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PRISCILLA BARBOSA FERREIRA SOARES

Tecido ósseo e implantes dentais – caracterização mecânica e biológica em condições de normalidade e submetidos à radiação ionizante.

Tese apresentada à Faculdade de Odontologia da Universidade Federal de Uberlândia, como requisito parcial para obtenção do Título de Doutor em Odontologia na Área de Concentração de Clínica Odontológica Integrada

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Banca Examinadora:
Orientador: Prof. Dr. Darceny Zanetta-Barbosa, UFU
Profa. Dra. Camilla Christian Gomes Moura, UFU
Profa. Dra. Paula Dechichi, UFU
Prof. Dr. Elcio Marcantonio Junior, FOAR-UNESP
Prof. Dra. Yara Teresinha Corrêa Silva Sousa, UNAERP

Suplentes
Prof. Dr. Alfredo Júlio Fernandes Neto, UFU
Prof. Dr. Roberto Sales e Pessoa, UNITRI

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Albert Einstein

SUMÁRIO

RESUMO

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O osso é um tecido conjuntivo resiliente em contínuo processo de modificação. Este tecido pode sofrer efeitos adversos frente ação da radiação ionizante, comprometendo o processo de reparo e a osseointegração. Integração esta que é modulada pela superfície de implantes dentais, Este trabalho tem como objetivo geral avaliar por meio da caracterização mecânica e histomorfométrica o tecido ósseo e a sua integração a implantes dentais em condições de normalidade e quando submetido à radiação ionizante. Este estudo foi dividido em cinco objetivos específicos; ***objetivo específico 1:*** gerar, por meio de ensaio dinâmico de indentação, protocolo para mensurar a dureza Vickers e o módulo de elasticidade do osso periimplantar, utilizando blocos composto por implante dental/osso extraídos de tíbias de coelhos; ***objetivo específico 2:*** avaliar a estabilidade e osseointegração de implantes com diferentes tratamentos de superfícies por meio da mensuração de frequência de ressonância e da análise histomorfométrica quantificando o contato osso/implante e fração de área ocupada pelo osso em implantes instalados em tíbias de coelhos, nos períodos de 2 e 4 semanas; ***objetivo específico 3:*** avaliar o efeito da radiação ionizante em tíbias de coelhos em função do período decorrido da aplicação da radiação ionizante nas propriedades de força, energia e rigidez do tecido ósseo por meio de ensaios mecânicos de resistência flexural, dureza Vickers e módulo de elasticidade por meio de ensaio de indentação dinâmica, espessura de cortical óssea, volume de tecido ósseo e percentual de porosidade por meio de microCT e quantificação das lacunas de osteócitos vazias, por meio de avaliação histológica; ***objetivo específico 4:*** avaliar o efeito da radiação ionizante sobre implantes previamente instalados e osseointegrado por meio de associação de metodologias envolvendo dureza Vickers e módulo de elasticidade mensurado por indentação dinâmica, resistência de integração implante/osso por meio de ensaio pull-out, espessura de cortical óssea, volume de tecido ósseo e percentual de porosidade por meio de microCT; ***objetivo específico 5:*** gerar síntese dos achados dos objetivos 1, 2, 3 e 4 em um artigo de comunicação aos clínicos brasileiros cumprindo a função social da geração do conhecimento. Após análise dos resultados pode-se concluir que o método de indentação dinâmica mostrou ser altamente útil na caracterização individualizada do tecido

ósseo periimplantar. As superfícies de implantes com diferente molhabilidade são capazes de produzir adequada integração osso/implante em condição normal de instrumentação do osso cortical. A radioterapia interfere nas propriedades mecânicas e estruturais do tecido ósseo. Em áreas reabilitadas com implantes a radioterapia resulta em redução parcial da integração osso/implante, reduzindo as propriedades mecânicas do osso periimplantar principalmente daquele localizado próximo à superfície do implante, o que pode comprometer o prognóstico de longo prazo.

ABSTRACT

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The bone is a connective resilient tissue in continuous modification process. This tissue may be adversely affected by action of ionizing radiation, compromising the healing process and osseointegration. The integration between bone and implant is modulated by the surface of dental implants. This study aimed to evaluate overall by mechanical and histomorphometric characterization of bone tissue and its integration to the dental implants in normal conditions and when subjected to ionizing radiation. This study was divided into five specific objectives; ***specific objective 1:*** generate, through dynamic indentation test protocol to measure the Vickers hardness and the modulus of elasticity of the periimplantar bone using blocks performed by bone/implant extracted by the rabbit's tibias; ***specific objective 2:*** to evaluate the stability and osseointegration of dental implants with different surface treatments by measuring the resonant frequency and histomorphometric analysis quantifying the contact bone/implant and area fraction occupied by bone implants installed in the tibia of rabbits after periods of 2 and 4 weeks; ***specific objective 3:*** to assess the effect of ionizing radiation on tibias of rabbits modulated by the time elapsed from the application of ionizing radiation on the force to fracture, work to failure and stiffness of bone tissue measured by 3-bending flexural strength test, on the Vickers hardness and elastic modulus calculated by means of dynamic indentation test, and on the cortical bone thickness, bone volume and bone porosity percentage measured by microCT and quantification of osteocytes lacunarity, measured by histological evaluation; ***specific objective 4:*** to evaluate the effects of ionizing radiation on previous installed and osseointegrated implants by association of different methods involving Vickers hardness and modulus of elasticity measured by dynamic indentation; implant/bone stability measured by pull-out test, the thickness of cortical bone, volume of bone tissue, and percentage of bone porosity measured by microCT; ***specific objective 5:*** generate manuscript that summarize the main findings of specific objective 1, 2, 3 and 4 designated to Brazilian clinical fulfilling the social function of knowledge generation. After analyzing the results it can be concluded that the dynamic indentation method proved to be highly useful for individualized characterization of the periimplantar bone tissue. The implant surfaces with different wettability are capable of

producing adequate integration bone / implant installed using instrumentation normal condition. Radiation therapy negatively affected the mechanical and structural properties of bone tissue. In areas rehabilitated with dental implant, the radiotherapy reduced partially the stability of implant/bone interface, thereby reducing the mechanical properties of the periimplantar bone tissue, mainly that bone tissue located close to implant surface, which can compromise the long-term prognosis.

INTRODUÇÃO E REFERENCIAL TEÓRICO

1. INTRODUÇÃO E REFERENCIAL TEÓRICO

O tecido ósseo é uma forma especializada de tecido conjuntivo constituído por células e por matriz extracelular, que possui a característica única de mineralizar (Katchburian & Arana-Chaves, 2004). A mineralização da matriz, constituída principalmente cálcio e fosfato organizado na forma de hidroxiapatita, confere a este tecido dureza e rigidez relativa (Boivin et al., 2008; Barth et al., 2011; Novitskaya et al., 2011). A parte orgânica do tecido ósseo, constituído pela matriz de colágeno proporciona-lhe importante resiliência e maleabilidade (Hamer et al., 1996; Barth et al., 2011; Novitskaya et al., 2011). Isto torna o tecido ósseo capaz de dissipar tensões e deformações frente a esforços mecânicos, sustentando e protegendo do órgão dental (Soares et al., 2011). O tecido ósseo apresenta estrutura organizada por meio de fibrilas colágenas como substância-base, componentes minerais, associada a componentes celulares, principalmente osteoblastos, osteócitos, osteoclastos e células indiferenciadas (Katchburian & Arana-Chaves, 2004). Este tecido possui importante capacidade de remodelação e adaptação ao esforço mecânico tornando-se mais denso (Katti, 2004).

O tecido ósseo é um tecido rígido particularmente resistente à compressão axial, possuindo ainda certo grau de flexibilidade e capacidade de absorção de impactos caracterizando assim um tecido resiliente e tenaz (Boivin et al., 2008; Barth et al., 2011). Quando o estado de tensão e deformação ultrapassa limite de resistência deste tecido, ocorre fratura ou dano permanente (Oosterwyck, 2003; Soares et al., 2011). O tecido ósseo, tal qual qualquer outro material composto, pode sofrer e acumular danos estruturais por fadiga, resultando em microfissuras causadas pelo carregamento não funcional ou produzidos pelo processo de fadiga (Oosterwyck, 2003). O osso possui por outro lado, a capacidade de detectar danos, possuindo mecanismos eficientes de adaptação fisiológica restaurando o estado inicial, ou seja, possui aptidão intrínseca de reparação (Katchburian & Arana Chaves, 2004). Esta capacidade de resposta do tecido ósseo é conseguida essencialmente por meio de processos de remodelação (Willey et al., 2008). Sendo que os estímulos mecânicos desempenham neste sentindo profunda influência. Mas para que este processo seja eficaz a saúde do tecido é pré-requisito.

Para avaliar processos de reparo e alterações fisiológicas e patológicas do tecido ósseo, olhares atentos aos componentes biológicos e mecânicos são imperativos. O ensaio de flexão tem sido empregado para obtenção da força máxima resistida pelo tecido, do trabalho necessário para gerar o dano e ainda a rigidez relativa frente à fratura (van Lenthe et al., 2008). Ensaio de indentação dinâmica por outro lado, tem sido empregado para obtenção da dureza e do módulo de elasticidade (Soares et al., 2014), que representam indiretamente a capacidade de deformação elástica e plástica do tecido ósseo (Kim et al., 2015). Estes dados fornecem indicativos importantes do estado de normalidade ou de alteração do tecido ósseo. Aliado a estas metodologias uma moderna e útil ferramenta não destrutível é a micro tomografia computadorizada de precisão (microCT) (Kim et al., 2015) Esta metodologia é capaz de avaliar parâmetros morfológicos como volume ósseo, espessura de cortical e porosidade do tecido que indiretamente representa o estado de saúde do tecido (Bouxsein et al., 2010; Kim et al., 2015) Este método tem sido recomendado como padrão ouro para análises *ex-vivo* por permitir a reconstrução tridimensional e por determinar parâmetros morfológicos que podem ser relacionados aos parâmetros histomorfométricos (Park et al. 2005). Outra ferramenta importante para avaliar o processo de evolução e reparo é a mensuração da lacunaridade do tecido ósseo, por meio de análise histomorfométrica (Dougherty & Henebry, 2001). Fica claro no estágio de evolução da ciência que o uso de associação de metodologias não destrutivas, como ensaios mecânicos e análises histomorfométricas podem melhor responder a importantes e relevantes questões de saúde do aparelho estomatognático.

O osso é uma estrutura altamente dinâmica, que se remodela e mantêm-se ativamente durante toda a vida, porém esta capacidade é influenciada negativamente por diversos processos patológicos e por agentes externos, tais como a radioterapia (Willey et al., 2011). O câncer de cabeça e pescoço acomete mais que meio milhão de pessoas anualmente em todo mundo (Jemal et al., 2011). A radioterapia é a modalidade terapêutica que utiliza as radiações ionizantes com o objetivo de destruir as células neoplásicas visando redução ou desaparecimento da neoplasia maligna (Ihde et al., 2009; Willey et al., 2011). Isoladamente ou associada à quimioterapia e cirurgia ressectiva, a radioterapia tem sido o tratamento recomendado para mais de 70% dos pacientes com tumores malignos (Ma & Shen, 2012). Até poucos anos a forma mais comum de emissão da radiação ionizante eram os aparelhos de Cobalto 60 (Ozen et al., 2005; Page et al., 2014). Este mecanismo de aplicação tem sido substituído gradualmente em países subdesenvolvidos, e praticamente não são mais utilizado no Brasil, e em países desenvolvidos, sendo hoje a primeira opção os aceleradores lineares. Estes aparelhos conseguem atuar em áreas mais específicas limitando maiores efeitos colaterais (Ozen et al., 2005; Page et al., 2014).

A radioterapia se constitui forma eficaz no tratamento do câncer bucal sendo um tratamento loco-regional, porém, causa alterações adversas visíveis nos tecidos adjacentes às áreas irradiadas. O sucesso ou falha do tratamento por radiação depende da dose liberada no volume inteiro do tumor e não deve variar mais do que 5% da dose prescrita (Niroomand-Rad et al., 1996). Este tipo de tratamento, quando aplicado na região da cabeça e pescoço, dependendo da dose de irradiação, tempo e volume do tratamento, dose de distribuição e uso concomitante de outras terapias, pode produzir alterações muitas vezes irreversíveis nos tecidos (Hopewell, 2003; Ihde et al., 2009). A radioterapia, não afeta apenas as células tumorais, ela pode também danificar em diferentes estágios células normais. A radiação ionizante em alta doses como a aplicadas rotineiramente no tratamento de tumores de cabeça e pescoço causam injúrias quase sempre irreversíveis ao esmalte e dentina (Soares et al., 2010; Soares et al., 2011), aos tecido musculares (Yachouh et al., 2010), e principalmente ao tecido ósseo (Hopewell, 2003; Ihde et al., 2009; Willey et al., 2011). No tecido ósseo são observados alterações no suprimento sanguíneo com resultante hipóxia interferindo negativamente na atividade de células osteoblásticas e osteócitos (Ihde et al., 2013; Pompa et al., 2015). Estes efeitos conduzem a hipovascularização, hipocelularidade e hipóxia óssea (Ihde et al., 2009; Pompa et al., 2015), reduzindo matriz extracelular e seu conteúdo mineral (Pelisser et al., 2007). A ação da radiação ionizante no tecido ósseo a nível molecular altera suas propriedades químicas e mecânicas pela degradação do colágeno e pela redução da densidade de ligações cruzadas intermoleculares (Barth et al., 2011; Willey et al., 2011). Todo este conjunto de eventos pode culminar com menor capacidade de osseointegração a implantes dentais e ainda mais severamente com a ocorrência de osteorradionecrose (Marx et al., 1983; Ozen et al., 2005).

As grandes alterações na matriz óssea após a irradiação são desenvolvidas progressivamente com o tempo, onde as mudanças iniciais são resultados de uma injúria ao sistema de remodelagem óssea, ou seja, os osteoblastos, osteócitos e osteoclastos, que tardiamente pode resultar em aumento da atividade da lise celular (Willey et al., 2011). Com isso, o processo de formação de matriz óssea é paralisado, impedindo o processo de mineralização, o que pode levar a fraturas ósseas espontâneas e à osteorradionecrose (Marx, 1983). Estruturalmente, a radiação afeta o colágeno por meio do aumento do seu grau de reticulação, o que resulta perda em relação à deformação, conduzindo à diminuição radical na resistência e ductilidade do osso (Maeda et al., 1988). As células endoteliais também são fortemente afetadas e a fibrose vascular resulta em diminuição da vascularização, afetando a vitalidade do osso e das células medulares, tornando a área suscetível a uma infecção e necrose, mesmo após um pequeno trauma (Hopewell, 2003; Willey et al., 2011) Essa interferência no metabolismo ósseo desequilibra a remodelação, favorecendo a reabsorção óssea, comprometendo as propriedades biomecânicas do osso e seu processo de reparação (Maeda et al., 1988). Porém poucos relatos na literatura associam estes eventos a análise sequencial de aspectos biológicos, morfométricos e mecânicos do tecido ósseo.

Quando o paciente perde um ou mais elementos dentais o protocolo restaurador hoje de primeira escolha é a instalação de implante (Ihde et al., 2009). Os índices de sucesso dos implantes dentais desde os preceitos descritos por Branemark são elevados e promissores. Porém as indústrias continuam progressivamente trabalhando no desenvolvimento de novas promissores tratamentos de superfícies dos implantes metálicos (Soares et al., 2015). A optimização da superfície do titânio pode favorecer o processo de osseointegração principalmente em situações clínicas nas quais o tecido ósseo alveolar possui reduzida densidade mineral e requer rápido processo de reparo ou frente a necessidade de carga imediata (Le Guéhennec et al., 2007). Vários mecanismos têm sido relatados para se obter tratamento de superfície como o uso de jato de plasma, jeteamento, ataque ácido ou anodização (Le Guéhennec et al., 2007; Mendonça et al., 2008; Novaes et al., 2010). Este tratamento pode resultar em alteração da composição topográfica e química, refletindo na molhabilidade da superfície (Le Guéhennec et al., 2007; Mendonça et al., 2008; Gittens et al., 2014). Podendo resultar em eventos benéficos de adsorção proteica, agregação e ativação plaquetária e retenção de fibrina (Kopf et al., 2015), melhorando a interação mais precocemente do implante ao tecido ósseo (Buser et al., 2004; Le Guéhennec et al., 2007; Mendonça et al., 2008; Gittens et al., 2014). Recentemente uma nova superfície superhidrofílicas de implantes dentais está disponível comercialmente Acqua® (Neodent, Curitiba, PR, Brasil), empregando método similar ao método desenvolvido por empresas sueca denominadas SLActive® (Instituto Straumann Ag, Basel, Suíca). Estudar estes novos mecanismos de tratamento de superfície que podem auxiliar os profissionais em condições de maior complexidade por limitações do tecido ósseo parece ser um desafio importante a ser vencido.

No Brasil, cerca de 800 mil implantes são instalados por ano no país, segundo levantamento da Associação Brasileira da Indústria Médica, Odontológico e Hospitalar (Abimo). Com isso estabelece-se um horizonte que é de pacientes vivendo mais e muitos deles com reabilitações dentais associadas a implantes. Pensar então em doenças que interagem com a vida adulta ou terceira idade e que tenha impacto na longevidade de implantes dentais é um horizonte importante para a pesquisa científica e deve ser entendida como real problema de saúde pública. Quando o paciente sofre tratamento radioterápico em áreas com implantes dentários a dose metal-tecido pode sofrer alterações de amplificação dos danos causados (Niroomand-Rad, 1995; Wang et al., 1996). Entre os oncologistas existe um consenso que enquanto a superdosagem pode aumentar o risco de necrose, e que a subdosagem pode comprometer a destruição do tumor (Niroomand-Rad, 1995; Granstrom, 2003). Dependendo da liga metálica ocorre maior espraiamento da radiação acentuando os efeitos deletérios da radioterapia. Porém não há consenso na literatura se os implantes dentais devem ser retirados previamente à radiação, pois este procedimento acaba sendo altamente mutilador. E ainda há necessidade de maior elucidação da efetividade clínica de implantes dentais já osseointegrados em pacientes com necessidades de tratamento radioterápico. A definição dos efeitos mecânico e biológico da radiação ionizante em áreas com implantes dentários deve ser estudada para prevenir e minimizar os danos causados pela radioterapia contribuindo com a melhora da qualidade de vida de pacientes.

A produção intelectual da Odontologia brasileira ocupa hoje a segunda posição mundial e tem se destacado tanto por indicadores quantitativos e qualitativos (Scariot et al., 2011; Scimago SJR, 2014). Porém os clínicos brasileiros que atuam, por exemplo, na área de periodontia e implantodontia não acompanham na mesma intensidade a divulgação de conhecimento com evidência científica em periódicos publicados em língua inglesa e livremente disponíveis no Brasil apenas para instituições com acesso ao Portal de Periódicos da CAPES. Por outro lado, os eventos científicos nacionais têm se tornado cada vez mais foco de divulgação mercantil de novos implantes que surgem a cada dia, produzidos muitas vezes por empresas com credibilidade duvidosa. Revistas nacionais, publicadas na língua portuguesa, de ampla tiragem que cheguem aos consultórios odontológicos deve ser um horizonte para que de forma complementar possa divulgar os principais achados científicos em área tão relevante econômica e socialmente como o foco deste estudo produzido no interior de uma Universidade Pública Brasileira.

Desta forma, parece pertinente utilizar associação de metodologias não destrutivas de análise morfométrica aliado a ensaios mecânicos e histomorfométricos para analisar de forma integradas e progressivas de diferentes fatores envolvidos no processo de integração de implantes dentais, no efeito da radioterapia sobre o tecido ósseo e principalmente na interação de implantes já integrados que necessitam sofrer radioterapia. Gerando com isso artigos já publicados e a serem submetidos aos periódicos de alto fator de impacto da odontologia mundial e ao mesmo tempo gerar síntese destes achados articulado em uma visão de educação continuada e popularização da ciência a ser submetido na forma de artigo de comunicação aos clínicos brasileiros cumprindo a função social da geração do conhecimento.

OBJETIVOS

2. OBJETIVOS

Objetivo Geral

Caracterizar as propriedades mecânicas de tecido ósseo e a sua integração a implantes dentais em condições de normalidade e quando submetido à radiação ionizante

Objetivos específicos

Objetivo específico 1

Capítulo 1 - *Measurement of Elastic Modulus a n d Vickers Hardness of Surround Bone Implant Using Dynamic Microindentation – Parameters Definition*

O objetivo deste estudo foi gerar protocolo para mensurar a dureza e o módulo de elasticidade do osso periimplantar, por meio do ensaio dinâmico de indentação.

Objetivo específico 2

Capítulo 2 - *Influence of Implant Surfaces on Osseointegration: A Histomorphometric and Implant Stability Study in Rabbits*

O objetivo deste estudo foi avaliar a estabilidade e osseointegração de implantes com diferentes superfícies empregando análise de frequência de ressonância (RFA) e histomorfometria (contato implante ósseo, BIC, e fração de área ocupada osso, BAFO), nos períodos de 2 e 4 semanas após a instalação em tíbias de coelhos.

Objetivo específico 3

Capítulo 3 – *Effect of after-therapy interval of ionizing radiation on bone: histomorphometric and biomechanical analysis*

O objetivo deste estudo foi avaliar o efeito da radiação ionizante na integridade biológica, estrutural e mecânica do osso em diferentes períodos após radiação.

Objetivo específico 4

Capítulo 4 - *Mechanical and morphological alterations produced by ionizing radiation on bone- dental implant interaction*

O objetivo deste estudo foi avaliar o efeito da radiação ionizante na integridade mecânica de implantes dentais previamente instalados à realização de radioterapia.

Objetivo específico 5

Capítulo 5 - *Interação da radioterapia e da reabilitação estético-funcional com implantes dentais – Abordagem clínica com evidência científica*

O objetivo deste trabalho foi gerar síntese dos achados dos objetivos específicos 1, 2, 3 e 4 compilados em um artigo de comunicação aos clínicos brasileiros cumprindo a função social da geração do conhecimento.

CAPÍTULOS

3. CAPÍTULOS

3.1 CAPÍTULO 1

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Measurement of Elastic Modulus and Vickers Hardness of Surround Bone Implant Using Dynamic Microindentation – Parameters Definition

Priscilla Barbosa Ferreira Soares1, Sarah Arantes Nunes2, Sinésio Domingues Franco3, Raphael Rezende Pires3, Darceny Zanetta-Barbosa2, Carlos José Soares4.

