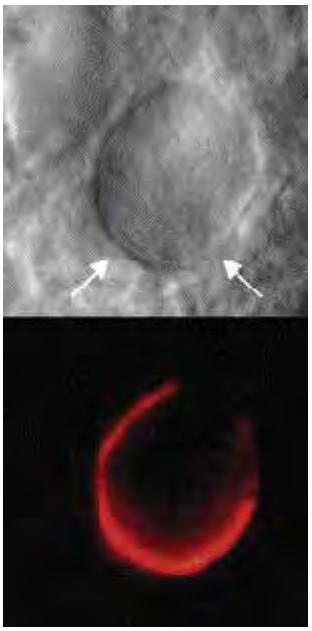
- Vector graphics containing fonts must have the fonts embedded in the files.
- Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

Line Art



- Definition: Black and white graphic with no shading.
- Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
- All lines should be at least 0.1 mm (0.3 pt) wide.
- Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
- Vector graphics containing fonts must have the fonts embedded in the files.

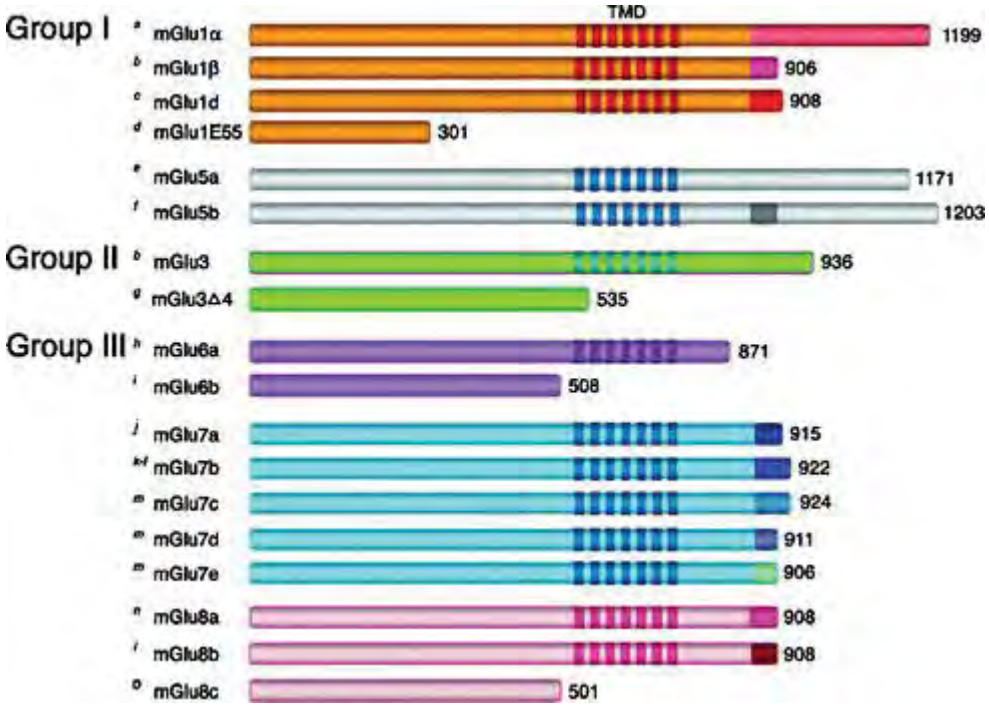
Halftone Art



- Definition: Photographs, drawings, or paintings with fine shading, etc.
- If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.

- Halftones should have a minimum resolution of 300 dpi.

Combination Art



- Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.
- Combination artwork should have a minimum resolution of 600 dpi.

Color Art

- Color art is free of charge for online publication.
- If black and white will be shown in the print version, make sure that the main information will still be visible. Many colors are not distinguishable from one another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent.
- If the figures will be printed in black and white, do not refer to color in the captions.
- Color illustrations should be submitted as RGB (8 bits per channel).

Figure Lettering

- To add lettering, it is best to use Helvetica or Arial (sans serif fonts).
- Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).
- Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.
- Avoid effects such as shading, outline letters, etc.
- Do not include titles or captions within your illustrations.

Figure Numbering

- All figures are to be numbered using Arabic numerals.
- Figures should always be cited in text in consecutive numerical order.
- Figure parts should be denoted by lowercase letters (a, b, c, etc.).
- If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures, "A1, A2, A3, etc." Figures in online appendices (Electronic Supplementary Material) should, however, be numbered separately.

Figure Captions

- Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.
- Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.

- No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.
- Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.
- Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

- When preparing your figures, size figures to fit in the column width.
- For most journals the figures should be 39 mm, 84 mm, 129 mm, or 174 mm wide and not higher than 234 mm.
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In order to give people of all abilities and disabilities access to the content of your figures, please make sure that

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- To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.

Audio, Video, and Animations

- Always use MPEG-1 (.mpg) format.

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- Submit your material in PDF format; .doc or .ppt files are not suitable for long-term viability.
- A collection of figures may also be combined in a PDF file.

Spreadsheets

- Spreadsheets should be converted to PDF if no interaction with the data is intended.
- If the readers should be encouraged to make their own calculations, spreadsheets should be submitted as .xls files (MS Excel).

Specialized Formats

- Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

Collecting Multiple Files

- It is possible to collect multiple files in a .zip or .gz file.

Numbering

- If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables.
- Refer to the supplementary files as “Online Resource”, e.g., "... as shown in the animation (Online Resource 3)", “... additional data are given in Online Resource 4”.
- Name the files consecutively, e.g. “ESM_3.mpg”, “ESM_4.pdf”.

Captions

- For each supplementary material, please supply a concise caption describing the content of the file.

Processing of supplementary files

- Electronic supplementary material will be published as received from the author without any conversion, editing, or reformatting.

Accessibility

In order to give people of all abilities and disabilities access to the content of your supplementary files, please make sure that

- The manuscript contains a descriptive caption for each supplementary material
- Video files do not contain anything that flashes more than three times per second (so that users prone to seizures caused by such effects are not put at risk)

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Online First

The article will be published online after receipt of the corrected proofs. This is the official first publication citable with the DOI. After release of the printed version, the paper can also be cited by issue and page numbers.

Anexo D. Normas para publicação do periódico Journal of the Mechanical Behavior of Biomedical Materials

Journal of the Mechanical Behavior of Biomedical Materials

Authors are requested to submit a cover letter that clearly states the novelty of the work presented in their manuscript.

Types of Contributions

Research Paper: A full-length article describing original research. There is no limit on the number of words, figures etc but authors should be as succinct as possible.

Review Article: An article which reviews previous work in a given field. Reviews are written by invitation only but the editor would welcome suggestions.

Technical Note: A short article describing a new experimental technique or analytical approach.

Short Communication: An article presenting new work in reduced form, which for some reason is not suitable for a full research paper. For example a case study.

Opinion Piece: A short article presenting the author's opinion on a particular question. Normally shorter and less comprehensive than a review article, making use of published and/or unpublished results.

Tutorial: An article of an educational nature, explaining how to use a particular experimental technique or analytical method. Normally written by invitation only but the editor welcomes suggestions.

Please ensure that you select the appropriate article type from the list of options when making your submission. Authors contributing to **special issues** should ensure that they select the special issue article type from this list.

The journal also accepts **letters**, which should be sent directly to the editor in chief for consideration.

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