1Department of Periodontology and Implantology, UFU - Federal University of Uberlandia, Uberlândia, MG, Brazil

2Department of Oral and Maxillofacial Surgery and Implantology, UFU - Federal University of Uberlandia, Uberlândia, MG, Brazil

3Department of Mechanical Engineering, UFU - Federal University of Uberlandia, Uberlândia, MG, Brazil

4Department of Operative Dentistry and Dental Materials, UFU - Federal University of Uberlandia, Uberlândia, MG, Brazil

Correspondence: Prof. Dr. Carlos José Soares - Avenida República do Piratini, S/N, Campus Umuarama, Bloco 4LA, Sala 4LA32, Uberlândia, Minas Gerais 38405-325, Brasil. e-mail: carlosjsoares@umuarama.ufu.br

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[1]Department of Periodontology and Implantology, UFU - Federal University of Uberlandia, Uberlândia, MG, Brazil
[2]Department of Oral and Maxillofacial Surgery and Implantology, UFU - Federal University of Uberlandia, Uberlândia, MG, Brazil
[3]Department of Mechanical Engineering, UFU - Federal University of Uberlandia, Uberlândia, MG, Brazil
[4]Department of Operative Dentistry and Dental Materials, UFU - Federal University of Uberlandia, Uberlândia, MG, Brazil

Correspondence: Prof. Dr. Carlos José Soares - Avenida República do Piratini, S/N, Campus Umuarama, Bloco 4LA, Sala 4LA32, Uberlândia, Minas Gerais 38405-325, Brasil. e-mail: carlosjsoares@umuarama.ufu.br

The clinical performance of dental implants is strongly defined by biomechanical principles. The aim of this study was to quantify the Vicker's hardness (VHN) and elastic modulus (E) surround bone to dental implant in different regions, and to discuss the parameters of dynamic microindantion test. Ten cylindrical implants with morse taper interface (Titamax CM, Neodent; 3.5 mm diameter and 7 mm a height) were inserted in rabbit tibia. The mechanical properties were analyzed using microhardness dynamic indenter with 200 mN load and 15 s penetration time. Seven continuous indentations were made distancing 0.08 mm between each other perpendicularly to the implant-bone interface towards the external surface, at the limit of low (Lp) and high implant profile (Hp). Data were analyzed by Student's t-test (α=0.05) to compare the E and VHN values obtained on both regions. Mean and standard deviation of E (GPa) were: Lp. 16.6 $\pm$ 1.7, Hp. 17.0 $\pm$ 2.5 and VHN (N/mm^2): Lp. 12.6 $\pm$ 40.8, Hp. 120.1 $\pm$ 43.7. No statistical difference was found between bone mechanical properties of high and low profile of the surround bone to implant, demonstrating that the bone characterization homogeneously is pertinent. Dynamic microindantion method proved to be highly useful in the characterization of the individual peri-implant bone tissue.

Key Words: bone, dental implant, elastic modulus, Vickers hardness, dynamic microindentation.

Introduction

The primary function of the stomatognathic system is preparation and processing of food through a biomechanical process of biting and chewing (1,2). This mechanism is based on the transfer of masticatory forces, mediated through the implant or intact and restored teeth. The integration between implant and bone depends on the tissue quality, and the inductive bone growing potential of the implant surface (2,3). The interactivity between the implant and surrounding bone is a typical repair process affected by the intensity and direction of the load applied over implant (4). The interface between bone and implant should be biologically integrated creating a unique body that is able to absorb the stress-strain generated by masticatory force (5). Titanium alloys have been extensively used as biomaterials in dental implantology due to their good mechanical performance and biocompatibility. Surface implant modifications using physical and chemical treatments have been used to increase the bone interaction to implants. (3,6).

Bone is a structure composites of an organic matrix (organized network of type I collagen fibrils) filled with a mineral component (apatite crystals bound to the collagen fibrils) (7,8). Trabecular bone has a porous structure and is located in medullary cavities (9,10). The effect of the implant parameters, bone quality and characteristic modulated by different aspects like aging, irradiation, tobaccoism, coffee consumption and alcohol consume, have been evaluated using several laboratory techniques (4,10-12). From a macro-biomechanical perspective, the anchorage of implants has been assessed using tensile tests (13), torque (14), pull-out (2) and push-in tests (2). Regarding micromechanical integration, the mechanical properties of surround bone to titanium implants have been evaluated using microindentation (4,15). Microindentation is a dynamic method that allows the determination of both the elastic modulus and the hardness of bone in the bone-implant interface, taking into account the anisotropic condition of the tissue structure (4,10,15,16).

The use of animal models has been useful to compare the treatment and to better explain the bone alteration-integration process (2). The advantage of the rabbit model is that it is a small animal and can accept dental implants in long bones, as the tibia and the femur (2,14). The reproducibility of animal model used is an important factor on the extrapolation of the results obtained for human health. Hansma et al. (17) related that have no instrument that can clinically measure the properties of bone in vivo. Additionally, the possibility to obtain the specific mechanical properties for each animal after euthanasia using microindentation may contribute for generation of the specific computational analysis of the influence the bone quality on the stress distribution using finite element models (18,19).

Limited description in the literature, to the best of our knowledge, of the parameters used to calculate the microindentation for animal samples has been described. (4,10,15-17,20) Therefore, using dynamic microindentation this study aimed: 1) to calculate the mechanical properties (elastic modulus and Vicker hardness) of the surround bone to dental implant immediately after insertion into the rabbit tibia; 2) to verify the influence of the implant-bone region on the bone mechanical properties.

Material and Methods

The research project was approved by the Ethics Committee of the Federal University of Uberlândia (Protocol #093/12). Five rabbit tibias were used. The residual soft tissue was removed and the tibias were stored in phosphate-buffered saline solution and frozen at -18 °C for 24 h before testing. For the dental implant insertion the tibias were removed from the frozen and stored on the bench for 2 h into the phosphate-buffered saline solution (21).

Sample Preparation

Two implants (3.5-mm diameter, 7.0-mm long dental implants; Titamax CM; Neodent, Curitiba, PR, Brazil) were inserted in the tibia diaphysis, with a distance of 10 mm from each other. The implants were inserted following the manufacturer's recommendation. The holes for the installation will be performed with 20:1 handpiece (Gearbox NSK E16R, Nakanishi, Shimohinata, Japan), mounted on electric motor (DrillerSmart, MIS Implant Technologies, Shlomi, Israel), with 800 rpm under copious irrigation with 0.9% saline solution. The tibia with two implants was sectioned using a water-cooled double-faced high-speed diamond disc longitudinally, reaching the center of the each implant and 5 mm from the other external limit of the implant, creating 2 samples for each tibia. The tibia was longitudinally sectioned reaching implant (Fig. 1A) using a precision saw (Isomet 1000, Buehler, Lake Bluff, IL, USA). The block consisted of the bone-implant sample was embedded using polyester resin (Instrumental Instrumentos de Medição Ltda, São Paulo, SP, Brazil). To perform the embedment process, the sample was positioned with the cutting implant surface downward over a glass plate and fixed using adhesive (Single Bond 2; 3M-Espe, St. Paul, MN, USA). The adhesive system was applied in a thin layer and the sample was stabilized using digital pressure followed by light activation for 20 s (Demetron; Kerr, Orange, CA, USA). A metallic tube (Metalon; Metalon Pooled Industries, Nova Iguaçu, RJ, Brazil) with 50 mm in length 30 mm in width and 10 mm in high was positioned around the sample and was fixed with wax. Three samples were embedded per block (Fig. 1B).

An important aspect that influenced on the methodology reproducibility was the position of the cutting performed on the implant-bone sample. During pilot study initially the cutting performed using precision saw was done into the center of the implant. During the finishing and polishing of the sample, several implants detached to the bone compromising the sample. Then, the position was modified by moving no more than 1.0 mm laterally to the center of the implant and the sample used to finish and polish was the side where is located the bigger amount of the implant. Therefore, when the finish and polish was finalized, the amount of the implant was sufficient to maintain the implant the correct position. After curing of the polyester resin, the surfaces were finished using 600-, 800-, 1200- and 2000-grit silicon-carbide papers (Norton, Campinas, SP, Brazil) and polished with metallographic diamond pastes (6, 3, 1, ¼ µm; Arotec, São Paulo, SP, Brazil). The metallic tube-implant was washed in an ultrasound bath (Cristofoli, Campo Mourão, PR, Brazil) with absolute alcohol for 3 times of 7 minutes each one to remove the debris.

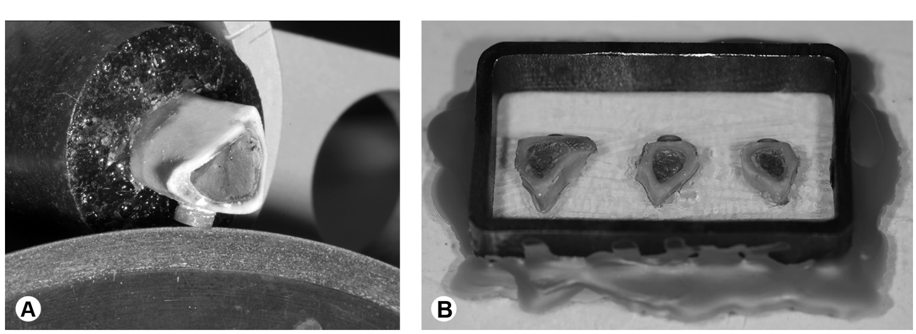

Figure 1. A. Cutting process of bone-implant preparation; B. Samples into the metallic tube fixed with wax.

Dynamic Indentation Test

Elastic modulus (E) and Vickers Hardness (VHN) of the bone was assessed by using a microhardness dynamic indenter (CSM Micro-Hardness Tester; CSM Instruments, Peseux, Switzerland). Seven continuous indentations were made distancing 0.08 mm between each other perpendicularly to the implant-bone interface towards the external surface, considering the low and high profiles. The test load was increased and decreased at a constant speed between 0 and 200 mN. The force increased from 0 to 200 mN in 60 s intervals, which was applied constantly for 15 s. Then the force was gradually removed from 200 mN to 0 mN in 60 s intervals. The load and the penetration depth of the indenter were continuously measured during the load–unload-hysteresis (22). The hardness is defined as the test force divided by the apparent area of the indentation at maximal force. From a multiplicity of measurements stored in a database supplied by the manufacturer, a conversion factor between hardness and VHN was calculated and implemented into the software, so that the measurements were expressed in Vickers hardness units:

$$VHN = \frac{P}{A}$$

where: *P* is the maximum load, *A* is the deep-sensing instrument.

The indentation modulus was calculated from the slope of the tangent of the indentation depth-curve at maximal force (Fig. 2) and is comparable with the elastic modulus of the material (*E*) expressed and using this formula:

$$E = \frac{1 - \nu_s^2}{\frac{1}{E_r} - \frac{1 - \nu_i^2}{E_i}}$$

where: *Ei* is the elastic modulus of the diamond indenter (1141 GPa), νi is the Poisson's ratio of the diamond indenter (0.07), *Er* is the reduced modulus of the indentation contact, and νs is the Poisson's ratio of the bone (0.3) (20). The typical course of measurement curve is shown in Figure 2.

Table 1. Mean and standard deviation of Elastic modulus (GPa) and Vickers Hardness (N/mm2) of low and high profile of surrounding bone to dental implant

Mechanical properties	Measurement location bone/ implant interface	
	Low profile	High profile
Elastic modulus (GPa)	16.6 ± 1.7[A]	17.0 ± 2.5[A]
Vickers hardness (N/mm²)	125.6 ± 40.8[A]	120.1 ± 43.7[A]

Letters represent statistical category within each mechanical property as a function of location around the steps of implant thread, defined by t-Student test (P<0,05) for each mechanical property.

Statistical Analysis

The E and VH data were tested for normal distribution (Shapiro-Wilk, p>0.05) and equality of variances (Levene's test, p>0.05), followed by parametric statistical tests. The pared *t*-Student test was performed the comparison between the data recorded in each bone region (low and high profiles). All tests employed a 0.05 level of statistical significance and all statistical analyses were carried out with the statistical package Sigma Plot version 13.1 (Systat Software, Inc., San Jose, CA 95110 USA).

Results

The E values in GPa for the bone structure in low and high profiles of implant interface are shown in Table 1. The t-Student test showed no difference between E recorded at low and high profile regions (P = 0.663). The E values recorded at different distance of the implant interface were plotted in Fig 3. The E values at all distances maintained stable irrespective of bone region.

The Vickers hardness in VHN for the bone structure in low and high profiles of implant interface are shown in Table 1. The Student's t-test showed no difference between E recorded at low and high profile regions (p = 0.777). The VHN values recorded at different distance of the implant interface are plotted in Figure 4. The VHN values at all distance maintained stable irrespective of bone region.

Discussion

In order understand the mechanical properties and the mechanisms involved in bone fragility, it is important to study the mechanical properties of its different components, and the relationships between them at the various levels of the structural organization (7,10). The dynamic indentation method described in this study showed very effective to

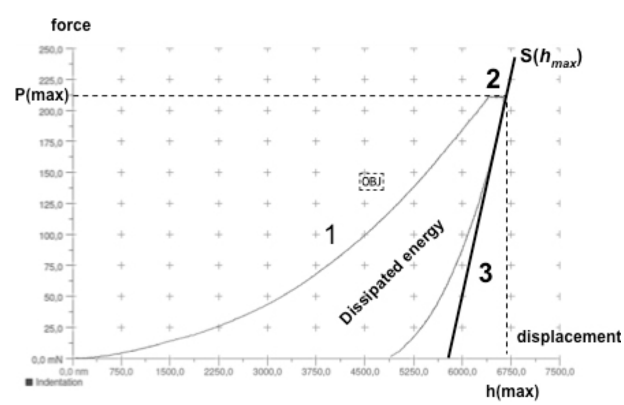

Figure 2. Force-displacement curve of a microindentation test: loading (1), holding (2), unloading (3) of an indenter tip. The third part leads to elastic recovery of the material and its initial slope is used to derive the elastic indentation modulus. The hysteresis represents the dissipated energy.

determine both important mechanical properties (E and VHN). The low variability of the data acquisition and the easy reproducibility of this methodology confirmed the validity of this method to study the bone/implant interaction.

The ratio between stress and strain that is resulted into the body in order to the force applied externally characterizes the elastic modulus of the structure. The modulus of elasticity of a material represents its stiffness and for most materials remains constant over a range of stress. The ratio of the longitudinal strain to the longitudinal stress in the linear part of the curve called Elastic modulus (15,17,20). The bone integrity around the implant may be inferred by the constant elastic modulus measured by indentation method in different regions.

The elastic modulus of bone and hardness represents and is greatly influenced by the proportion of the anisotropic type I collagen fiber and the hydroxyapatite crystal composite (7,8). According to Rho et al. (7) and Leng et al. (8), the differences in elastic modulus have been speculated to be in close relation to the turnover of the bone, ie, the maturity of the individual osteons, moreover, the distribution of their types. In general, it has been suggested that in the bone, the elastic modulus correlates with hardness (4,9,10).

The elastic modulus reduction of the surround bone to implant has been correlated to the poor bone integration of the dental implant (2,15). In the present study was analyzed the E and VHN on low and high profiles of the surround bone aiming to test the immediate negative effect caused by specific drills used during hole implant preparation. The last drill used during whole preparation has 2.8 mm in diameter and the implant has 3.5 mm in diameter. Thus, the stress caused by implant insertion may cause damage on the area due to the high profile of the bone (2,15). However, no significant difference was found between low and high profile regions, and then it can be speculate that no damage was caused by this procedure.

Indentation techniques have been widely used to study bone tissue micromechanical properties (4,9,10,15,16,20). However, interpreting microhardness values is complicated because this parameter is related to both elastic and plastic deformations (4,9,10,15,20). Hansma et al. (17) developed an innovative technique to measure directly, on small indented areas, both elastic modulus (E) and contact hardness (Hc). Contact hardness corresponds to the ratio between indentation load and the contact area of the indentation print. Elastic modulus was calculated from the slope of the tangent of the indentation depth-curve at maximal force and is comparable with the elastic modulus of the material (E), which this method the calculation take into account more realistic the viscoelasticity of the bone tissue.

The second purpose of this study was to better establish the parameters of this methodology for testing the E and VHN for rabbit tibia used to test dental implant model. This experimental method involved the frozen bone stored for 24h, however the frozen storage bone containing implants at -20° C has no effects on the biomechanical properties of Bone/ Implant interface (23). During pilot study were performed different methods to embed the bone-implant sample into polystyrene resin. The use of the metallic tube to embed the sample is mandatory to result in better flattening of samples during finishing and polishing procedures (18). When the samples were embedded only into polystyrene resin, during the finishing procedure the surface is not flat because no stronger material can limit the finishing process. During the indentation test, firstly is performed a reference indentation to determine the distance of the surface to the indenter (2,18). When the surface is not real flat the equipment is not able to perform the second indentation and the test consumed much more time and the data recorded resulting in high variability.

Elastic modulus and Vickers hardness of the rabbit

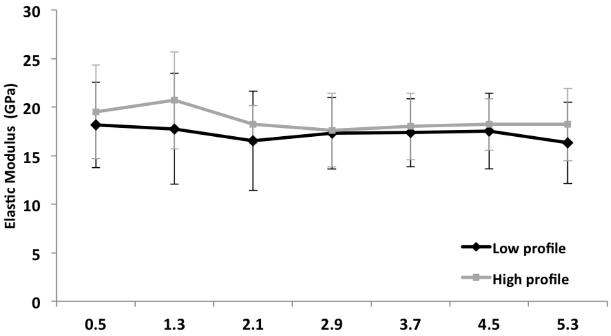

Figure 3. Elastic modulus bone values for all distances measured at low and high implant profiles.

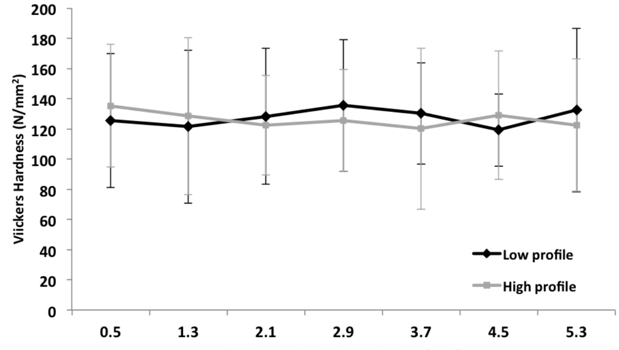

Figure 4. Vickers hardness bone values for all distances measured at low and high implant profiles.

bone is very similar to human bone and the cost of the experiment is much lower than for dog experiments. Posterior maxilla is very important region for implant strategy for oral rehabilitation, the possibility to find an animal model with bone characteristic similar is very important for research field. Seong et al. (1) calculate the elastic modulus of posterior human maxilla bone and found results that range from 13.1 to 16.4, results very similar that were found in the current study. Additionally, the turnover of the rabbit is 3 times lower than human, than other advantage is the lower time consuming for complex experiments (24). It was confirmed that rabbit tibia is a useful model to test the different aspects involved with dental implant protocols (2,14,24). Additionally, the tibia rabbit bone is a suitable model to test dental implant integration in different experimental designs and the dynamic indentation model is a very accurate method to test E and VHN, however the methodological parameters used is this study like to use the metallic recipient for sample embedment and the position of the section of the sample is important to determine the reproducibility of this methodology.

In conclusion, there is no difference between bone mechanical properties, E and VHN, of high and low profiles of the surround bone to implant. Dynamic microindantion method proved to be highly useful in the characterization of the individual periimplant bone tissue, demonstrating that the computational analysis is pertinent.

Resumo

O desempenho clínico de implantes dentais é fortemente definido por princípios biomecânicos. Este trabalho objetivou quantificar a Dureza Vickers (VHN) e módulo de elasticidade (E) do osso periimplantar e discutir parâmetros metodológicos de ensaio dinâmico de indentação. Foram utilizados 10 implantes de corpo cilíndrico com interface cone morse, (Titamax CM; Neodent, Curitiba, PR, Brasil), diâmetro de 3.5 mm e altura de 7 mm inseridos em tíbia de coelho recém obtidas após abate dos animais. As propriedades mecânicas foram analisadas usando penetrador dinâmico de microdureza Vickers (CSM Micro-Hardness Tester; CSM Instruments, Peseux, Switzerland) com carga de 200 mN e tempo de penetração de 15s. Foram feitas 7 indentações no osso cortical na base da rosca (Br) e na ponta da rosca (Pr) na direção perpendicular ao implante, com distância entre elas de 0,08 mm perpendicular a interface osso implante em direção a superfície esterna. Os dados foram analisados por meio de teste t-Student ($P<0,05$). O valores médios e desvio padrão de E (GPa) foram: Br. 16,6 ± 1,7A; Pr. 17,0 ± 2,5A e VHN (N/mm^2): Br. 125,6 ± 40,8A; Pr. 120,1 ± 43,7A. Não houve diferença significativa entre as propriedades mecânicas avaliadas no osso na base e na ponta da rosca do implante, demonstrando que a caracterização desta estrutura de forma homogênea em análises computacionais é pertinente. O método de indentação dinâmica mostrou ser altamente útil na caracterização individualizada do tecido ósseo periimplantar.

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CAPÍTULOS

3.2 CAPÍTULO 2

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Influence of Implant Surfaces on Osseointegration: A Histomorphometric and Implant Stability Study in Rabbits

Priscilla Barbosa Ferreira Soares1, Camilla Christian Gomes Moura2, Marcela Claudino3, Valessa Florindo Carvalho4, Flaviana Soares Rocha1, Darceny Zanetta-Barbosa1.

1Department of Oral and Maxillofacial Surgery and Implantology, UFMG - Universidade Federal de Uberlândia, Uberlândia, MG, Brazil

2Department of Endodontics, UFMG - Universidade Federal de Uberlândia, Uberlândia, MG, Brazil

3Department of Dentistry, UEPG - Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil

4Department of Operative Dentistry and Dental Materials, UFMG - Universidade Federal de Uberlândia, Uberlândia, MG, Brazil

Correspondence: Prof. Dr. Darceny Zanetta-Barbosa, Avenida Pará S/N, Campus Umuarama, Bloco 4T, Bairro Umuarama, 38400- 902 Uberlândia, MG, Brasil. Tel./ Fax: +55-34-3225-8148. email: darceny_@hotmail.com

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[1]Department of Oral and Maxillofacial Surgery and Implantology, UFMG - Universidade Federal de Uberlândia, Uberlândia, MG, Brazil
[2]Department of Endodontics, UFMG - Universidade Federal de Uberlândia, Uberlândia, MG, Brazil
[3]Department of Dentistry, UEPG - Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil
[4]Department of Operative Dentistry and Dental Materials, UFMG - Universidade Federal de Uberlândia, Uberlândia, MG, Brazil

Correspondence: Correspondence: Prof. Dr. Darceny Zanetta-Barbosa, Avenida Pará S/N, Campus Umuarama, Bloco 4T, Bairro Umuarama, 38400- 902 Uberlândia, MG, Brasil. Tel./ Fax: +55-34-3225-8148. email: darceny_@hotmail.com

The aim of this study was to evaluate the stability and osseointegration of implant with different wettability using resonance frequency analysis (RFA) and histomorphometric analysis (bone implant contact, BIC; and bone area fraction occupied, BAFO) after 2 and 4 weeks in rabbit tibiae. Thirty-two Morse taper implants (length 7 mm, diameter 3.5 mm) were divided according to surface characteristics (n=8): Neo, sandblasted and dual acid-etched; and Aq, sandblasted followed by dual acid-etched and maintained in an isotonic solution of 0.9% sodium chloride. Sixteen New Zealand rabbits were used. Two implants of each group were installed in the right and left tibiae according to the experimental periods. The RFA (Ostell®) was obtained immediately and after the sacrifice (2 and 4 weeks). The bone/implant blocks were processed for histomorphometric analysis. Data were analyzed using two-way ANOVA followed by Tukey's test and Pearson's correlation for ISQ, BIC and BAFO parameters ($p=0.05$). No significant effect of implant, period of evaluation or interaction between implant and period of evaluation was found for BIC and BAFO values ($p>0.05$). Only period of evaluation had significant effect for RFA values at 4 weeks ($p=0.001$), and at 2 weeks ($p<0.001$). RFA values were significantly higher at the final period of evaluation compared with those obtained at early periods. There was a significant correlation between BIC values and BAFO values ($p=0.009$). Both implant surfaces, Aq and Neo, were able to produce similar implant bone integration when normal cortical bone instrumentation was performed.

Key Words: dental implant, resonance frequency, histomorphometry, wettability, rabbits

Introduction

In the past twenty years, optimization on titanium implant surfaces has been advocated for improving the osseointegration process. This aspect impacting mainly in specific clinical situations with alveolar bone has reduced mineral density or is required rapid healing for early loading rehabilitation (1,2). Several methods have been developed to obtain different implant surfaces such as plasma spray, grid blasting, acid etching and anodization (1,3,4), which may result on variations of the topography and chemical composition (1,3,5). The implant surface determined by these treatments may affect the protein adsorption, platelet activation and aggregation, fibrin retention (6), cell surface interaction, and cell tissue development at implant/bone interface (1,3,5,7).

The surface topography of the implant is another characteristic that may interfere on the bone biological response (4,8-10). Moderately microroughness surface have proven to be superior to smooth counterparts (8,9), improving parameters as bone-implant contact, new bone formation and removal torque (11,12). Surface chemistry is also an important characteristic for implant performance since its affects on surface energy and wettability (5,7). The implant surface energy measured indirectly by the liquid-solid contact angle (CA) affects the initial blood-implant interactions, the initial stages of cell adhesion, proliferation and differentiation (5,13). Generally, CA ranges from 0 to 180°, values above 90° characterizes the hydrophobic surface, while values lower than 90° are designated hydrophilic surfaces, and values very close to 0° are considered superhydrophilic surfaces (5).

Wetting is reduced on microroughned surfaces created by acid etched, sandblasting or anodization (4,5). Nowadays, most implant surfaces clinically evaluated are of hydrophobic type (5,14,15). SLActive® (Institut Straumann Ag, Basel, Switzerland) was introduced on the market as a superhydrophilic titanium implant surface, which is produced by sandblasting followed by etching using a mixture of HCl and H_2SO_4 followed by storing in NaCL solution (12). SLActive has been evaluated *in vitro* (6,12) and *in vivo* (7,11,12,16). Recently, a new superhydrophilic implant was commercially available, Acqua® (Neodent, Curitiba, PR, Brazil), which is produced by a similar method than SLActive®, resulting in similar microroughness and contact angle (17). No study has tested the biological and surface parameters of this new implant technology.

In this way, the aim of this study was to investigate the new commercially available dental implant on osseointegration by a histomorphometric evaluation of bone-implant-contact (BIC), bone area fraction occupied (BAFO) and resonance frequency analysis (RFA) after 2 and 4 weeks in rabbit tibiae. The null hypothesis was that the implant surface modification employed on Acqua implants has no effect of histomorphometric parameters.

Material and Methods

Thirty-two morse taper implant junctions (Titamax CM; Neodent, Curitiba, PR, Brazil), measuring 3.5 mm in diameter and 7 mm in length, were divided into the following 2 groups (n = 8) according to surface treatment: sandblasting with abrasive particles followed by acid etching (Neo; Neoporos) and Neo maintained in an 0.9% sodium chloride isotonic solution (Aq; Acqua). After installation of the implant, the groups Neo and Aq were divided according to the experimental periods of 2 and 4 weeks.

Surgical Procedure

Sixteen New Zealand white rabbits weighing between 3.0 and 3.5 kg were included in this study. The experimental protocol was evaluated and approved by the Ethics Committee for Animal Research (Protocol #093/12, Universidade Federal de Uberlândia, Brazil). The guidelines of the Brazilian College of Animal Experimentation were followed in all animal protocols.

Prior to surgery, the legs of animals were shaved and the tibiae area was cleaned with a 0.2% chlorhexidine solution (Rioquimica, São José do Rio Preto, SP, Brazil). The animals were anaesthetized with an intramuscular injection of a combination of 0.25 mg of ketamine/kg of body weight (Ketamina Agener; Agener União Ltda., São Paulo, SP, Brazil) and 0.5 mg of xylazine/kg of body weight (Rompum® Bayer S.A. São Paulo, SP, Brazil). The infiltration of anesthesia was applied using 2% lidocaine and 1:100,000 epinephrine (Alphacaine 0.5 - 1 mL/site, DFL, Rio de Janeiro, RJ, Brazil) to reduce stimulation during surgery, generating vasoconstriction.

Incisions of 3 cm in length were performed in the both rabbits' tibias. The soft tissue and periosteum were removed, and a sharp subperiosteal dissection exposed the proximal tibia. Implants were placed using a progressive sequence of drills, under constant irrigation with saline, according to the manufacturer's instructions. All drilling procedures were conducted at 1200 rpm. One implant was installed on the proximal site of each tibia (n=8). The soft tissues were sutured in separate layers using an interrupted suture (#5.0 nylon sutures Ethicon®; Johnson & Johnson Medical Ltd., Blue Ash, Ohio, United States). To prevent infection, daily intramuscular injections of Cefazolin (Yuhan Company; 250mg) were given for 1week. To prevent pain, a dose of an anti-inflammatory Meloxicam® 0.3 mg/kg (Ourofino, São Paulo, SP, Brazil) were administrated. Each rabbit was maintained in individual cages and received food and water.

Resonance Frequency Analysis

Values of implant stability quotient (ISQ) were obtained immediately after implant placement (primary stability) and after 2 or 4 weeks (secondary stability), according to experimental group. For every series of RFA measurements, the ISQ values were recorded using a specific device (Osstell; Integration Diagnostics, Göteborg, Sweden) in two different directions: buccal and palatal. A transducer (Smartpegs) was attached to the implant, and ISQ ranging from 1 to 100 was recorded. The Osstells was brought into very close contact with the Smartpegs, although without touching it, until an audible signal confirmed that the measurement had been taken.

Histological Procedures

The animals were euthanized randomly after 2 and 4 weeks by an intramuscular injection of high dose of the anesthetic solution and the tibiae containing the implants were removed. Tissue blocks containing the implant were fixed in 10% buffered formalin solution for 24 h and washed in running water for 24 h. These bone/implant blocks were dehydrated in an increasing ethanol series (70%, 80%, 90% and 100%) with 7 days for each phase at 5 °C. Following dehydration, the samples were embedded in a methacrylate-based resin (LR White hard grade, London Resin Company, Theale, Berkshire, UK) according to the manufacturer's instructions. After polymerization, the specimens were sectioned along the longitudinal axis with a precision diamond disk (Struers, Ballerup, Hovedstaden, Denmark), resulting in two sections with approximately 300 µm thickness. The sections were fixed on the acrylic plates using cyanoacrylate adhesive (Super bonder Loctite, São Paulo, SP, Brazil). The slices were finished using abrasive papers sequence (#120, 220, 320, 500, 1200 and 2000 µm) (Struers, Ballerup) in a polishing machine (TegraSystem, Struers, Ballerup) under water irrigation. The sections, reduced to a final thickness of 30 µm, were stained with toluidine blue and observed under optical microscope.

Histomorphometric Analysis

All histological sections were identified with a random numerical sequence in order to codify experimental periods and groups, by independent evaluator. Histomorphometric evaluation was performed using an optical microscope (Axion Imager A1M, Carl Zeiss, Germany) attached to a digital camera (Axiocam ICc3, Carl Zeiss, Germany). The acquired digital images were analyzed by a single and calibrated blind examiner for both experimental groups and both periods. Osseointegration process was evaluated using the bone-to-implant contact (BIC) and bone area fraction occupancy (BAFO) parameters quantified using software Image Tool 3.0 (San Antonio Dental School, University of Texas Health Science, TX, USA). The regions of bone-to-implant contact (BIC) along the implant perimeter were subtracted from the total implant perimeter and the calculations were performed to determine the BIC. For bone area fraction occupancy (BAFO), firstly was obtained the total area of threads and the area occupied by space or no-bone, and after was determine the percentage of total area of threads occupied by bone tissue.

Statistical Analysis

The BIC, BAFO and ISQ data were tested for normal distribution (Kolmogorov-Smirnov) and equality of variances (Levene's test), followed by parametric statistical tests. All data were analyzed by two-way ANOVA (Implant surface and period of evaluation) followed by Tukey's test. Pearson's correlations test was used to verify the correlation between BIC and BAFO values. All statistical analyses were carried out with the statistical package Sigma Plot version 13.1 (Systat Software, Inc., San Jose, CA, USA) using a significance level of α=0.05.

Results

Histomorphometric Values

Two-way ANOVA showed no significant effect of implant (p=0.699), period of evaluation (p= 0.10) or the interaction between implant and period of evaluation (p=0.542). For Acqua implant the mean BIC after two weeks was 56.6±16.6% and after 4 weeks was 71.2±11.7%. For Neoporos implant the mean BIC after two weeks was 60.0±16.5% and after 4 weeks was 63.7±15.7%.

Two-way ANOVA showed no significant effect of implant (p=0.683), period of evaluation (p=0.653) and the interaction between implant and period of evaluation (p=0.436). For Acqua implant the mean BAFO after two weeks was 67.7±10.2% and after 4 weeks was 75.1±11.7%. For Neoporos implant the mean BAFO after two weeks was 69.7±19.5% and after 4 weeks was 68.6±8.1%.

RFA Values

Means and standard deviation values of implant stability quotient for animals sacrificed after 2 weeks are shown on Figure 1A. Two-way ANOVA showed significant effect for period of evaluation (p<0.001), however no significance was found for implant (p=0.827), or for the interaction between implant and period of evaluation (p=0.713). For Acqua implant the mean IQF values measured immediately was 51.9±10.8 N/cm and after 2 weeks was 73.6±13.5 N/cm. For Neoporos implant the mean IQF values measured immediately was 52.7±13.2 N/cm and after 2 weeks was 70.5±13.0 N/cm.

Means and standard deviation values of implant stability quotient for animals sacrificed after 4 weeks are shown on Figure 1B. Two-way ANOVA showed significant effect for period of evaluation (p=0.001), however no significance was found for the implant (p=0.118), or the interaction between implant and period of evaluation (p=0.745). For Acqua implant the mean IQF values measured immediately was 51.9±7.1 N/cm and after 4 weeks was 65.0±5.7 N/cm. For Neoporos implant the mean IQF values measured immediately was 57.3±10.3 N/cm and after 4 weeks was 68.3±3.0 N/cm.

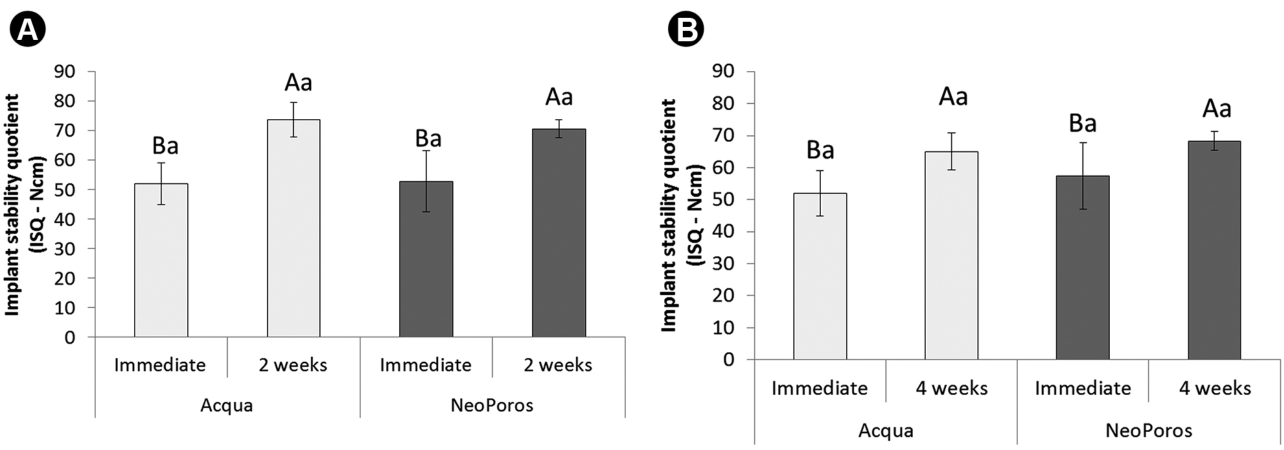

Figure 1. A: Implant stability quotient values for 2 weeks. Different letters represent significant difference, uppercase letter for periods of evaluation and lower case letters for implant type comparison; B: Implant stability quotient values for 4 weeks. Different letters represent significant difference, uppercase letter for periods of evaluation and lower case letters for implant type comparison.

Correlations

The Pearson correlations between different parameters are shown on Figure 2A-C. There was statistically significant correlation between BIC values and BAFO values (Pearson correlation coefficient: 0.541, p=0.009). Individual BIC values and BAFO values had no significant correlations with ISQ values (BIC: Pearson correlation coefficient: 0.0914, p=0.686; BAFO: Pearson correlation coefficient: 0.329, P=0.135).

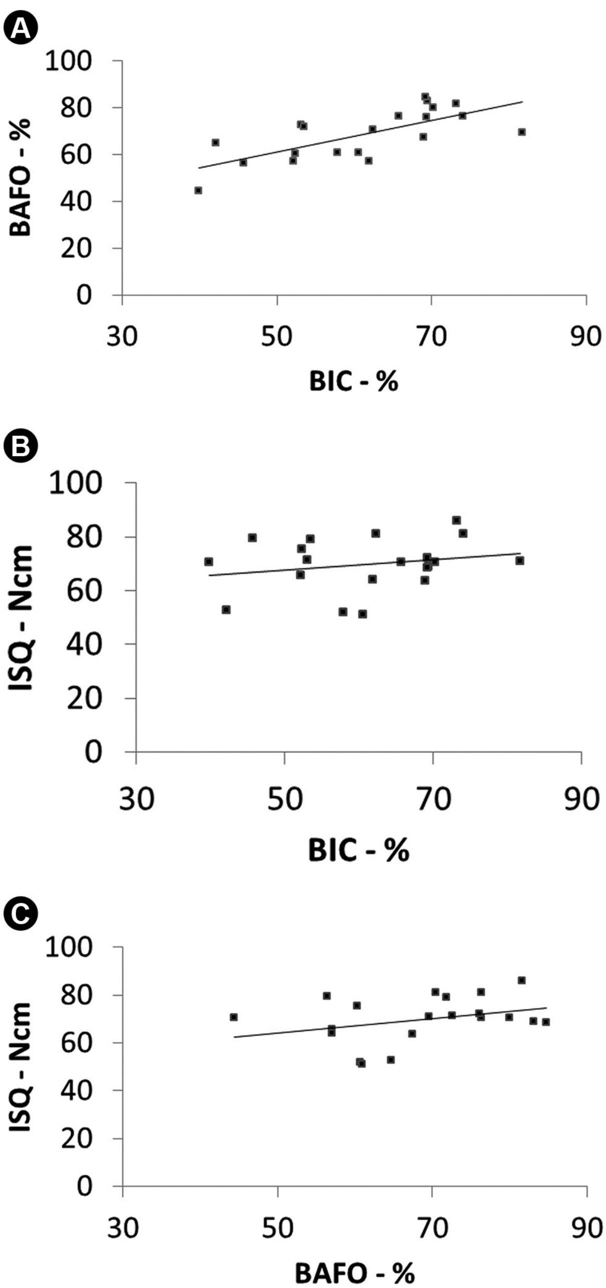

Figure 2. A: Correlation of BIC and BAFO values; B: correlation of BIC and ISQ values; C: correlation of BAFO and ISQ values.

Histological Observations

Qualitative microscopic evaluation demonstrated new bone formation, visible as blue stain, adjacent to the implant surface in all of the samples. The threads were tightly lodged in surrounding cortical bone. After 2 weeks (Figs. 3A and 4A), new bone matrix was interposed between the implants and bone walls indicating contact osteogenesis. There were no signs of massive resorption. After 4 weeks (Figs. 3B and 4B), both implants surfaces were surrounded by newly formed bone with trabeculae of immature bone, increasing in thickness of the cortical bone in contact with the implant and more resorption e substitution.

Discussion

It was the aim of this animal study in rabbits to compare the osseointegration performance of two microrough commercially implants at 2 and 4 weeks after installation of dental implants with an identical shape and geometry. The implants were evaluated histologically by means BIC and BAFO and RFA. The null hypothesis was accepted once the implant surface modification employed on Acqua implants has no effect of histomorphometric parameters.

BIC and BAFO are long established measures for osseointegration in scientific literature (7,8,10,11,16-20). BAFO reflects the bone occupancy rate, which can be filled by newly formed bone via distance osteogenesis or contact osteogenesis, such as for bone fragments compressed between bone wall. BIC shows new bone formation in contact with implant surface, which has been related to contact osteogenesis. However, the proportion of BIC depends on a number of factors including surgical technique, site of implantation, time and implant design. The present study was delineated to minimize the effect of these variables, as the effect of surface energy/wettability was the focus.

Several *in vitro* studies have demonstrated that hydrophilic surfaces tend to enhance osteoblast adhesion, proliferation, differentiation and bone mineralization compared to hydrophobic surfaces (5,13). In vivo studies have also been demonstrated that higher hydrophilicity surface correlates positively with faster osteogenesis (7,16). However, despite the greater hydrophilicity presented by surface Aq compared to Np surface (17), the BIC values were not significantly different between the two groups, and did not vary as a function of period of evaluation. These findings differ from other studies in which hydrophobic surfaces and highly hydrophilic surfaces were compared (12,16). Those studies compared implants with surface SLA and SLActive, which resemble the surfaces tested regarding the roughness and wettability. Similarly to implants SLActive, the surface Aq was obtained by sandblasted and acid-etched treatment followed by storage in ampules containing isotonic NaCl solution (17). The submersion of the implant in isotonic solution appears to protect the Ti surface from atmospheric contamination, thus preserving a chemically reactive surface (21). X-ray photoelectron microscopic analysis showed a lower carbon concentration and high oxygen values on both SLActive (7,10,12) and Aq surfaces (17), promoting a super-hydrophilic surface. Data from previous researches confirm that contact angle of SLActive (12) and Aq (17) are similar, with values <5º. Despite of the similarities between Aq and SLActive surfaces, differences in BAFO and BIC parameters compared to the studies using SLActive may be related to experimental design.

The implant design, the healing chamber dimensions and type of bone (cortical or trabecular) exert strong effect on osseointegration over time (20). It is recognized that drilling protocol (oversized, intermediate or undersized)

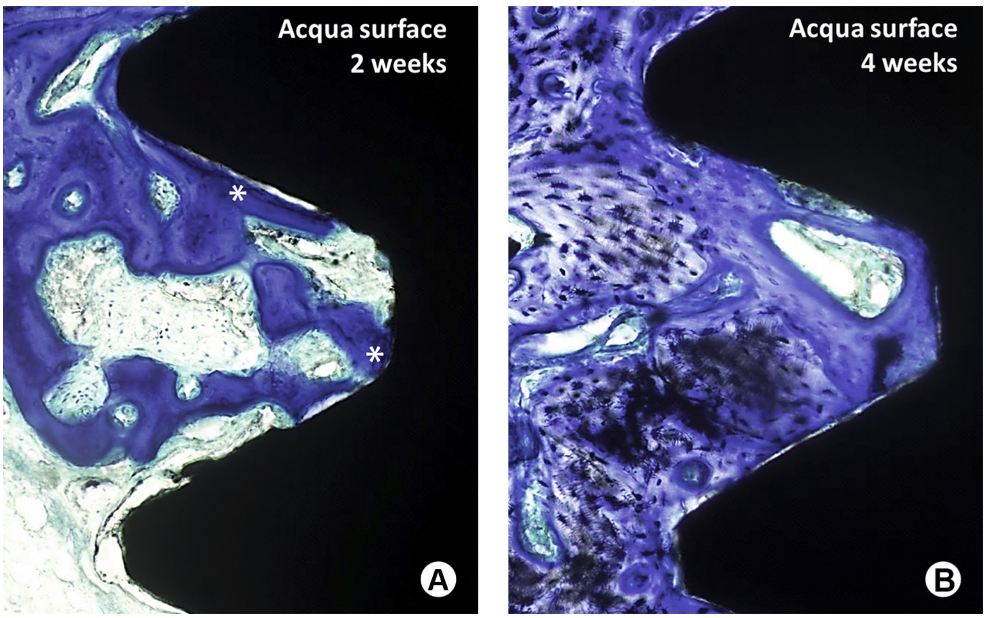

Figure 3. Sections of Acqua Ti implants and the surrounding tissue; A: after 2 weeks; B: after 4 weeks. At 2 weeks, thin layer of newly formed bone (*). At 4 weeks, similar conditions as those of 2 weeks were observed, with active remodeling of old bone structures.

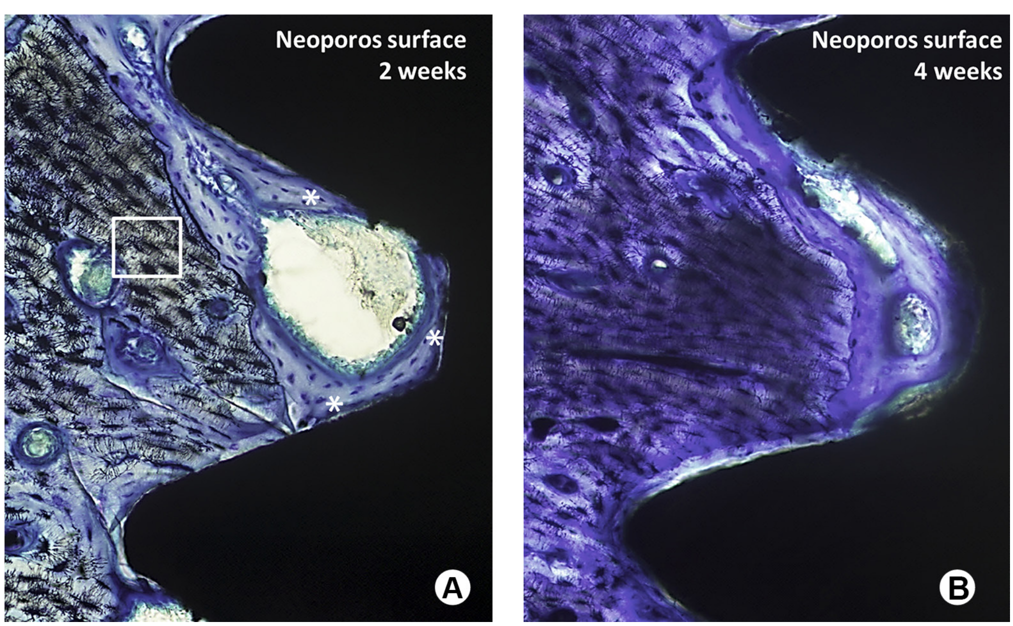

Figure 4. Sections of Neoporos Ti implants and the surrounding tissue; A: after 2 weeks; B: after 4 weeks. At 2 weeks, thin layer of newly formed bone (*), which in some areas was connected to trabecular of lamellar bone (square) in intimate contact with both surface. At 4 weeks, were observed similar conditions as 2 weeks, with active remodeling of old bone structures.

result in different biological responses with higher BIC and BAFO values for intermediate drilling (22). This fact seems to be related to the blood's clot ability to fill the space between the bone wall and the implant threads which facilitates intramembranous like bone formation at bone interface (20). The current study did not created healing chamber, generating a press-fit condition in the bony walls with a little space between the implant and bone. It is possible to speculate that implant macrogeometry and insertion technique had reflected in the lack of differences in BIC and BAFO values between for Neo and Aq surfaces. Furthermore, old bone should be resorbed before new bone formation in areas of close contact between bone and implant surface (16). This assumption may explain why the present findings agree partially with Sartoretto et al. (17). Those authors also observed no statistically significant differences in the BIC values and BAFO between the Aq and Np at 14 days, although they have found in the 28-day period. In the same way as presented in this study, the authors had no detected increasing of the percentage of BAFO along the time. It is also important to note that such study did not specify in which region BIC and BAFO analyses were performed; if they were on the cortical or medullar, or on both, since this factor may impair on the outcome. These findings corroborate the positive effects of a highly hydrophilic surface, such as Aq, may have been minimized by the implant insertion conditions.

The present study also evaluated mechanical implant stability by RFA method. ISQ values obtained in RFA analysis allow measuring the primary and secondary stability (19). Primary stability measured immediately after implant installation have been related to a tight-fitting between the implant surface and marginal or apical bone. On the other hand, secondary stability is consequence of new bone formation and remodeling process (19,23), which was evaluated on 14 and 28 days post implant installation. Considering that Aq and Neo possess the same macrogeometry and were installed on the same region of tibia, was expected a lack of difference between the groups for primary stability. This lack of differences in ISQ values between the groups Aq and Np was maintained for all experimental periods. Though some studies have shown that secondary stability is correlated to the surface properties of dental implants (19), the present findings did not support this theory. Other factors, such as strong bone anchorage (18,24), stiffness of the surrounding bone (2,18,19), type of implant used and surgical technique (18,25) may support the current results. Nevertheless, the increases in RFA values that occur during implant healing and have been attributed to increased bone anchorage cannot be explained by histomorphometric data (18,25). As observed in this study, no correlations between histomorphometric parameters of osseointegration and ISQ values could be identified by other authors (2,18,24,25). Considering that histological sections are two-dimensional images, and do not represent the entire implant-bone contact around the implant, and also that sections does not indicate the mechanical strength, it is not surprising the lack of correlation between these parameters (25). The authors recognized a limitation of this study, since it was not tested either the implant surface on cortical bone under superinstrumentation conditions during implant installation. Under these conditions, the Aq surface tends to induce more bone neoformation, due the superhidrophilic of Aq surface.

In conclusion, morse taper implants junction installed in cortical bone with same roughness but opposed wettability characteristics did not result in differences in new bone formation or implant stability on initial periods, indicating that in this bone site the chemical alterations on implant surface had no effect on short period of implant bone integration. Both implant surfaces, Aq and Neo, were able to produce similar implant bone integration when normal cortical bone instrumentation was performed.

Resumo

O objetivo deste estudo foi avaliar a estabilidade e osseointegração de implantes com superfícies com diferentes molhabilidades empregando análise de frequência de ressonância (RFA) e histomorfometria (contato implante ósseo, BIC, e fração de área ocupada osso, BAFO), nos períodos de 2 e 4 semanas em tíbias de coelhos. Trinta e dois implantes cone Morse (comprimento 7mm, diâmetro 3,5 mm), foram divididos de acordo com tratamento de superfície (n = 8): Neo, superfície jateada e condicionada com ácido; e Aq, superfície jateada e condicionada com ácido e mantida em solução isotônica de cloreto de sódio a 0,9%. Dezesseis coelhos tipo Nova Zelândia foram utilizados neste estudo. Dois implantes de cada grupo foram instalados nas tíbias direita e esquerda de acordo com os períodos experimentais. Os valores de RFA (Ostell®) obtidos imediatamente e após o sacrifício (2 e 4 semanas). Os blocos ósseos / implante foram processados para análise histomorfométrica. Os dados foram analisados usando ANOVA fatorial seguido pelo teste de Tukey e também por meio de correlação de Pearson para os fatores RFA, BIC e BAFO (P=0,05). Nenhum efeito significativo dos fatores implante, período de avaliação e da interação entre o implante e período de avaliação foram observados para os valores de BIC e BAFO. Apenas o período de avaliação resultou em efeito significativo para valores RFA após 2 semanas (p=0,001), e 4 semanas (p<0,001). Os valores de RFA valores foram significativamente mais elevados no final do período de avaliação em comparação com os obtidos em inicialmente. Houve correlação significativa entre os valores BIC e BAFO (p=0,009). Ambas as superfícies de implantes, Aq e Neo, são capazes de produzir adequada integração osso/ implante em condição normal de instrumentação do osso cortical.

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CAPÍTULOS

3.3 CAPÍTULO 3

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Effect of after-therapy interval of ionizing radiation on bone: histomorphometric and biomechanical analysis

Priscilla Barbosa Ferreira Soares[1], Carlos José Soares[1], Rainde Naiara Rezende de Jesus[1], Paula Dechichi[2], Rubens Spin-Neto[3], Darceny Zanetta-Barbosa[1]

[1] School of Dentistry, Federal University of Uberlândia, Uberlândia, MG, Brazil
[2]Institute of Biomedical Sciences, Federal University of Uberlândia, Uberlândia, MG, Brazil
[3]School of Dentistry, Aarhus University, Denmark.

Corresponding author:
Darceny Zanetta-Barbosa, DDS, MSc, PhD,
Avenida Pará s/n°, Campus Umuarama, Bloco 4T, Departamento de Cirurgia e Traumatologia Buco-Maxilo-Facial, Bairro Umuarama, Uberlândia, Minas Gerais, Brazil, CEP: 38.400–902, Fax and Telephone: 55-34-3218-2636, E-mail: darceny_@hotmail.com

Effect of after-therapy interval of ionizing radiation on bone: histomorphometric and biomechanical analysis

Priscilla Barbosa Ferreira Soares[1], Carlos José Soares[1], Rainde Naiara Rezende de Jesus[1], Paula Dechichi[2], Rubens Spin-Neto[3], Darceny Zanetta-Barbosa[1]

[1] School of Dentistry, Federal University of Uberlândia, Uberlândia, MG, Brazil
[2]Institute of Biomedical Sciences, Federal University of Uberlândia, Uberlândia, MG, Brazil
[3]School of Dentistry, Aarhus University, Denmark.

Running title: Radiation effect on bone.

Keywords: ionizing radiation; bone; biomechanics; histomorphometric; micro-computed tomography.

Corresponding author:
Darceny Zanetta-Barbosa, DDS, MSc, PhD,
Avenida Pará s/n°, Campus Umuarama, Bloco 4T, Departamento de Cirurgia e Traumatologia Buco-Maxilo-Facial, Bairro Umuarama, Uberlândia, Minas Gerais, Brazil, CEP: 38.400–902, Fax and Telephone: 55-34-3218-2636, E-mail: darceny_@hotmail.com

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Abstract

Objective: Radiotherapy may damage the bone tissue, which impair the bone formation resulting in significant biomechanical alterations. This study aimed to evaluate the radiotherapy effects on biomechanical, histomorphometric and microstructural parameters of bone, in different periods compared with intact bone tissue. **Materials and Methods:** Eighteen adults male New Zealand rabbits were treated with radiotherapy (single dose of 30Gy). The animals were divided into 6 groups: GNoIr, control group- no radiation; and 5 irradiated groups sacrificed after: GIr24h 24 hours; GIr7d, 7 days; GIr14d, 14 days; GIr21d, 21 days; GIr28d, and after 28 days. After these periods, the animals were sacrificed and the tibias (n = 6) were tested using three-point bending test to calculate: ultimate force, work to failure and stiffness. Dynamic indentation test were used to quantify Vickers hardness and elasticity modulus. microCT was used to analyze the cortical volume (CtV), cortical thickness (CtTh) and porosity (Ct.Po). Data were analyzed by one-way ANOVA, Kruskall-Wallis \followed by Tukey, Dunnet and Dunns tests (P<.05). **Results:** The force, work to failure, stiffness, elastic modulus and Vickers hardness values of irradiated bone were significantly lower that non-irradiated bone. The irradiated bone had significantly lower CtTh and CtV values and higher CtPo than non-irradiated bone. No significant difference was found for lacunarity between non-irradiated bone and irradiated bone. **Conclusion:** Ionizing radiation had negative effect on mechanical and micro-architecture parameters. The damage on bone tissue is more significantly observed after 14 to 21 days after radiotherapy.

Introduction

Head and neck cancer accounts for more than half a million cases annually worldwide [1]. Male subjects are affected significantly more than females with an average ratio of 3:1. In males, the incidence rate exceeds 0.2 per 1000 in regions such as Central, Southern, and Eastern Europe, Brazil, and among African Americans in the Unites States [1]. Mouth and tongue cancers are more common in the Indian subcontinent, nasopharyngeal cancer is more common in Hong Kong, and pharyngeal and/or laryngeal cancers are more common in other populations [2].

In the last many decades, radiotherapy has become the preferred treatment, or at least an essential part of a comprehensive treatment plan, for something close to 70% of patients with malignant tumors [3]. Radiotherapy not only can cure cancer, but also relieve symptoms and improve quality of life. However, radiotherapy kills not only cancer cells, but also damages normal cells and affects their function [3]. Radiation possesses adverse effects on bone causing injures in the small blood vessels with persistent hypoxia reducing the quantity and activity of osteoblasts and osteocytes [4,5]. The undesirable effect of radiotherapy conduces to the hypocellularity, hypovascularization and bone hypoxia [3-6], which may increase the occurrence of osteoradionecrosis [7].

There are several mechanisms involved in that function breakdown. Radiation reduces cellular activity, blood supply, and partial oxygen pressure in bone, compromising its quality and quantity [3]. In the eighties, a model which became known as the “3H concept”, proposed the mechanism of irradiation damage on bone was due to endarteritis obliterans followed hypoxia, hypocellularity, and hypovascularity [8]. Another important factor to be considered is that radiation compromises bone regeneration in a dose-dependent manner [6].

One of the first signs of radiation-related side effects in the maxilla-facial bones is osteoradionecrosis or post-radiation osteomyelitis [9]. Despite the considerable decrease in the last 50 years, the risk of osteoradionecrosis still fluctuates between 5% and 9%, being a considerable therapeutic problem [10]. However, reports on the effects of radiotherapy on bone strength, density, or bone-mineral content are more variable [11,12], and one could speculate that further studies, especially considering diverse phases of bone development, are needed.

An experimental model which could be used to provide better information on the bone-cells / radiation therapy interaction is the rabbit tibiae model. This is a very well established model for assessing bone biological and biomechanical characteristics, which would be impaired after large radiation doses from radiation therapy [13,14]. This model brings insight not only regarding bone healing, but also on the integration of implants, which will also be affected by radiation [6,15,16]. To the present, not many studies on the relevance of the time after radiation therapy on bone healing can be found in the literature. Therefore, the aim of this study was to evaluate the effects of ionizing radiation on bone tissue in diverse periods, by means of histomorphometric and biomechanical analysis, testing the null hypotheses that there is no effect of the radiotherapy on bone tissue and, that the period after radiation therapy would not impair on bone-tissue biological and biomechanical properties.

Material & Methods

Animal care

Eighteen adults' male New Zealand White rabbits, weighting 3.0–3.5 kg, were used in this study, assigned to 6 groups with three animals in each group. The animal experiment protocol was approved by Bioethics committee for animal experimentation (CEUA # 093/12) at the Universidade Federal de Uberlândia, Brazil and followed the ethical code for animal experimentation of the Council for International Organization of Medical Sciences. All animals were maintained with food and water ad libitum. The animals were randomly divided into 6 groups containing 3 rabbits that results on 6 tibiae each (n = 6): GNo, without radiation; GIr24h, radiation and sacrificed 24 hours after; GIr7d, radiation and sacrificed 7 days after; GIr14d, radiation and sacrificed 14 days after; GIr21d, radiation and sacrificed 21 days after; GIr28d, radiation and sacrificed 28 days after.

Radiation

During the sessions the animals (Groups Ir) were under general anesthesia with an intramuscular injection of a combination of 0.25 mg of ketamine/kg of body weight (Ketamina Agener®; Agener União, São Paulo, SP, Brazil) and 0.5 mg of xylazine/kg of body weight (Rompum® Bayer. São Paulo, SP, Brazil). The left and right hind legs of each rabbit were subjected to a single dose of 30Gy irradiation. A dose of irradiation was delivered with a source–skin distance of 60 cm and the field of size was 15 x 15 with direct electron beam of 9 MeV electron (Varian 600-C® Varian Medical Systems, Palo Alto, California, EUA). The dose rate was 400 cgy/min. After radiation, the skin, hair, weight, and appetite of the rabbits were closely monitored. The application of 30Gy caused a significant reduction of the bone regeneration capacity but acute effects that were well supported by the animal. When a single dose of 30Gy was compared to clinical practice it would correspond to approximately 50-70Gy applied in fractioned radiotherapy [3,6,10].

The animals were sacrificed by an overdose of anesthetic after 24 hours, 7, 14, 21, and 28 days after radiation. The overlying soft tissues were removed and the tibias were stored in phosphate buffered saline solution and frozen at -20 °C in plastic tubes.

Mechanical testing

The samples were stored frozen at -20 °C for 48 h. Each tibia was subjected to a three-point bending test until failure, using a materials testing machine (EMIC DL 2000, EMIC Equipamentos e Sistemas de Ensaio Ltda, Sao José dos Pinhais, Brazil). Each specimen was positioned horizontally on the two holding fixtures of the machine, with the tibial tuberosity facing outwards, while the upper loading fixture applied the load from lateral to medial at a loading rate of 1.0 mm/min (Fig 1.A). The span length between holding fixtures was 40 mm and the load was applied at the center of the lengthened area. Load and displacement data were recorded and subsequently, load vs. displacement curves were plotted. Maximum load values were derived from data (Fmax; N), work to failure (mJ) and stiffness values (S; N/mm) were calculated as the slope of the initial linear uploading portion of the curves. After the three-point bending test, the portion of the tibia diaphysis was sectioned with a diamond disk with constant irrigation for obtain two bone segments of 6 mm.

Dynamic indentation test

The samples preparations and experimental protocol were performed as described previously by Soares et al. [19]. The bone sample was embedded into polyester resin (Instrumental Instrumentos de Medição Ltda, São Paulo, SP, Brazil). To perform the sample was positioned with the cutting implant surface downward over a glass plate and fixed using adhesive (Single Bond 2, 3M-Espe, St. Paul, MN, USA). The adhesive system was applied in a thin layer on the implant surface and the sample was stabilized using digital pressure, followed by light activation for 20 seconds (Demetron, Kerr, Orange, CA, USA). Three samples were embedded per block. The metallic tube (Metalon Indústrias Reunidas, Nova Iguaçu, RJ, Brazil) 50 mm in length, 30 mm in width and 10 mm in high was positioned around the sample and was fixed with wax (Wilson, Polidental, Cotia, Brazil). After curing of the polyester resin the surfaces were finished using silicon-carbide papers (#600, 800, 1200, 2000; Norton, Campinas, SP, Brazil) and polished with metallographic diamond pastes (6, 3, 1, ¼ μm; Arotec, São Paulo, SP, Brazil). The metallic tube/implant was washed in an ultrasound (Cristofoli, Campo Mourão, PR, Brazil) with absolute alcohol for 3 times of 7 minutes to remove the debris. Elastic modulus (E) and Vicker's hardness (VHN) of the bone was assessed by using a microhardness dynamic indenter (CSM Micro-Hardness Tester; CSM Instruments, Peseux, Switzerland), made five continuous indentations were distancing 0.08 mm between each other (Fig 2.A). The indentation was carried out with controlled force, whereby the test load was increased or decreased at a constant speed ranging between 0 and 200 mN in 60-second intervals. The maximum force of 200 mN was held for five seconds. The load and the penetration depth of the indenter were continuously measured during the load-unload hysteresis. The universal hardness is defined as the applied force divided by the apparent area of the indentation at the maximum force. The measurements were expressed in VH units by applying the conversion factor supplied by the manufacturer. The indentation modulus was calculated from the slope of the tangent of the indentation depth curve at the maximum force and is comparable to the E of the material.

MicroCT evaluation

For assessment of the bone micro-architecture in the experimental groups, the tibia diaphysis was examined using a desktop microCT system, commercially available as SkyScan 1272 (Bruker, Kontich, Belgium). During scanning, the tibia was placed in the polyethylene tube and immobilized inside the tubes by means of soft modeling clay.

The scanning parameters were 15 μm pixel size, 50 kV X-ray voltage, 160 mA electric current and 0.5 mm Al filter. Subsequently, the reconstructed 3D data sets were quantified using CTAn automated image analysis system, Fig 3.A (Bruker, Kontich, Belgium). For this, the volume of interest (VOI) for cortical analyses was selected and extending totally 300 slices. Cortical architecture was assessed in the diaphysis and was characterized by cortical volume (CtV), cortical thickness (CtTh) and porosity (Ct.Po) according to standard procedures [17].

Histomorphometric analysis - lacunarity

After microCT analyses, the bone was decalcified in 10% formic acid solution for 15 days. Then, decalcified specimens were processed and embedded in paraffin wax. The specimens were sectioned longitudinally along the axial plane and embedded in paraffin. From the central region of the bone, six non-serial thin sections with 5 µm of thickness were obtained and stained with hematoxilin and eosin.

Eighteen histological images of bone per group were captured at ×10 magnification, using a binocular microscope Nikon Eclipse E2000 (Nikon, São Paulo, Brazil) coupled with camera Moticam Pro 252B and the software Motic Live Imaging Module (Motic®, British Columbia, Canada). The screen shots, Fig. 4.A, were merged and the soft tissue erased using Photoshop CS6 software (Adobe®, Adobe System Inc., San Jose, CA, USA). Each image was analyzed separately; the bone channels were segmented and called regions of interest (ROI) Fig. 4.B, and finally this regions were converted to binary images with HL Image 2005++, Fig 4.C (Western Vision, Salt Lake City, UT, USA). The ROI features were analyzed by Lacunarity. The lacunarity is able to characterize the texture of images, with characterization of the spatial organization of an image, including the average size of any structural subunit(s) within an image [18].

Statistical Analysis

The maximum force (Fmax), work to failure (Wf) stiffness values (S), cortical volume (CtV), cortical thickness (CtTh), porosity (Ct.Po), lacunarity, elastic modulus (E), Vickers hardness (VHN) data were tested for normal distribution (Shapiro-Wilk) and equality of variances (Levene’s test), followed by parametric statistical tests. One-way analysis of variance (ANOVA) was performed for all data. Multiple comparisons were made using Tukey's test for comparison between irradiated groups on different periods and Dunnet test was used for comparison between control group and irradiated groups. All tests employed $\alpha=0.05$ significance level and all analyses were carried out with the statistical package Sigma Plot version 13.1 (Systat Software Inc, San Jose, CA, USA).

Results

Three bending flexural test – force, work to failure and stiffness.

The force (N), work to failure (mJ) and stiffness (N/mm) mean and standard deviation values for the non-irradiated and irradiated bone structure from animals sacrificed after different periods are shown in Fig. 1.B, 1.C and 1.D. Irradiated bone tended to reduce the force, work to failure and stiffness compared with non-irradiated bone, with significant difference observed for animals sacrificed after 14 days for Force (N) and 21 days for work to failure (mJ) and stiffness (N/mm) compared with control group. The force, work to failure and stiffness values of irradiated bone structure of animals sacrificed after 21 and 28 days were significantly lower than the values obtained from animals sacrificed after 24h.

Dynamic microindentation - Elastic Modulus and Vickers Hardness

The E values in GPa for the non-irradiated and irradiated bone structure from animals sacrificed after different periods are shown in Fig.2C. Irradiated bone tended to reduce the E values compared with control group, however the significant difference was observed for animals sacrificed after 14 days. The E values of irradiated bone structure of animals sacrificed after 21 and 28 days were significantly lower than the values obtained from animals sacrificed after 24h.

The VHN values in N/mm2 for the non-irradiated and irradiated bone structure from animals sacrificed after different periods are shown in Fig.2D. Irradiated bone tended to reduce the VHN values compared with non-irradiated bone, with significant difference observed for animals sacrificed after 7, 14, 21 and 28 days compared with control group. The VHN values of irradiated bone structure of animals sacrificed after 21 and 28 days were significantly lower than the values obtained from animals sacrificed after 24h.

MicroCT analysis – morphologic parameters

The mean values and standard deviation of CtV cortical volume (mm^3), CtTh, cortical thickness (mm) and Ct.Po bone porosity (%) are shown on Fig. 3.B, 3.C and 3.D respectively. Irradiated bone tended to reduce the CtV values compared with control group, however the significant difference was observed for animals sacrificed after 21 days. No difference was observed of CtV values among the irradiated bone structure irrespective of period of sacrificing. Irradiated bone tended to reduce the CtTh values compared with control group, however the significant difference was observed for animals sacrificed after 21 days. The CtTh values of irradiated bone structure of animals sacrificed after 21 and 28 days were significantly lower than the values obtained from animals sacrificed after 24h. Irradiated bone tended to increase the CtPo values compared with control group, however the significant difference was observed for animals sacrificed after 14 days. The CtPo values of irradiated bone structure of animals sacrificed after 28 days were significantly higher than the values obtained from animals sacrificed after 24h.

Lacunarity

The lacunarity mean and standard deviation values for the non-irradiated and irradiated bone structure from animals sacrificed after different periods are shown in Fig. 4.D. No significant difference was found for lacunarity between non-irradiated bone and irradiated bone irrespective of period of sacrificing.

Discussion

In the present study, we speculated that ionizing radiation after different therapy intervals determine negative effects of diverse on bone tissue. Both the null tested hypotheses were rejected; the radiotherapy produced significantly deleterious effect on the biomechanical and morphometric parameters, which are more observed after same period after radiation therapy.

Radiotherapy, besides its extraordinary action to treat and cure head and cancer, causes damages functional cells, affecting their function [3]. As a slowly proliferating tissue, bone exhibits a relatively high resistance to ionizing radiation. However, high radiation doses such as those delivered for head-and-neck cancers have been reported to strongly increase the risk of osteoradionecrosis [21]. This effect on bone tissue can impair bone remodeling in significant levels, by reducing cellular activity, blood supply, and oxygen supply to the tissue [3]. This effect is organizing in three sequential events that happen in bone tissue following radiation: hypoxia, hypocellularity, and hypovascularity [8]. The deleterious effects of radiation on bone tissue depend on the time, dose per fractions, number of fractions and total dose [4,21]. Due to the difficulties inherent to animal models studies, the animals were exposed to a single dose radiation enough to cause inhibition of the bone regeneration [6]. This single dose was based on protocols reported by previous studies, which ranging between 25 Gy and 35 Gy [6,15].

The primary effect of radiation on bone is atrophy, which leads the reduction the number of functioning structural components in the tissue. This aspect starts with vascular changes, affecting the usual cells resident in the tissue, and finally the production and maintenance of the bone matrix [22]. Considering these changes to the level of the narrow Haversian channels will lead to the formation of sclerotic connective tissue within the marrow cavity [11]. At later periods, sub-intimal fibrosis and hyaline thickening of the media of the blood vessel can also lead to a narrowing of the vascular lumen, as it was already described more than half a century ago [23]. The reduction in the number of osteoblast cells following irradiation is also well described in the literature, and this is associated with decreased collagen production and alkaline phosphatase activity [24], leading to decreased bone-tissue mineralization and osteopenia [22]. This aspect can also explain the decreasing on mechanical parameters of bone tissue.

Instrumented indentation is a powerful method to measure mechanical properties of bone tissue at various structural levels [25,26]. Bone tissue present anisotropic ratio with direction-dependent hardness and elastic modulus values [26,27]. Therefore during sample preparation was observed carefully the same orientation during bone cutting and polishing process [26]. Elastic modulus and Vickers hardness account for the ability of bone tissue to resist elastic and plastic deformations, respectively [28]. The effect of irradiation on bone mechanical properties is not completely understood; however, we may theorized that irradiation most likely affects the collagen and its interaction with the mineral [29]. This structural degradation may explain the reduction on elastic modulus and Vickers hardness values. The alteration on elastic and plastic bone behavior, determined by proteinaceous phase of bone, reduce the viscoelasticity and crack resistant [29,30].

It is known that a strength and plastic behavior of the bone depends of the interaction between the collagen and mineral fraction [13]. In this regard, damage to the collagen matrix induced by irradiation can be extremely problematic to the biomechanical properties of bone, in particular through the formation of collagen cross-links. Loss in plastic deformation could be related to a suppression of the prevailing plasticity mechanism in bone of fibrillar sliding, which results principally from a change in the proportion of the three types of collagen cross-linking caused by the irradiation damage [14].

By following these mechanisms in which bone remodeling is impaired by radiation, it is clear that, due to its indirect action, the effects of ionizing radiation in the bone tissue should take place some period after the radiation exposure, rather than immediately. This is related to the time lapse between the moment in which the cellular and vascular alterations occur (immediately) and the first signs and symptoms of this impairment can be seen in terms of structural changes in bone tissue morphology and structure [11]. This is clear from the results of the present study, in which alterations in bone tissue structure and morphology were noticed only 14 days after the end of radiation therapy, and became more evident after 21 and 28 days, respectively. The present results corroborate those findings, with irradiated bone showing reduced force, work to failure, and stiffness compared with non-irradiated bone, especially considering the longer evaluation periods evaluated. These findings were also corroborated by the results of the microCT analysis, in which irradiated bone tended to reduce the CtV values compared with control group, for animals sacrificed after 21 days. The reduced E values found for the irradiated bone were also in agreement with this line of thinking, and took place only after 21 days. The same was seen for VHN, but this was already observed after 7 days, getting to a more visible scenario the longer the evaluation period. The only test which did not follow the expected tendencies was the lacunarity assay, since the non-irradiated and irradiated groups showed no differences. The fact that the results became more evident just after 21 days could be related not only to the progressive damage to the microcirculation, as suggested on the 3H concept, but also on the “timing” of the rabbit physiologic bone remodeling.

The mechanical alterations observed in this study may impair on several clinical situations such as bone defect repair on oral cavity and implant osseointegration [31]. The present findings showed that irradiated bone, impairs negatively on biomechanical and micromorphology parameters, this changes are normally seen after longer periods, such as 14 to 21 days, and not immediately after radiation therapy, suggesting that this is an indirect outcome which starts with vascular alterations.

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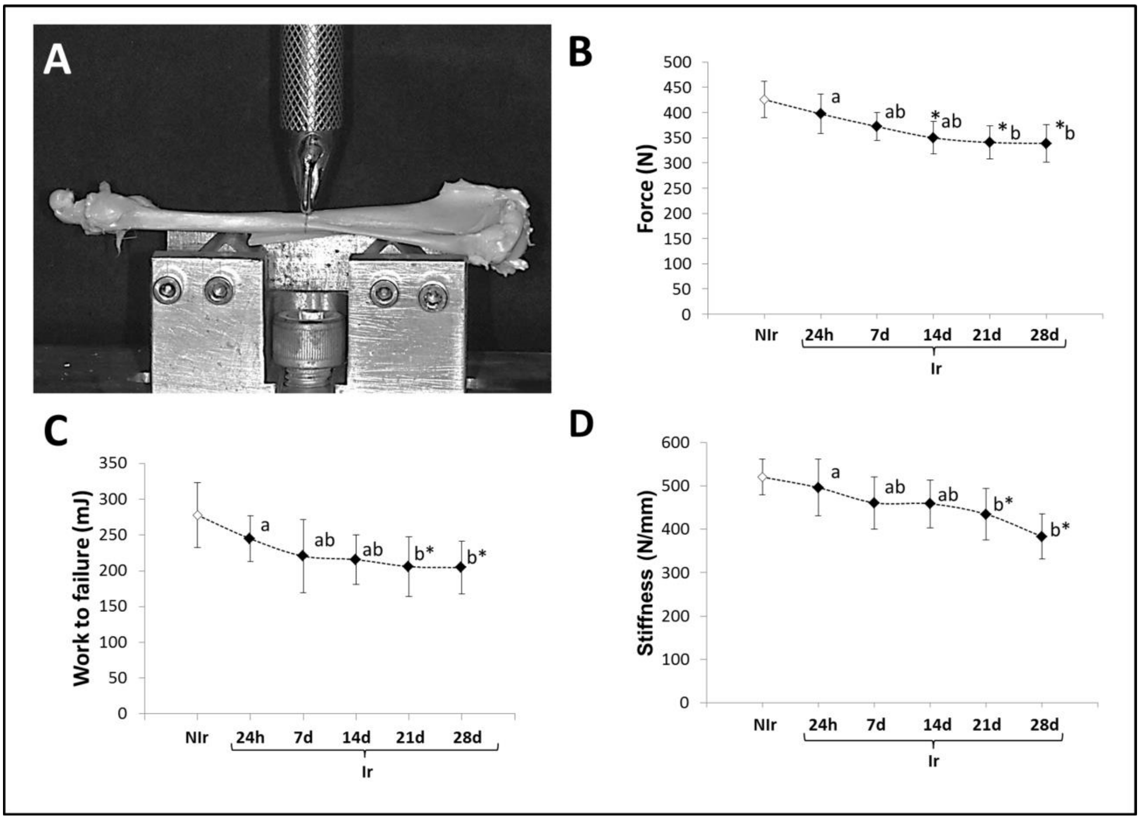

Fig. 1. A. Three-bending flexural test of rabbit tibia bone; B. Mean and standard deviation values mechanical parameters for non-irradiated bone (control group) and irradiated bone from animals sacrificed after different periods (24h, 7, 14, 21 and 28d): B. Force (N); C. work to failure (mJ); D. stiffness (N/mm2) analyzed by Tukey and Dunnet test ($P < 0.05$).

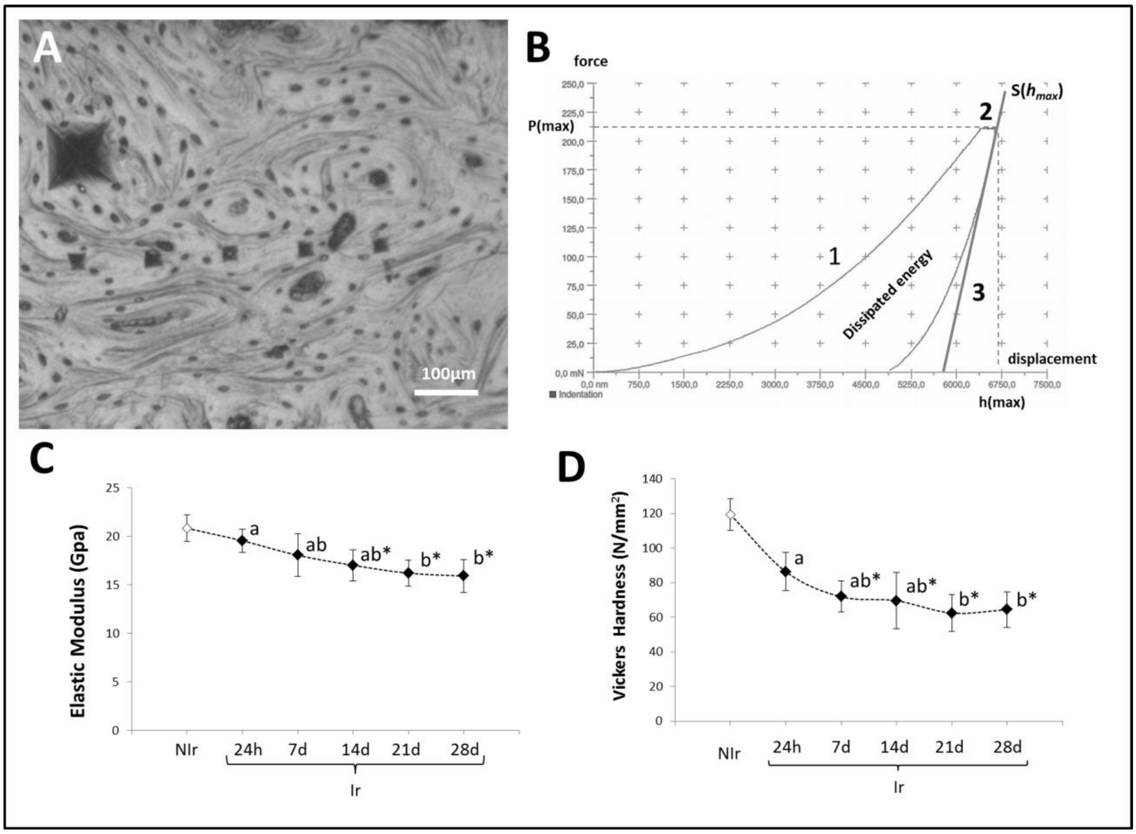

Fig. 2. A. Microindentation of bone structure, large indentation for calibration and 5 indentation for parameters calculation; B. Force-displacement curve of a microindentation test: loading (1), holding (2), unloading (3) of an indenter tip. The third part leads to elastic recovery of the material and its initial slope is used to derive the elastic indentation modulus. The hysteresis represents the dissipated energy; C. Mean and standard deviation values of mechanical parameters for non-irradiated bone (control group) and irradiated bone from animals sacrificed after different periods (24h, 7, 14, 21 and 28d); C. Elastic Modulus, (GPa); D. Vickers Hardness (N/mm2), analyzed by Tukey and Dunnet test (P < 0.05).

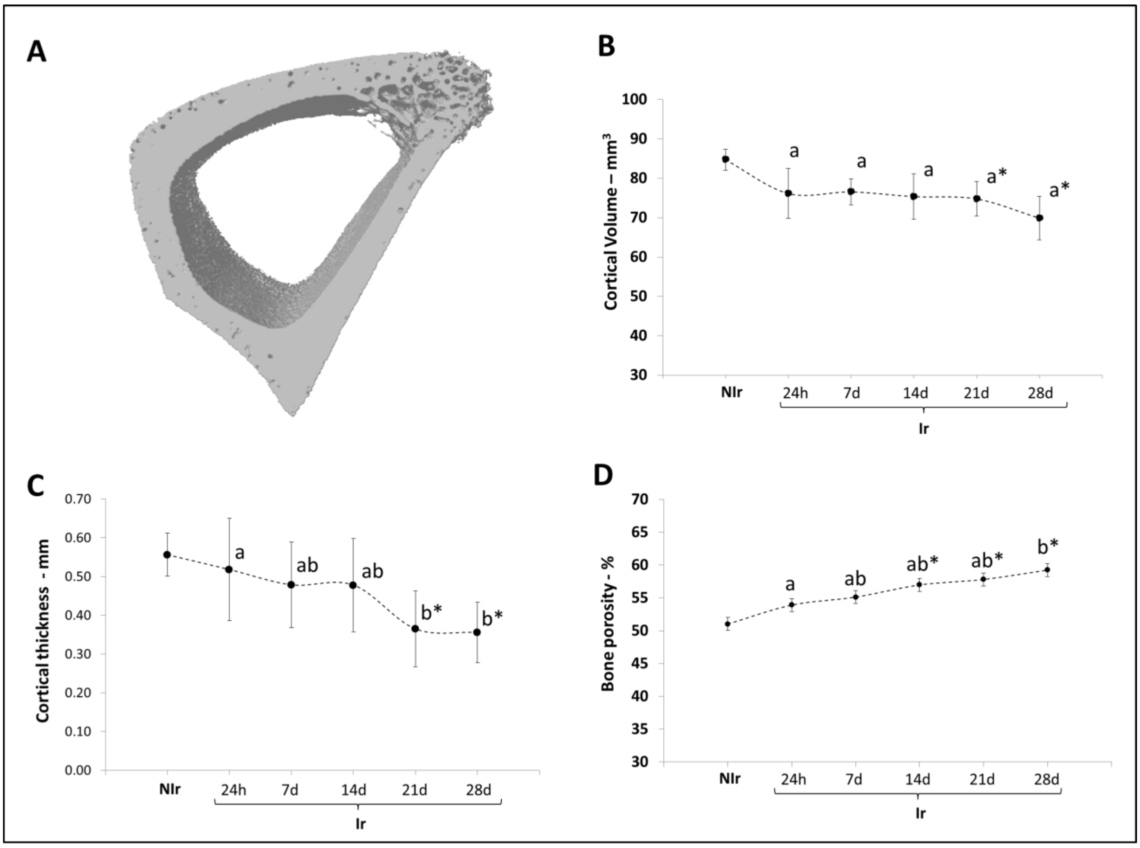

Fig. 3. A. microCT image of bone structure; B. Mean and standard deviation values of mujtorphologic parameters measured by microCT for non-irradiated bone (control group) and irradiated bone from animals sacrificed after different periods (24h, 7, 14, 21 and 28d): B. cortical volume – CtV in mm^3; C. cortical thickness – CtTh in mm; D. bone volume – CtPo in %, analyzed by Tukey and Dunnet test ($P < 0.05$).

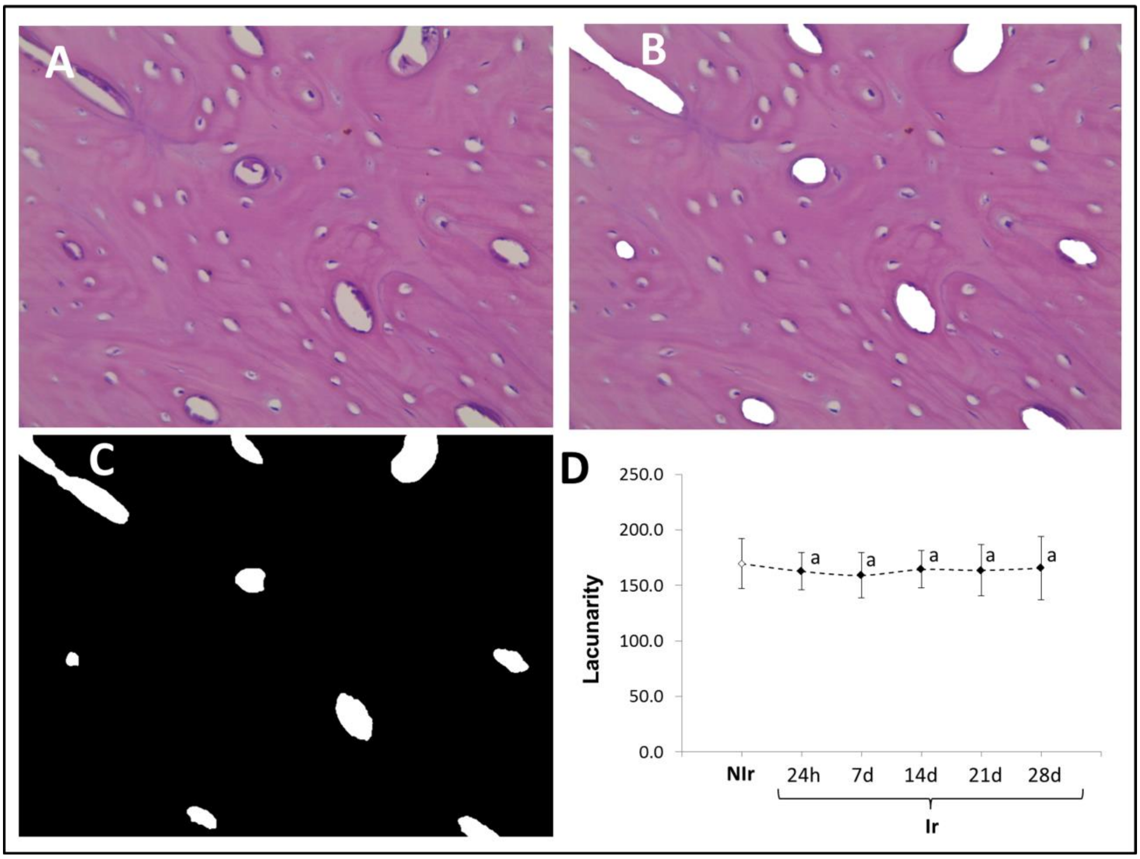

Fig. 4. A. Fig. 1. Photomicrograph of the specimen illustrating the steps of the methodology applied (HE - Original Magnification 100). A: Digitalized histological image; B: Histological image with segmented bone channels (ROI); C: Binary image obtained through algorithms developed in the SCILAB mathematical environment. Bone channels were called region of interest (ROI); D. Mean and standard deviation values of lacunarity values for non-irradiated bone (control group) and irradiated bone from animals sacrificed after different periods (24h, 7, 14, 21 and 28d) analyzed ANOVA one-way.

CAPÍTULOS

3.4 CAPÍTULO 4

Artigo a ser enviado para publicação no periódico Clinical Oral Implants Research

Mechanical and morphological alterations produced by ionizing radiation on bone- dental implant interaction

Priscilla Barbosa Ferreira Soares[1], Carlos José Soares[1], Vitor Carvalho Lara[2], Paula Dechichi[1], Camilla Christian Gomes Moura[1], Darceny Zanetta-Barbosa[1]

[1] School of Dentistry, Federal University of Uberlândia, Uberlândia, MG, Brazil

[2] School of Medicine – Federal University of Triângulo Mineiro, Uberaba, MG, Brazil

Corresponding author:

Darceny Zanetta-Barbosa, DDS, MSc, PhD,

Avenida Pará s/n°, Campus Umuarama, Bloco 4T,

Departamento de Cirurgia e Traumatologia Buco-Maxilo-Facial, Bairro Umuarama, Uberlândia, Minas Gerais, Brazil, CEP: 38.400–902,

Fax and Telephone: 55-34-3218-2636,

E-mail: darceny_@hotmail.com

Mechanical and morphological alterations produced by ionizing radiation on bone- dental implant interaction

Priscilla Barbosa Ferreira Soares[1], Carlos José Soares[1], Vitor Carvalho Lara[2], Paula Dechichi[1], Camilla Christian Gomes Moura[1], Darceny Zanetta-Barbosa[1]
[1] School of Dentistry, Federal University of Uberlândia, Uberlândia, MG, Brazil
[2] School of Medicine – Federal University of Triângulo Mineiro, Uberaba, MG, Brazil

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Corresponding author:
Darceny Zanetta-Barbosa, DDS, MSc, PhD,
Avenida Pará s/n°, Campus Umuarama, Bloco 4T,
Departamento de Cirurgia e Traumatologia Buco-Maxilo-Facial, Bairro Umuarama, Uberlândia, Minas Gerais, Brazil, CEP: 38.400–902,
Fax and Telephone: 55-34-3218-2636,
E-mail: darceny_@hotmail.com

Mechanical and morphological alterations produced by ionizing radiation on bone- dental implant interaction

Abstract

Objective: Radiotherapy may be necessary to treat patients with osseointegrated implants that have been development head and neck cancer, which impair the implant secondary stability and also the surrounding bone mechanical properties. This study aimed to evaluate the radiotherapy effect on implant retention and additionally on the biomechanical, histomorphometric and microstructural parameters of bone close and distant of the dental implant.

Materials and Methods: Twenty adults male New Zealand rabbits received three implant of 3.5mm in diameter (Titamax CM, Neodent) and were divided into 2 groups: NoIr, control group- no radiation; Ir, the animals were irradiated 2 weeks after implant installation with 30Gy in single dose. After 4 weeks of the implant installation the animals were sacrificed and the implant/bone blocks were used for each experimental test (n = 10). Pull-out test using axial loading at 1.0mm/s crosshead speed was used for measuring the implant retention. Dynamic indentation test were used to quantify Vickers Hardness (VHN) and elasticity modulus (E) of bone tissue close and distant of implant surface. MicroCT was used to analyze the bone cortical volume (CtV), cortical thickness (CtTh) and porosity (Ct.Po) close (Ir-CIm) and distant (Ir-dIm) of implant surface. Data were analyzed by using t-student test and one-way ANOVA followed by Tukey test ($P<.05$).

Results: The implant shear retention values for Ir group was significant lower than NIr group. The bone of Ir-cIm group had significantly lower E, VHN, CtTh and CtV values and higher CtPo for than for Ir-dIm. The Ir-dm group had significantly lower E, VHN, CtTh values and higher CtPo than NIr group.

Conclusion: Ionizing radiation over osseointegrated implant had negative effect on mechanical; on bone implant shear retention and micro-architecture parameters of bone mainly close to implant surface.

Introduction

Radiotherapy associated with chemotherapy and surgery is a treatment choice for head and neck cancer treatment. However, the irradiation possesses adverse effects on bone causing injures in the small blood vessels with persistent hypoxia reducing the quantity and activity of osteoblasts and osteocytes (Ihde et al. 2009; Pompa et al. 2015). The undesirable effect of radiotherapy conduces to the hypocellularity, hypovascularization and bone hypoxia (Ihde et al. 2009; Li et al. 2014; Pompa et al. 2015), which may increase the occurrence of osteoradionecrosis (Ozen et al. 2005). These effects have impaired on the best moment to install implants after irradiation (Scherpers et al. 2006; Pompa et al. 2015). This context has stimulated the supporting therapies to improve osseointegration and to reduce the chance of osteoradionecrosis. Several studies have been performed to evaluate the implant survival in previously irradiated area (Anderson et al. 2013; Pompa et al. 2015). Additionally, is also persecuted the use of implant surface modification, laser and hyperbaric oxygen on irradiated bone aiming to improve bone integration. However, few studies have been evaluated the implications of the presence of osseointegrated implants in irradiated bone areas (Scherpers et al. 2006; Pompa et al. 2015).

The presence of a metal in the radiation field may results in a dosage enhancement at the tissue metal interface caused by the interaction of ionizing radiation with the atoms of the metal (Wang et al. 1996; Ozen et al. 2005). Radiation dose increasing generated by backscattered radiation of high-energy photons and electrons at tissue metal interface might increase the radiomorbidity, impairing on bone repair, resulting in osteoradionecrosis (Ozen et al. 2005; Anderson et al. 2013). However there is no consensus regarding the impact of radiotherapy on the functionality and survival of primary placed implants located in radiation field. Considering that most of patients with head and neck cancer are 50 years or older (Ozen et al. 2005), and also that world population tends to have more life time, associated with the increasing insertion of dental implants, the clinicians may be faced with the question of whether to remove or maintain the osseointegrated implants before cancer treatment. Furthermore, recently in head and neck tumors, has been advocated to include dental implants during ablative surgical session, if possible, with postoperative radiotherapy (Schoen et al. 2004; Pompa et al. 2015).

The bone morphology parameters such as cortical bone thickness, bone volume and porosity, and also mechanical properties, such as elastic modulus and Vickers hardness, associated with bone/implant integration resistance are important parameters to predict the effect of radiation on osseointegrated bone implant. Elastic modulus and Vickers hardness account for the ability of bone tissue to resist elastic and plastic deformations, respectively (Kim et al. 2015). The secondary stability, expressed by shear strength required to detach the implant from bone, is correlated with clinical implant outcomes (Cha et al. 2015). Little integrated information are available regarding the effects of radiotherapy and scattered radiation in regions with implants this study aimed to analyze the effect of radiation on osseointegrated bone implant on mechanical bone properties, bone morphological aspects and bone/implant interaction. The null tested hypothesis was that the radiation on osseointegrated implant area would no affect the morphology and mechanical parameters of implant peripheral bone and bone/implant integration resistance.

Materials and Methods

The experimental protocol was evaluated and approved by the Ethics Committee for Animal Research (Protocol #093/12, Universidade Federal de Uberlandia, Brazil) and supported by the Brazilian College of Animal Experimentation guideline. Twenty New Zealand white rabbits weighing between 3.0 and 3.5 kg were included in the study. During the experiment, rabbits were kept in individual cages with free access to food and water. The animals received three implant on both tibias and were randomly divided into two groups (n = 10): NoIr (control group), animals were keep free of irradiation and Ir, animals received external irradiation on both tibias 2 weeks after the implant installation surgery.

Surgical procedure

Twelve hours before surgery, animals were fasted. For sterilize preparation of the surgical site, the legs of animals were shaved and the tibiae area was cleaned with a 0.2% chlorhexidine solution (Rioquimica, São José do

Rio Preto, SP, Brazil). The animals were anaesthetized through intramuscular injection of a combination of 0.25 mg of ketamine/kg of body weight (Ketamina Agener®; Agener União Ltda., São Paulo, SP, Brazil) and 0.5 mg of xylazine/kg of body weight (Rompum® Bayer S.A. São Paulo, SP, Brazil). The infiltration of anesthesia was applied using 2% lidocaine and 1:100,000 epinephrine (Alphacaine® 0.5 - 1 ml/site, DFL, Rio de Janeiro, RJ, Brazil), to reduce stimulation during surgery and generate vasoconstriction. Incisions of 3 cm in length were performed in the both rabbits' tibias. The soft tissue and periosteum were removed, and a sharp subperiosteal dissection exposed the proximal tibia. Implants were placed using a progressive sequence of drills, under constant irrigation with 0.9% sodium saline solution, according to the manufacturer's instructions. All drilling procedures were conducted at 1200 rpm. Three Morse taper implants 3.75mm in diameter and 7.0mm in length (Titamax CM, Neodent®, Curitiba, PR, Brazil) were inserted into each animal (one on the left tibia and two on the right tibia) at diaphysis region, which contain mainly cortical bone. The soft tissues were sutured in separate layers using an interrupted suture (#5.0 nylon sutures Ethicon®; Johnson & Johnson Medical Ltd., Blue Ash, Ohio, United States). To prevent infection, daily intramuscular injections of Cefazolin (Yuhan Company; 250mg) were given for 1week. To prevent pain, a dose of an anti-inflammatory Meloxicam® 0.3 mg/kg (Ourofino, São Paulo, SP, Brazil) were administrated. Each rabbit was caged individually and received food and water. After surgery, animals were randomly divided into NoIr and Ir groups.

Radiation protocol

During the radiation sessions the animals (Ir group) were maintained under sedation by an intramuscular injection with a combination of 1.3 ml of ketamine (100mg/ml) and xilazyne chlorate (7mg/kg per body weight). The both hind legs of each rabbit were subjected to a radiation using single dose of 30 Gy. A 5-mm bolus was applied to ensure full build-up. This total dose is considered suitable for producing compromised surgical bed, simulating the clinical situation in rabbits similarly that damage observed in humans with radiation superior at 60Gy (Ma & Shen, 2012). The tibial metaphysis region of the hind leg was designated as the zone for irradiation. A single dose of irradiation was delivered with a source–skin distance of 60 cm and the field of size was 15 x 15 with direct electron beam of 9 MeV electron (Varian 600-C® Varian Medical Systems Inc, Palo Alto, California, USA). The dose rate was 400cgy/min. A 5-mm bolus was applied to ensure full build-up. After radiation, the skin, hair, weight, and appetite of the rabbits were monitored.

Animals sacrificing and sample preparations

After 4 weeks of the implantation, the animals were anesthetized with thiopental 2.5% and sacrificed under anesthesia overdose with an intravenous injection of potassium chloride 19% (Ariston Chemical and Pharmaceutical Industry Ltda. São Paulo, SP). The overlying soft tissues were removed and the specimens were fixated in 10% buffered formalin solution. The tibias were stored in plastic tubes containing phosphate buffered saline solution and frozen at -20°C before testing. The implant installed on the left tibia was used for pull out test, one implant installed on the right tibia diaphysis was used for the microCT analysis and the other one was used for dynamic indentation test (Fig. 1).

MicroCT analysis

The bone/implant samples (n = 10) were scanned at energy of 90 kV and intensity of 278 mA with a resolution of 9 mm pixel using Cu 0.1mm filter (Skyscan-1272 X-ray microtomograph, Skyscans, Kontich, Belgium). The reconstructed 3D data sets were quantified using CTAn automated image analysis system (Bruker, Kontich, Belgium). The volume of interest (VOI) for cortical analyses was selected around the implant and defined as a column from implant axis with a radius of 1.5 mm within cortical bone and extending totally 200 slices. The implant was selected based on its threshold level and this region was circumferentially expanded, creating a 0.55mm zone around the implant. For comparing the effect of metal effect on radiation enhancement the same bone volume affected by radiation were measure 2.5mm far from the external limit of the first measurement. The following microarchitecture was assessed in VOI images: cortical volume (CtV), cortical thickness (CtTh) and porosity (Ct.Po) according to standard procedures (Brouxsein et al. 2010).

Dynamic Indentation Test

Elastic modulus (E) and Vickers hardness (VHN) of the bone samples (*n* = 10) were assessed by using a microhardness dynamic indenter (CSM Micro-Hardness Tester; CSM Instruments, Peseux, Switzerland). The samples preparations were previously described in Soares et al. (2014). Using a Vickers indenter seven continuous indentations were made distancing 0.08 mm between each other. Indentations were performed also far from the implant interface. The indentation was carried out with controlled force, whereby the test load was increased or decreased at a constant speed ranging between 0 and 200 mN in 60-second intervals. The maximum force of 200 mN was held for five seconds. The load and the penetration depth of the indenter were continuously measured during the load-unload hysteresis. The universal hardness is defined as the applied force divided by the apparent area of the indentation at the maximum force. The measurements were expressed in VH units by applying the conversion factor supplied by the manufacturer The indentation modulus was calculated from the slope of the tangent of the indentation depth curve at the maximum force, which is comparable to the E of the bone structure (Zysset 2009; Soares et al. 2014).

Pull-out Test

The tibia/implant sample (*n* = 10) was mounted in a customized device during the pull-out tests. The device was adjusted to aligned with the load-cell. This mechanical test consisted of applying an increasing vertical force along the fixture axis until the bone-fixture interface was broken. A mechanical testing (EMIC DL 2000; EMIC, São José dos Pinhais, PR, Brasil) machine fitted with a calibrated load-cell of 1000N was used to perform the pull-out tests. Crosshead speed range was set to 1.0 mm/min. Using Finite element analysis were checked the stress distribution of the pull-out test to confirm the area where the stresses are most concentrated. Data were graphed as force versus displacement and the failure load was calculated from the graph. In addition to failure load, the displacement up to the failure point and interface stiffness was also calculated from the graph.

Statistical Analysis

The cortical volume (CtV), cortical thickness (CtTh) and porosity (Ct.Po), Elastic modulus (E), Vickers hardness (VHN), and pull-out data were tested for normal distribution (Shapiro-Wilk, $p>0.05$) and equality of variances (Levene's test), followed by parametric statistical tests. One-way analysis of variance (ANOVA) was performed for CtV, CtTh, Ct.Po, E and VHN values. Multiple comparisons were made using Tukey's test. *t*-Student test was performed for pull-out data. All tests employed $\alpha=.05$ significance level and all analyses were carried out with the statistical package Sigma Plot version 13.1 (Systat Software Inc, San Jose, CA, USA).

Results

MicroCT analysis – Morphologic parameters

The cortical volume CtV in mm^3, cortical thickness CtTh in mm, and bone porosity CtPo in % measured by microCT analysis for non-irradiated and irradiated groups are shown in Table 1. The Tukey test showed the non-irradiated group measured close to implant surface had significantly higher CtV ($P = 0.022$), and lower CtPo values ($P = 0.002$) than irradiated group. The Ir-dIm group had higher CtV ($P = 0.032$), and lower CtPo values ($P = 0.025$) than Ir-cIm group. However no significant difference was found between groups for CtTh values ($P = 0.412$), irrespective of the area measured.

Dynamic Indentation Test – E and VH

The elastic modulus - E in GPa, and Vickers hardness - VHN in N/mm^2 measured by dynamic indentation test for non-irradiated and irradiated groups are shown in Table 2. The *t*-Student test showed the non-irradiated group had significantly significant higher E ($P < 0.001$), and also higher VHN values ($P = 0.001$) than irradiated group near to implant surface. The E and VHN values of irradiated bone far from the implant surface were significant higher that non-irradiated group and significantly lower than irradiated group near to implant surface ($P = 0.034$).

Pull-out test – secondary implant/bone structure stability

The failure load in N, displacement in mm, and stiffness in N/mm measured by the pull-out test for non-irradiated and irradiated groups are shown in Figure 1. The stress distribution is concentrated on the cortical bone tissue, area where all the measurements were performed. The *t*-Student test showed the non-irradiated group had significantly higher failure load ($P = 0.002$), higher displacement ($P < 0.001$), and higher stiffness values ($P = 0.019$) than irradiated group.

Discussion

The current study evaluated the effects of radiation in bone/implant interface mimicking a clinical situation where the implants were installed, and presented bone integration before the radiotherapy. There are two situations in which the presence of metallic implant in the irradiation field is a concern aspect: (i) when the patient previously rehabilitated with implant has a head and neck tumor and will be submitted to radiotherapy or (ii) when the tumor resection is done during ablative surgery session with implant rehabilitation prior to radiotherapy (Schoen et al. 2004; Schepers et al. 2006). In both cases the dosage enhancement at tissue metal interface produced by the ionizing spreader effect is increased (Wang et al. 1996). The bone damage and possible induce osteoradionecrosis is enhanced in this situation (Ozen et al. 2005; Ihde et al. 2009). To date there is no consensus regarding the effects of the dental implants on radiation dose distribution, and if it may cause significant increasing on tissue radiomorbity. Removal or maintenance of the titanium implants from patients that receive radiation on the implant bone area is not clear clinical decision (Granström 2003; Ozen et al. 2005). The scarce information available on the literature regarding the effects of radiation on biomechanical parameters in the bone/implant interface after ionizing radiation stimulated the evaluation of the secondary stability by means pull-out test, the elasticity modulus and hardness of the bone tissue near to the implant interface in sites in which the implants were installed and osseointegrated before the radiotherapy.

Based on the previous knowledge about the effects of radiation on bone tissue in sites without metallic implants (Granström 2003; Ihde et al. 2009), it was hypothesized that the presence of titanium implants in irradiation field would result in reduction of the elasticity modulus and the Vickers hardness of the bone, and decrease the force required to detach the implant from bone, These hypothesis were accepted as demonstrated by the results of pull-out and dynamic indentation tests. The bone elastic modulus and Vickers hardness measured by indentation test were significantly lower after radiation compared with control group. Additionally the secondary stability of dental implant inserted into tibia decreased significantly after rabbit was choose as study model because this is the smallest animal that can accept commercially available dental implants in long bone (Park et al. 2005). However, the adverse effects of radiation on bone tissue are dependent of factors as time, dose per fractions, number of fractions and total dose (Ihde et al. 2009; Anderson et al. 2013). Due to the difficulties inherent to animal models studies, the rabbits were exposed to a single dose radiation enough to cause maximal inhibition of the bone regeneration (Li et al. 2014). This single dose was based on protocols reported by previous studies, which ranging between 25 Gy and 35 Gy (Jacobsson et al. 1985; Li et al. 2014). The irradiation by linear accelerator was chosen because nowadays the standard treatment for head and neck tumors uses the referred device. Another concern about the experimental design was the delimitation of the same area for analysis in all tests. Considering that most of altered dose from backscattering was limited to 1 mm away from the implant/bone interface (Li et al. 2014; Fang et al. 2014) the area of interest of the study should be restricted to this region.

Microindentation was adopted to investigate elastic modulus and Vickers hardness. In this study the samples were embedding in acrylic resin to prevent bending deformation, which result in minor or no increase on hardness of compact bone (Soares et al. 2014), not influencing the results as the same conditions were established for control and experimental groups. The measurement of hardness is used to assess the resistance of a material deformation that is the ability of surface to resist indentation (Boivin et al. 2008). Elastic modulus and Vickers hardness account for the ability of bone tissue to resist elastic and plastic deformations, respectively (Kim et al. 2015). In current study this parameter showed lower values in irradiated group, which demonstrated the ability of irradiation irreversibly and dramatically, modify bone properties. It is known that a strength and plastic behavior of the bone depends of the interaction between the collagen and mineral fraction (Hamer et al. 1996). In this regard, damage to the collagen matrix induced by irradiation can be extremely problematic to the biomechanical properties of bone, in particular through the formation of collagen cross-links. The present findings are in agreement from the Barth et al. (2011), which observed major losses in bone strength, ductility and fracture resistance following exposures in excess of 30 Gy. These authors state that loss in plastic deformation could be related to a suppression of the prevailing plasticity mechanism in bone of fibrillar sliding, which results principally from a change in the proportion of the three types of collagen cross-linking caused by the irradiation damage. It was observed in this study a decreasing in the elasticity modulus in the irradiated bone, which can be explained at the molecular level by the methods used in this study once microindentation measurement is suited to test mechanical properties at the intermediate level of organization of bone. The response of irradiated bone close to implant was significantly more evidenced than when radiation was applied only on bone tissue, demonstrating that the metallic implant increased the radiation effect.

For measuring the failure load and interface stiffness was used the pull-out test. No consensus is related to the better methodology for testing bone/implant integration using push-in or pull-out test (Chang et al. 2010; Seong et al. 2013). The general loading capacity of the interface (or interfacial shear strength) is calculated by dividing the maximum force by the area of implant in contact with the host bone (Berzins et al. 1997; Chang et al. 2010). Pull-out tests is better applicable for non-threaded cylinder type implants, because their interfacial failures are solely dependent on shear stress without any consideration for either tensile or compressive stresses (Brunski et al. 2000). However pull-out methodology test more intensively the area near to neck of the implant, represented by cortical bone area, where the stress concentration is predominantly (Fig. 2). Additionally, very lower stress concentration is observed on thread sites. It is possible to add more information connect with the mechanical and morphometric calculated on the same area. The lower values of failure load, displacement and stiffness for irradiated group are probably related to modifications promoted by radiation on noncollanous proteins present on bone/implant interface. The alteration of the mechanical properties of the cortical bone tissue located near to neck of the implant, where the higher stress is concentrated, may also reflect on the interface resistance. The decreasing of elastic modulus and hardness reflect the reduction of the bone capacity of elastic and plastic deformation requesting more of the interface.

In addition, we also evaluated micro-structural changes of bone around implant using µCT assessment. This method has been recommended as the gold standard for ex vivo analysis because it allows 3-dimensional measurements and good correlation with histomorphometric data (Park et al. 2005; Swain & Xue, 2009; Fang et al. 2014). Although the use of µCT for studies involving implants may generated the blurred border around the implant due to metal artifacts caused by an scatter noise, this artifact can be circumvented by making expansion of the implant during the analysis (Ozen et al. 2005; Li et al. 2014). The reduction in volume and an increase in porosity observed in the present study are in agreement with the literature (Ihde et al. 2009). The primary effect of radiation in bone is atrophy which involves reduction in the number of structural components to the tissue (Hopewell, 2003). Radiation-induced changes in bone include vascular changes, bone matrix and cellular changes. The decreased in number of osteoblasts and osteocytes, increased number of oteoclasts resulting in higher bone resorption (Brogniez et al. 2002) as the reduced number of capillaries also be related to micro-structural alterations observed in µCT. The presence of mettalic implant also increased the alteration of the morpholic bone parameters when compared of the irrradiated bone far from the implant. Demonstranting that the implant really impar on the bone mechanical properties and secundary staility.

In the presence of head and neck cancer that need to be treated with radiation the observation of the previous installed implant should be a important aspect. The limitation of the radiation area as possible should avoid the implant location and if is not necessary the return for frequent appoitment for analysis of the implant stability is recommended. For futhers studies is mandatory to study new local or systemic apporach that may reduce the negative effect on bone-implant interface.

Conclusion

Considering the limitations of this study, such as the fact that there is no load on the implants, and that were installed only in the cortical bone, we can concluded that radiotherapy in previously rehabilitated regions decreased the secondary dental implant stability measured by pull-out bond test, and also is able to alter the mechanical and morphologic properties of bone around implants which that can compromise the long-term prognosis.

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Table 1. Mean and standard deviation values Cortical volume CtV in mm^3, cortical thickness CtTh in mm, and porosity CtPo in % measured by microCT analysis for non-irradiated and irradiated groups.

Groups		CtV (mm^3)	CtTh (mm)	CtPo (%)
NoIr, non-irradiated implants		6.9 ± 0.3^A	0.30 ± 0.06^A	65.9 ± 1.4^A
Ir, Irradiated implants	***IrcIm, measured close to the implant surface***	6.1 ± 0.3^C	0.33 ± 0.03^A	71.9 ± 1.7^C
	IrdIm, measured distant to the implant surface	6.5 ± 0.3^B	0.31 ± 0.03^A	68.4 ± 1.1^B

Letters represent significant difference within each morphological parameters, defined by Tukey est ($P < 0.05$).

Table 2. Mean and standard deviation values of elastic modulus in GPa and Vickers hardness in N/mm^2 measured by dynamic indentation test for non-irradiated group and irradiated group measured close and distant from the implant surface.

Groups		Mechanical properties	
		Elastic modulus (GPa)	**Vickers hardness (N/mm^2)**
NoIr, non-irradiated implants		20.8 ± 3.2^A	115.9 ± 32.5^A
Ir, Irradiated implants	***IrdIm, measured distant to the implant surface***	18.3 ± 2.5^B	91.5 ± 32.0^B
	IrcIm, measured close to the implant surface	16.1 ± 2.5^C	69.7 ± 27.2^C

Letters represent significant difference within each mechanical property, defined by *t*-Student test ($P < 0.05$).

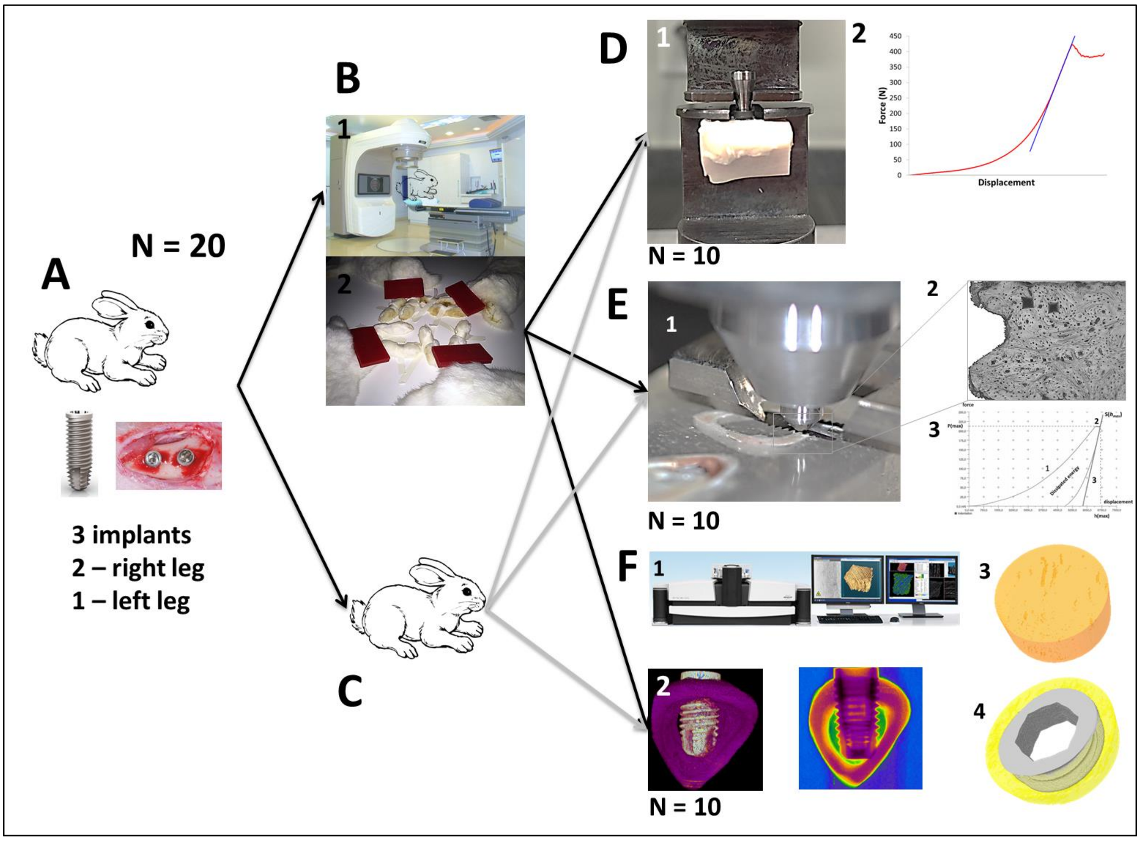

Fig. 1. A, Implant installation on rabbit legs – 3 implants per animal; B, Animals that were submitted to radiotherapy by single dose of 30Gy; C, Animals free of the radiotherapy; D, Pull-out test for measuring the implant/bone stability for non-irradiated and irradiated groups; E, dynamic indentation test for measuring the E and VHN; F, microCT analysis for measuring CtV, CtTh and CtPo for non-irradiated and irradiated close and distant of implant surface groups.

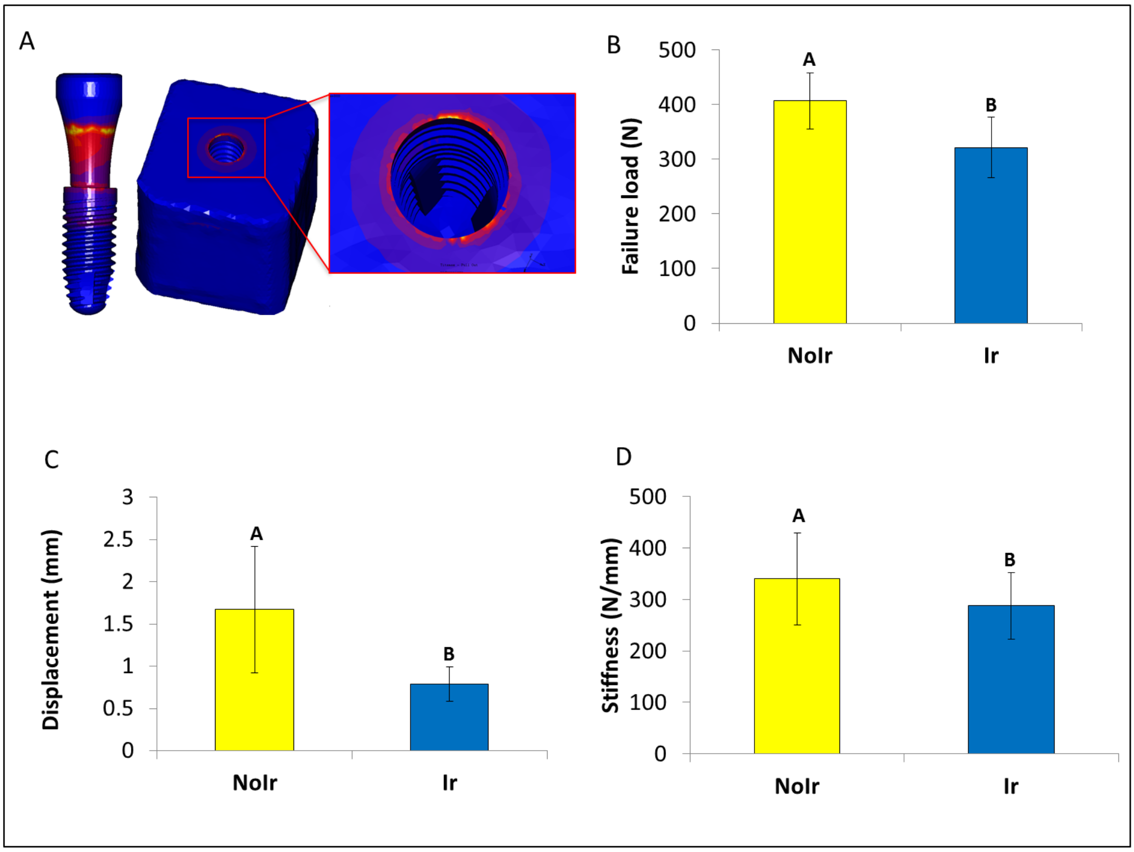

Fig. 2. A, Finite element model of pull-out shown the homogeneous stress distribution on the cortical bone close to the neck of the implant; B, Failure load in N; C, displacement in mm; D, stiffness in N/mm, measured by the pull-out test for non-irradiated and irradiated groups.

CAPÍTULOS

3.5 CAPÍTULO 5

Artigo a ser enviado para publicação no periódico ImplantNews

Interação da radioterapia e da reabilitação estético-funcional com implantes dentais – Abordagem clínica com evidência científica

Priscilla Barbosa Ferreira Soares[1]
Flávio Domingues das Neves[2]
Darceny Zanetta-Barbosa[3]

[1] Doutoranda em Clínica Odontológica, Área de Cirurgia e Traumatologia Buco-Maxilo-Facial e Implantodontia, Faculdade de Odontologia, Universidade Federal de Uberlândia, MG, Brasil.
[2] Professor Titular Área de Cirurgia e Traumatologia Buco-Maxilo-Facial e Implantodontia, Faculdade de Odontologia, Universidade Federal de Uberlândia, MG, Brasil.
[3] Professor Associado 4 da Área de Oclusão, Prótese Fixa e Materiais Odontológicos, Faculdade de Odontologia, Universidade Federal de Uberlândia, MG, Brasil.

Autor de Correspondência:
Darceny Zanetta-Barbosa, DDS, MSc, PhD,
Avenida Pará s/n°, Campus Umuarama, Bloco 4T,
Departamento de Cirurgia e Traumatologia Buco-Maxilo-Facial, Bairro Umuarama, Uberlândia, Minas Gerais, Brasil, CEP: 38.400–902,
Fone e Fax: 55-34-3218-2636,
E-mail: darceny_@hotmail.com

Interação da radioterapia e da reabilitação estético-funcional com implantes dentais – Abordagem clínica com evidência científica

Priscilla Barbosa Ferreira Soares[1]
Flávio Domingues das Neves[2]
Darceny Zanetta-Barbosa[3]

[1] Doutoranda em Clínica Odontológica, Área de Cirurgia e Traumatologia Buco-Maxilo-Facial e Implantodontia, Faculdade de Odontologia, Universidade Federal de Uberlândia, MG, Brasil.
[2] Professor Titular Área de Cirurgia e Traumatologia Buco-Maxilo-Facial e Implantodontia, Faculdade de Odontologia, Universidade Federal de Uberlândia, MG, Brasil.
[3] Professor Associado 4 da Área de Oclusão, Prótese Fixa e Materiais Odontológicos, Faculdade de Odontologia, Universidade Federal de Uberlândia, MG, Brasil.

Autor de Correspondência:
Darceny Zanetta-Barbosa, DDS, MSc, PhD,
Avenida Pará s/n°, Campus Umuarama, Bloco 4T,
Departamento de Cirurgia e Traumatologia Buco-Maxilo-Facial, Bairro Umuarama, Uberlândia, Minas Gerais, Brasil, CEP: 38.400–902,
Fone e Fax: 55-34-3218-2636,
E-mail: darceny_@hotmail.com

Interação da radioterapia e da reabilitação estético-funcional com implantes dentais – Abordagem clínica com evidência científica

Resumo

O uso de implantes dentais constitui alternativa conservadora e popular para reabilitar pacientes desdentados parciais e totais, devolvendo acima de tudo qualidade de vida. As indústrias tem desenvolvido novas superfícies de implantes visando otimizar a osseointegração. Muitos destes pacientes que necessitam ou que receberam implantes podem ser acometidos por câncer de cabeça e pescoço, que é tratado com radioterapia. Este trabalho tem por objetivo associar evidências científicas reportadas em diversos estudos sequenciais do nosso grupo de pesquisa com relato de caso clínico de tratamento cirúrgico-reabilitador de paciente com câncer na região de cabeça e pescoço que possuía implantes dentais. Embora novas superfícies de implantes tenham desempenho semelhantes em pacientes normais esta estratégia pode ser mais um aliado na melhora de desempenho de implantes em pacientes irradiados. A radioterapia reduz as propriedades mecânicas e estruturais do osso e pode comprometer o processo de osseointegração. A liga de Titânio dos implantes mantidos em áreas que recebem radioterapia atua como fator que potencializa a radiação que atinge o osso circunvizinho ao implante, embora os implantes continuem mantendo desempenho satisfatório. Os clínicos devem estar atentos a estes novos e desafiadores cenários na reabilitação implanto suportada.

Palavras chaves: Implante dental; radioterapia; tratamento de superfície; osseointegração.

Abstract

The use of dental implant is nowadays a viable, popular and conservative alternative that for rehabilitating partial or total edentulous patients, recovering mainly the life quality. The manufactures have been innovated with new implant surfaces aiming to optimize the osseointegration. Patients that need to receive or that have received dental implants may be affected by head and neck cancer in the future, which is treated with radiotherapy. Therefore the aim of this study was to associate the scientific evidences produced by our research group with the clinical case report of surgery and implant supported prosthesis rehabilitation of patient with dental implants and developed head and neck

cancer and was treated with radiotherapy. Although the implant surface innovation had relative improvement of osseointegration performance for normal patients, it may be an additional tools for patients that received radiotherapy. The radiotherapy reduces the mechanical and structural properties of bone tissue, which may impair on implant bone integration. The radiation applied over previous installed implant increase the radiation effect around metallic dental implant. The clinicians should be alert for these new and challenge scenarios for implant supported oral rehabilitation.
Keywords: dental implants; radiotherapy; implant surface treatment; osseointegration.

Introdução

A vida média da população mundial e no Brasil tem aumentado de forma crescente e contínua, ao mesmo tempo a taxa de natalidade diminui marcantemente, resultando em progressivo envelhecimento da população[1]. Esta mudança no perfil populacional resulta em olhar cada vez mais aprofundado aos cuidados com a saúde na terceira idade. O aumento de tempo de vida tem sido acompanhado pelo aumento progressivo de reabilitação de desdentados parciais ou totais por meio de implantes buscando acima de tudo maior qualidade de vida[2].

A qualidade do tecido ósseo, dos protocolos cirúrgicos e características do implante utilizado norteiam o sucesso destes procedimentos reabilitadores[3]. Desta forma buscar meios de melhorar a produção industrial de implantes com desenvolvimentos de geometrias inovadoras, superfícies que estimulem a integração primária, associada a constantes e progressivas investigações de aspectos mecânicos e biológicos devem nortear a conduta da implantodontia moderna[4]. A qualidade superficial dos implantes dentais tem correlação direta com a evolução da regeneração óssea[5]. A indústria tem buscado desenvolver superfícies bioativas ou bioindutoras que potencializem o processo de osseointegração precoce, possibilitando reabilitações imediatas com excelentes resultados clínicos[5,6].

No Brasil, cerca de 800 mil implantes são instalados por ano no país, segundo levantamento da Associação Brasileira da Indústria Médica, Odontológica e Hospitalar (Abimo). Com isso estabelece-se um horizonte que é de pacientes vivendo mais e muitos deles com reabilitações dentais associada a implantes. Pensar então em doenças que interagem com a vida adulta ou terceira idade e que tenha impacto na longevidade de implantes dentais é um horizonte importante para a pesquisa científica e deve ser entendida como real problema de saúde pública.

Evidencia-se na população mundial o crescente aumento da ocorrência de câncer na região de cabeça e pescoço[7]. O câncer da cavidade oral representa 40% de todos os tumores malignos da região da cabeça e do pescoço, sendo geralmente tratado com cirurgia associado ou não à radioterapia[8]. Ambas as modalidades têm efeitos adversos sobre os tecidos saudáveis moles e duros da cavidade oral[9,10]. Para tratar este tipo de tumor a radioterapia associada à cirurgia tem sido a estratégia terapêutica frequentemente utilizada, tanto no aspecto paliativo quanto definitivo no tratamento do câncer. A radioterapia é o uso da radiação ionizante na destruição de tumor cancerígeno pela absorção da energia da radiação incidente, tendo como principio maximizar o dano ao tumor e minimizar o dano aos tecidos vizinhos. No entanto, doses de radiação na região de cabeça e pescoço são elevadas, o que pode causar efeitos indesejáveis aos tecidos normais adjacentes afetando o tecido muscular[11], esmalte e dentina[12,13] e tecido ósseo[14,15]. O tecido ósseo afetado pela radiação, particularmente quando associados com cirurgias, são severamente desvascularizados, reduzindo significativamente a capacidade reparadora pela angiogênica deficiente e consequente hipóxia[16]. O objetivo principal do tratamento de pacientes de oncologia oral não pode estar focado apenas na eliminação do tumor, a reabilitação estético-funcional deve ser alcançada buscando recuperação física e psicológica dos pacientes. Portanto este trabalho tem por objetivo associar evidências científicas reportadas em diversos estudos sequenciais do grupo de pesquisa Biaor (Biomecânica Aplicada a Odontologia Restauradora/CNPq), que envolvem tratamento de superfícies de implantes e efeitos da radioterapia sobre o tecido ósseo e sobre implantes osseointegrados. Por meio de relato de caso clínico de tratamento cirúrgico-reabilitador de paciente com câncer na região de cabeça e pescoço que possuía implantes dentais, visa popularizar conhecimentos a cerca deste tema a clínicos, ampliando o olhar para este novo e desafiador horizonte da odontologia reabilitadora.

Relato de caso Clínico

Paciente APS, 63 anos de idade, sexo masculino, foi encaminhado a Eikon Odontologia Especializada (Uberlândia, MG), para avaliar a possibilidade de reabilitação oral, pois não se sentia realizado com o estado estabelecido após tratamento de câncer acometido na região da maxila. Ao levantar o histórico odontológico do paciente evidencia-se que o mesmo havia finalizado em março de 2008, reabilitação implanto-retida realizada com instalação de 3 implantes de 3,75 mm de diâmetro (Titamax HE, NeoPoros, Neodent, Curitiba, PR, Brasil) na região anterior (Figura 1). Sobre estes implantes havia sido realizada prótese implanto-retida reabilitando a região anterior. Em março de 2014, decorrido 6 anos da instalação dos implantes, foi diagnosticado tumor acometendo o seio maxilar e assoalho de órbita. Após a biópsia foi fechado o diagnóstico de carcinoma epidermóide de mucosa do seio maxilar, com invasão óssea, da musculatura orbitária e filetes nervosos do lado direito. Como tratamento foi proposto e realizado cirurgia ressectiva envolvendo maxilectomia com extensão da cavidade orbitária direita e parte da arcada direita envolvendo dois dos 3 implantes. Como tratamento complementar foi prescrito 6 doses 1 vez por semana de Cisplatina na dosagem de 30mg/m^2 com a finalidade de potencializar a ação da radioterapia. A radioterapia realizada por acelerador linear (Varian 6EX, Palo Alto, CA, EUA), iniciou em abril de 2014 e finalizou em junho do mesmo ano, envolvendo 35 sessões de segunda a sexta com dose diária de 1,8Gy, totalizando 63Gy. A área irradiada foi focada na região da maxila, envolvendo o implante remanescente na maxila. Para isolar a maxila da mandíbula foi utilizado abaixador de língua.

Após a terapia cirúrgica e durante o procedimento radioterápico o paciente não utilizou nenhum tipo de prótese. O paciente mostrava-se extremamente insatisfeito com sua condição bucal. Tendo restrições alimentares, dificuldades de fonação e com autoestima muito baixa, considerando-se mutilado e alheio ao convívio social (Figura 2). Em virtude do aprimoramento das técnicas de reabilitação e do desejo do paciente de ter sua condição oral e facial melhoradas, foi proposta prótese implanto retida e muco suportada, tipo “overdenture” parcial, vedando a comunicação buco-sinusal, protocolo viável frente à avaliação radiográfica favorável (Figuras 3A, 3B e 3C). Este implante remanescente estava instalado em área que havia sido acometido pelo tratamento radioterápico. Para sucesso da reabilitação foi imprescindível que o implante remanescente fosse incluído como pilar de estabilização e retenção da prótese, o mesmo recebeu um encaixe Equator – Neodent®, que tem como principal característica ter bom desempenho sem ocupar tanto espaço na parte interna da prótese (Figuras 4A e 4B).

Prótese esta que é reabilitadora e obturadora da comunicação resultante da cirurgia. Ao final do tratamento, o paciente manifestou excelente aceitação e adaptação com a prótese realizada considerando-se bem reabilitado tanto no aspecto funcional como estético (Figuras 5A e 5B). Aproximadamente um ano de uso da prótese o paciente foi reavaliado, demonstrando sucesso do procedimento realizado com o mesmo relatando extrema satisfação, nesta ocasião a borracha interna do Equator – Neodent® foi trocada melhorando a retenção e estabilidade da prótese.

Discussão

O maior tempo de vida, aliado a popularização da reabilitação empregando implantes dentais e a crescente ocorrência de câncer de cabeça e pescoço torna cada vez mais frequente dois cenários para o clínico: 1) a necessidade de avaliar a sobrevida de implante osseointegrado envolvido na área delimitada para receber radioterapia; e 2) a necessidade de instalação de implantes em osso submetido a radioterapia para reabilitar perdas dentais e reparar cirurgias ressectivas. Este dois cenários estão envolvidos neste relato de caso e suportam uma discussão reflexiva importante sobre conceitos e aspectos que hoje já devem ser tratados como problema de saúde pública. Frente a este contexto diversos questionamentos surgem no momento de se planejar a ação terapêutica e reabilitadora tanto do tumor de cabeça e pescoço como do protocolo reabilitador a ser implementado. O tecido ósseo sofre alteração mecânica, biológica e estrutural quando submetido à radioterapia? Esta alteração interfere no processo de osseointegração? A liga metálica constituinte do implante potencializa o efeito da radiação e com isso pode significar efeito negativo na permanência de implante osseointegrado submetido à radioterapia? Novos tratamentos de superfícies dos implantes podem ser vistos como potencializadores de osseointegração primária e assim servir de perspectiva para implantes instalados nestas condições? Estes tópicos devem ser discutidos no horizonte do cirurgião dentista que cada vez mais irá deparar com condições semelhantes.

A radioterapia é sem dúvida uma potente e importante ferramenta terapêutica para o tratamento de tumores e vem sofrendo avanços marcantes[17]. Para realização de radioterapia aparelhos de Cobalto 60 e Aceleradores lineares são chamados de megavoltagem, pois têm energia bem alta e, por isso, não há interação da radiação com os primeiros milímetros da pele, minimizando sequelas actínicas[18]. Embora os aparelhos de radiação, constituídos a base de Cobalto 60, tenham tratado milhares de pessoas pelo mundo, eles resultam em maiores efeitos colaterais por resultar em maior espraiamento do feixe de radiação e assim afetando maior área de tecido sadio não incluído na região de atenção[19]. O acelerador linear empregado neste paciente é uma grande conquista da ciência e traz melhorias fundamentais na maior colimação da radiação e com isso minimiza este dano residual[19].

O sucesso da radioterapia depende entre outros fatores da dose liberada no volume inteiro do tumor, a qual não deve variar mais do que 5% da dose prescrita[20]. A superdose pode aumentar o risco de necrose e a subdose pode comprometer a destruição do tumor devendo ser avaliada. Muitas vezes não há como reduzir a radiação total por motivos óbvios de que a eliminação de células tumorais residuais é mais relevante que os danos residuais causados. Porém este fato deve ser discutido e levado em consideração para que o mínimo necessário para a melhor resposta da radioterapia seja empregado e assim não se tenha danos adicionais que possam ser evitados. O uso da associação de quimioterapia, com a prescrição de Platistine® CS (Cisplatina 1mg, Bentley, Austrália) tem efeitos positivos com aumento de cerca de 6,5% no sucesso de tratamento de cânceres de cabeça e pescoço[21]. Outro aspecto que cabe mais atenção e que requer estudos futuros é a interação do uso de drogas quimioterápicas que visam potencializar o efeito da radioterapia no tecido ósseo.

A radioterapia resulta em efeito adverso ao tecido ósseo, reduzindo suas propriedades mecânicas especialmente reduzindo tanto o módulo de elasticidade, que reflete a capacidade de deformação elástica, quanto na dureza, que reflete a capacidade de deformação plástica do osso, determinando o potencial de alteração no processo de reparo[22]. A radiação causa dano nos vasos sanguíneos e com isso reduz o aporte nutricional afetando na atividade das células do tecido ósseo[23]. Tudo isso favorece a ocorrência de osteoradionecrose[24]. A osteoradionecrose é dependente da dosagem de radiação e da capacidade de reparo do organismo e pode resultar em danos irreversíveis reduzindo a capacidade de reparo do tecido ósseo[25]. Estes efeitos podem impactar negativamente na osseointegração de implantes dentais e acaba por modular o momento ideal de instalação de implantes dentais[23]. Portanto o clínico deve estar atento ao receber pacientes que tenham sido submetidos a radioterapia e principalmente deve estar pronto a atuar na orientação e prevenção dos danos aos tecidos dentais e de suporte para seus pacientes que forem diagnosticados com câncer de cabeça e pescoço. No caso apresentado cogitou-se a instalação de implantes temporários, porém com caráter definitivo[26] com o'rings – Intra-lock, no osso palatino, favorecendo ainda mais a estabilidade protética, entretanto o serviço de oncologia desaconselhou por receio de osteoradionecrose. O limite entre reabilitar o pacientes como ele se encontra e buscar melhorar os aspectos de retenção, correndo o risco de piorar a situação clínica ainda apresenta prognóstico obscuro, neste caso a prudência deve prevalecer. Por isto o grupo contentou-se com um único implante para reter a prótese, contando com a maior área chapeável possível para melhor estabilidade da mesma.

Outro aspecto evidenciado neste relato de caso foi a permanência do implante mencionado, já osseointegrado e que foi envolvido na área de foco do tratamento radioterápico. Não há consenso na literatura a cerca da melhor estratégia para manutenção ou remoção destes implantes[23,27]. A presença do metal na cavidade bucal pode resultar em aumento da dosagem de radiação próximo ao artefato metálico[24]. Esta influência é dependente do tipo de liga constituinte do metal[28]. Em relação aos implantes dentais dados mostram incremento de aproximadamente 18% da radiação próximo ao implante[28]. O que resulta em redução da qualidade do tecido ósseo próximo ao implante com redução da dureza e do módulo de elasticidade do tecido ósseo. Eventualmente, a remoção do implante é traumática e alcança o edentulismo do paciente. Entretanto o mesmo deve ficar ciente que a longevidade do tratamento é dependente da sobrevida do implante, e não é possível mensurar o quanto o osso do entorno foi acometido, e tampouco quanto tempo este implante suportará o carregamento funcional, sendo necessário acompanhamento periódico. Fica evidente a importância para a ciência odontológica o acompanhamento de um maior número de casos destes protocolos terapêuticos para melhor evidência científica.

Considerações Finais

O osso irradiado é sem dúvida um enorme desafio para o cenário de instalação de implantes dentais. Os clínicos devem se ater a este aspecto e buscar analisar respostas individuais frente a cada tratamento. O sucesso da reabilitação de pacientes irradiados com próteses implanto suportadas depende de uma interação multiprofissional buscando superar as respostas multifatoriais. Pompa et al., 2015 em um estudo retrospectivo de reabilitações implanto suportadas concluíram que o carregamento tardio pode melhorar o prognóstico de implantes dentais em pacientes irradiados. Este cuidado mostra que este cenário ainda requer maior atenção da comunidade científica na busca por meios de prevenir e minimizar os danos causados pela radioterapia, por inovações tecnológicas e protocolos regenerativos que potencializem a osseointegração. Com isso a odontologia poderá mais ainda contribuir com a melhora da qualidade de vida de pacientes como o da abordagem do relato, devolvendo satisfação pessoal e autoestima.

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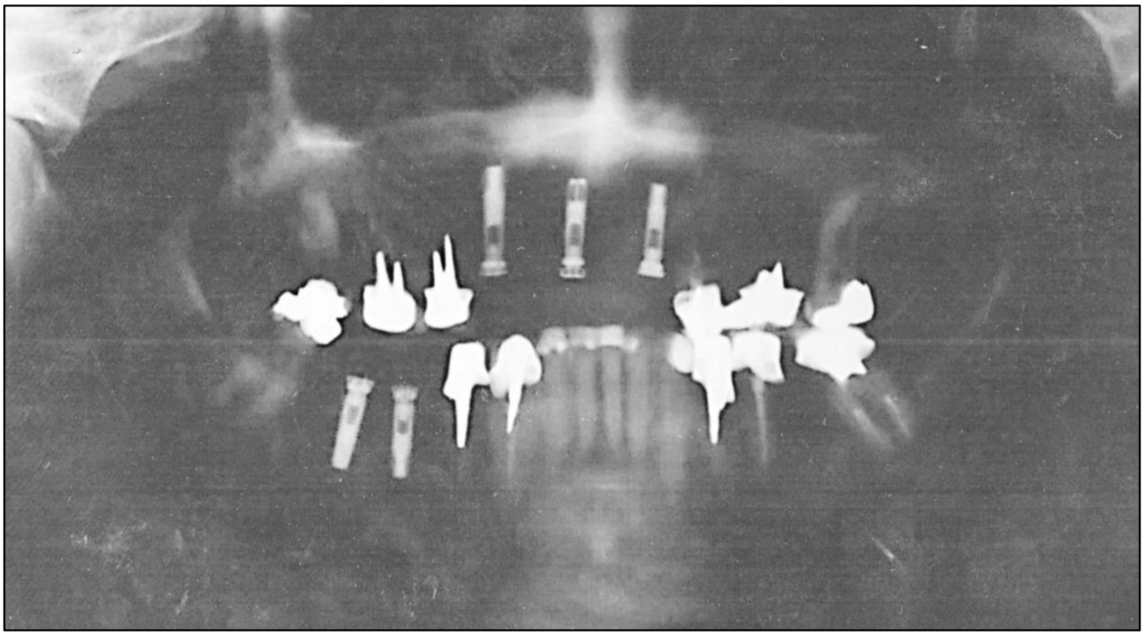


Figura 1. Radiografia panorâmica realizada no ano de 2008 demonstrando a presença de 3 implantes na região anterior superior.

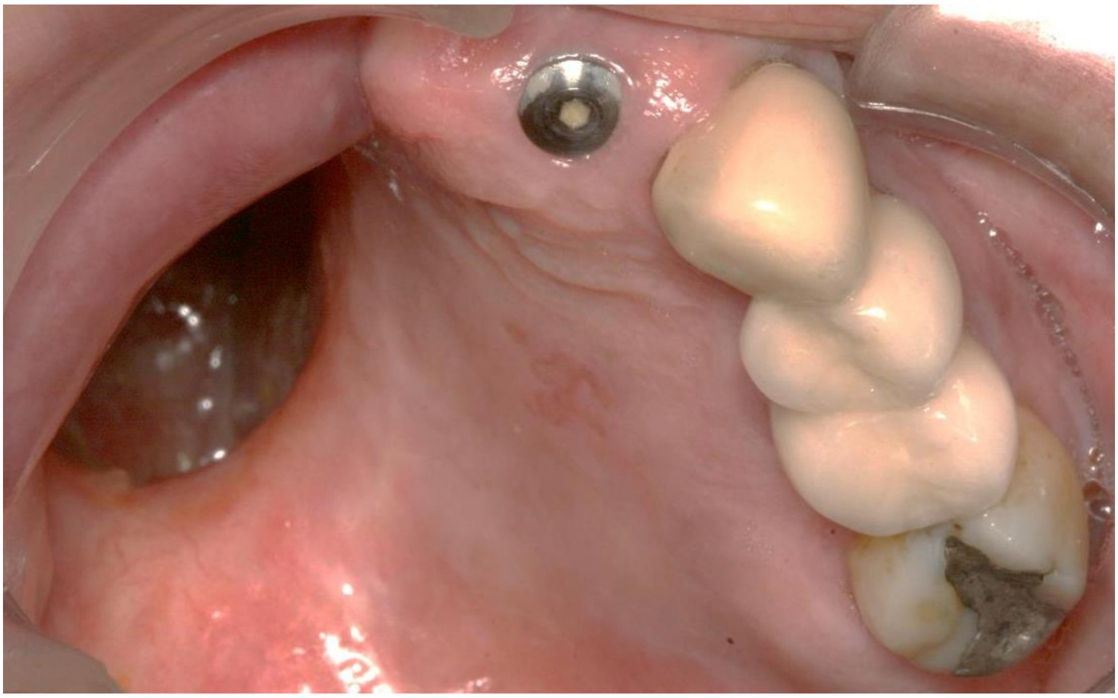
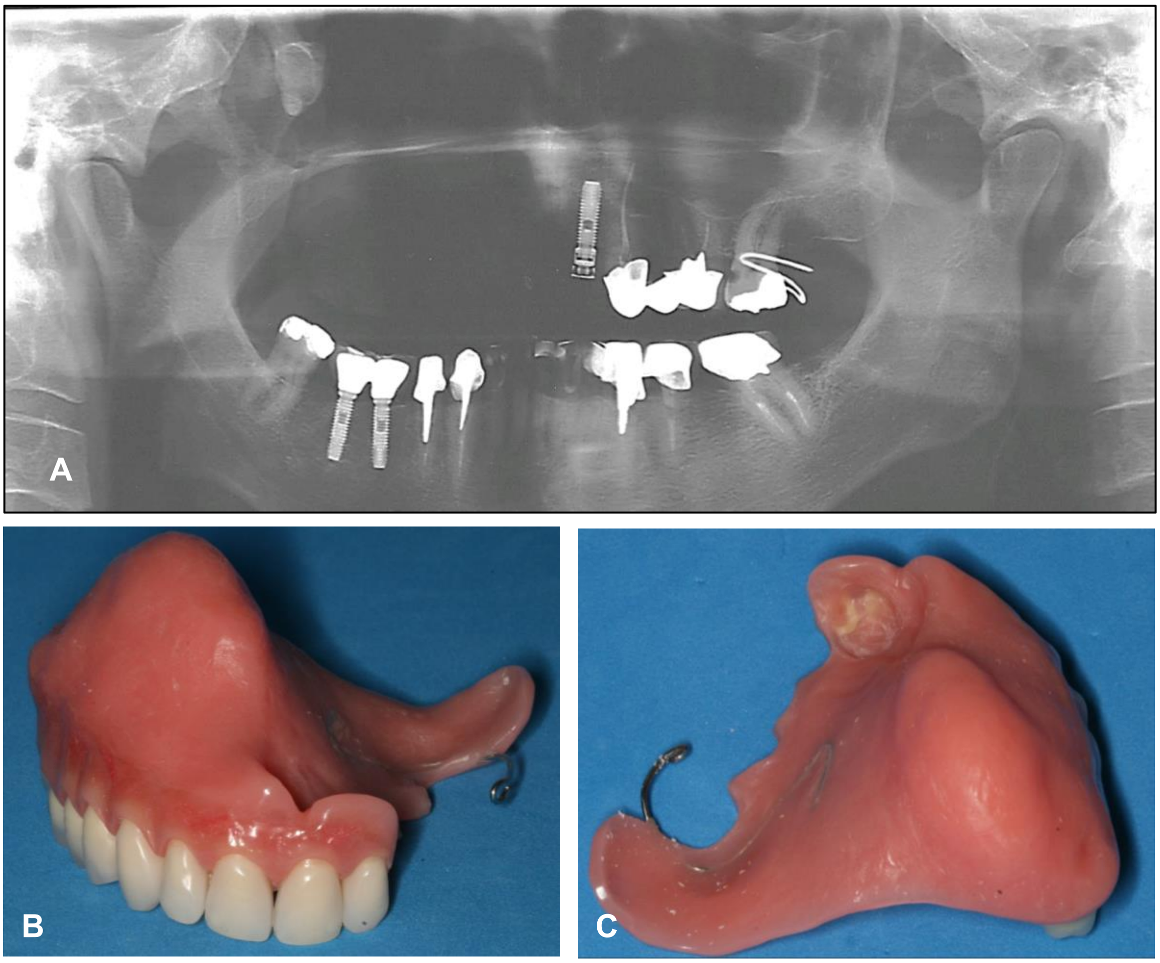
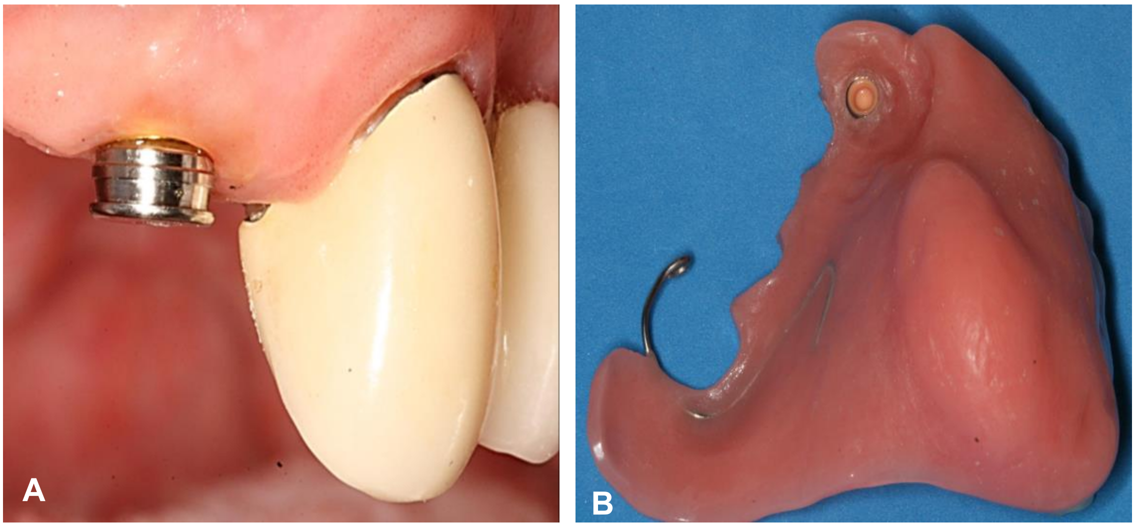


Figura 2. Aspecto intra oral da destacando comunicação buco-sinusal resultante da cirurgia ressectiva para remoção do tumor e presença de implante na região anterior remanescente.



Figuras 3. A, Radiografia panorâmica pós-cirurgia ressectiva demonstrando implante superior no limite de circunscrição da lesão que foi envolvido na área irradiada; B, Vista vestibular da prótese implanto retida e muco suportada, tipo “overdenture” parcial; C, Vista palatina da prótese.



Figuras 4. A, Implante remanescente envolvido na área irradiada mantido como pilar de estabilização e retenção da prótese com encaixe Equator, Neodent®; B, aspecto palatino da captura do encaixe.

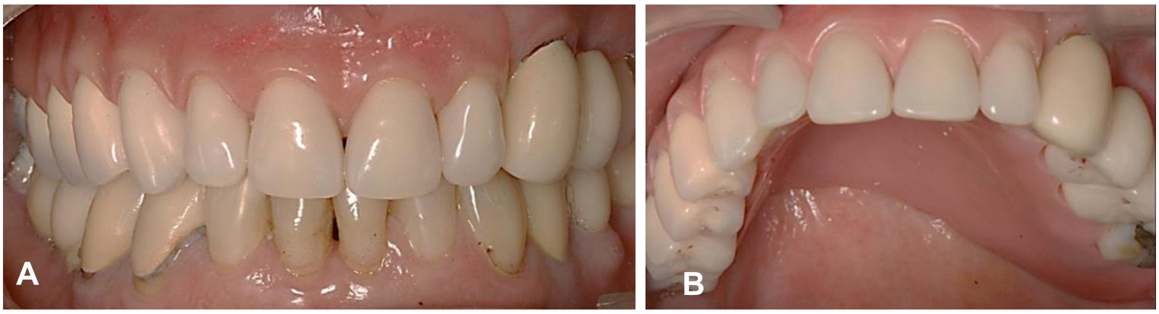


Figura 5. A, Aspecto final do tratamento reabilitador; B, Prótese reabilitadora e obturadora da comunicação resultante da cirurgia ressectiva do tumor.

CONCLUSÕES

4- CONCLUSÕES

Dentro das limitações metodológicas impostas pelo delineamento experimental destes estudos que envolveu 1 estudo laboratorial, 3 estudos *in vivo* em modelo animal e 1 relato de caso clínico pode-se concluir-se que:

- O método de indentação dinâmica mostrou ser altamente útil na caracterização individualizada do tecido ósseo periimplantar;
- Não há variação do módulo de elasticidade e dureza Vickers do tecido ósseo próximo ou distantes às roscas do implante;
- As superfícies de implantes hidrofílicas (Neoporos) e superhidrofílicas (Acqua) são capazes de produzir adequada integração osso/implante em condição normal de instrumentação do osso cortical;
- A radiação ionizante teve efeito negativo sobre os parâmetros mecânicos e microarquitetura e histomorfométricos do tecido ósseo de coelhos;
- O dano no tecido ósseo irradiado foi mais significativamente observado após 14 e 21 dias após a radioterapia aplicada no modelo animal testado;
- A radiação ionizante tem efeito negativo na estabilidade de implantes osseointegrado reduzindo a resistência da interface osso implante;
- A radiação ionizante aplicada em área com implantes osseointegrados afeta negativamente os parâmetros mecânicos e microarquitetura do tecido ósseo sendo o efeito mais evidenciado naquele tecido próximo à superfície do implante;
- Reabilitar pacientes acometidos por câncer de cabeça e pescoço que foram submetidos a protocolos cirúrgicos e radioterápicos requer intervenção multiprofissional e avaliações periódicas por apresentar ainda prognóstico com relativa evidência científica.

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ANEXOS

ANEXOS

Parecer do Comitê de ética

Universidade Federal de Uberlândia
Pró-Reitoria de Pesquisa e Pós-Graduação
Comissão de Ética na Utilização de Animais (CEUA)
Avenida João Naves de Ávila, nº. 2160 - Bloco A, sala 224 - Campus Santa Mônica - Uberlândia-MG –
CEP 38400-089 - FONE/FAX (34) 3239-4131; e-mail:ceua@propp.ufu.br; www.comissoes.propp.ufu.br

ANÁLISE FINAL Nº 156/12 DA COMISSÃO DE ÉTICA NA UTILIZAÇÃO DE ANIMAIS PARA O PROTOCOLO REGISTRO CEUA/UFU 093/12

Projeto Pesquisa: "Análise do efeito da radioterapia e oxigenoterapia hiperbárica na interação biológica e mecânica de implantes dentais".

Pesquisador Responsável: Darceny Zanetta Barbosa

O protocolo não apresenta problemas de ética nas condutas de pesquisa com animais nos limites da redação e da metodologia apresentadas.

SITUAÇÃO: PROTOCOLO DE PESQUISA APROVADO.

OBS: O CEUA/UFU LEMBRA QUE QUALQUER MUDANÇA NO PROTOCOLO DEVE SER INFORMADA IMEDIATAMENTE AO CEUA PARA FINS DE ANÁLISE E APROVAÇÃO DA MESMA.

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Uberlândia, 03 de Dezembro de 2012

Profa. Dra. Ana Elizabeth Iannini Custódio
Vice Coordenadora *Pro tempore* da CEUA/UFU

Release para Imprensa

Modalidade: Pesquisa Científica.

Assunto: Tese defendida no Programa de Pós-Graduação em Odontologia – Faculdade de Odontologia, UFU.

Autores: Priscilla Barbosa Ferreira Soares; Orientador: Prof. Dr. Darceny Zanetta Barbosa.

A incidência de câncer de cabeça e pescoço é uma doença que cresce em toda população mundial, afetando principalmente adultos e idosos. Com o aumento do tempo de vida da população brasileira e mundial aliado a maiores acessos a tratamentos odontológicos como o uso de implantes dentais, um senário desafiador se estabelece: pacientes vivendo mais com implantes dentais instalados que desenvolvem câncer de cabeça e pescoço e necessitam de tratamento radioterápico. Pacientes acometidos por câncer apresentam marcante redução na qualidade de vida e quando são submetidos a radioterapia os efeitos colaterais parecem ser ainda maiores. Nosso grupo de pesquisa comprovou por meio de estudos em coelhos que a radioterapia afeta negativamente o tecido ósseo reduzindo sua resistência tornando-o menos propenso a reparar danos sofridos. Outra pesquisa também desenvolvida nesta tese mostrou que os implantes dentais produzidos por empresa nacional possuem adequada qualidade de osseointegração, aspecto importante para a maior longevidade clínica. E finalmente, outro achado importante é que pacientes que no passado receberam implantes dentais e que venham a desenvolver câncer de cabeça e pescoço e por isso necessitam de radioterapia sofrem com efeitos negativos da radioterapia tanto na superfície do implante como no osso ao redor da área que foi afetada pela radiação. A presença do metal no interior do osso acaba por aumentar o efeito negativo da radioterapia. Por tudo isso a busca constante por meios preventivos dos danos da radioterapia no osso e nos implantes dentais devem ser estimulados buscando criar mecanismos de aumentar a longevidade destes procedimentos, melhorando a qualidade de vida e a autoestima destes indivíduos que tanto sofrem pela luta contra o câncer.

Contato: Secretaria PPG Odontologia, Sra. Maria das Graças, Fone: (34) 32258115