UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE ODONTOLOGIA DE ARARAQUARA

ANA EMÍLIA FARIAS PONTES

AVALIAÇÃO DAS ALTERAÇÕES DOS TECIDOS AO REDOR DE IMPLANTES INSERIDOS EM DIFERENTES NÍVEIS EM RELAÇÃO À CRISTA ÓSSEA. ESTUDO CLÍNICO, RADIOGRÁFICO, E HISTOMÉTRICO EM CÃES

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Tese apresentada ao Programa de Pós-graduação em Periodontia da Faculdade de Odontologia de Araraquara, Universidade Estadual Paulista para obtenção do título de Doutor em Periodontia.

Orientador:
Prof. Dr. Elcio Marcantonio Junior
Co-orientador:
Prof. Dr. Joni Augusto Cirelli

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Araraquara, 01 de março de 2007.

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DEDICATÓRIA

À minha família, em especial aos meus pais, **Jaime Pontes da Silva** e **Hermelisa Farias Pontes**, minha gratidão eterna pelas demonstrações de amor e apoio, que há anos são o alicerce para vencer os desafios e continuar na busca dos meus ideais, superando assim os percalços da distância física; e aos meus irmãos e cunhados, **Angelisa Farias Pontes** e **Gerson Farias Borges**, **Jaime Pontes da Silva Filho** e **Rooselane Belchior Lima**, com quem sempre poderei contar...

Ao **Fernando Salimon Ribeiro**, dedico este trabalho que foi fruto de sua inteligência e esforço. Agradeço imensamente pela sua dedicação, a qual foi absolutamente imprescindível em todas as fases, desde a concepção da idéia, passando pela elaboração do projeto, realização da fase experimental, e análise dos resultados. Agradeço pelo privilégio da sua convivência, o que rendeu muitos momentos produtivos juntos, e que faz parte de um projeto muito maior, de estudo e de vida. Sou muito agradecida também à sua família, principalmente ao **Dr. José do Amaral Ribeiro** e à **Sra. Arlete Miriam Salimon Ribeiro**, que sempre me acolheram maravilhosamente bem. Muito obrigada.

AGRADECIMENTOS ESPECIAIS

Ao **Prof. Dr. Elcio Marcantonio Junior**, pelo exemplo de competência e profissionalismo. Agradeço por ter assumido a orientação deste estudo, me proposto novos desafios, e me transmitido confiança, que foram fundamentais para meu aprimoramento profissional no decorrer deste curso de Doutorado.

Ao **Prof. Dr. Joni Augusto Cirelli**, pela orientação deste estudo, e por me ter direcionado, e ajudado a aperfeiçoar minha argumentação. Agradeço ainda por ter aceitado o desafio de desenvolver esta tese em uma linha de pesquisa nova para ambos.

À **Profa. Dra. Rosemary Adriana Chiérici Marcantonio**, por ser um símbolo de retidão e capacidade profissional, e por seus sorrisos sempre sinceros.

Ao **Prof. Dr. Benedicto Egbert Corrêa de Toledo**, por ter me aceitado neste Programa ainda como estagiária, e por ter confiado em mim durante todos estes anos.

Ao **Prof. Dr. Adriano Piattelli**, exemplo a ser seguido de generosidade, dedicação à pesquisa científica, e capacidade de liderança. Agradeço pela atenção durante minha estada na Itália, e pelas constantes demonstrações de confiança.

Al Professore Adriano Piattelli, un esempio di generosità, dedicazione alla ricerca scientifica, e capacità di direzione. La ringrazio per l'attenzione durante il mio soggiorno in Italia, e per le costanti dimostrazioni di fiducia.

Às colegas **Vanessa Camila da Silva** e **Daniela Leal Zandim** pelas inúmeras demonstrações de apoio e incentivo no decorrer de toda a fase experimental desta tese.

Ao **Dr. Rogério Margonar**, excelente colega e profissional, que foi responsável pela fase protética deste estudo. Muito obrigada pelo seu apoio e disponibilidade.

À **Dra. Giovanna Iezzi**, minha querida amiga, e competente colega de laboratório, venho demonstrar toda minha gratidão e admiração.

Alla mia cara amica, e brava collega di laboratorio, Dottoressa Giovanna Iezzi, vorrei esprimere tutta la mia gratitudine e ammirazione.

Aos amigos **Beatriz Maria Valério Lopes**, **Eduardo de Paula Ishi**, e **Ricardo Vieira Garcia**, doces presenças nos momentos solenes e nas situações mais corriqueiras. Vocês estão em meu coração.

AGRADECIMENTOS

À Faculdade de Odontologia de Araraquara, Universidade Estadual Paulista (UNESP), na pessoa de sua Diretora Profa. Dra. Rosemary Adriana Chiérici Marcantonio, e Vice-Diretor Prof. Dr. José Cláudio Martins Segalla, por ter permitido o desenvolvimento desta pesquisa em suas instalações.

Ao Programa de Pós-Graduação em Periodontia da Faculdade de Odontologia de Araraquara - UNESP, na pessoa de seu Coordenador Prof. Dr. Carlos Rossa Júnior, e Vice-Coordenador Prof. Dr. Elcio Marcantonio Junior, pela minha aceitação no quadro de alunos, e pelo apoio à realização desta pesquisa.

Aos professores do Programa de Pós-Graduação em Periodontia da Faculdade de Odontologia de Araraquara - UNESP, Prof. Dr. Benedicto Egbert Corrêa de Toledo, Prof. Dr. Carlos Rossa Junior, Prof. Dr. Elcio Marcantonio Junior, Prof. Dr. Joni Augusto Cirelli, Prof. Dr. José Eduardo Cezar Sampaio, Prof. Dr. Ricardo Samih Georges Abi Rached, Profa. Dra. Rosemary Adriana Chiérici Marcantonio, e Profa. Dra. Silvana Regina Perez Orrico, pelo exemplo de competência e dedicação à ciência.

Aos professores do Programa de Pós-Graduação em Periodontia da Faculdade de Odontologia de Ribeirão Preto, Universidade de São Paulo, Prof. Dr. Arthur Belém Novaes Júnior, Profa. Dra. Daniela Bazan Palioto Bulle, Prof. Dr. Márcio Fernando de Moraes Grisi, Prof. Dr. Mário Taba Júnior, e Prof. Dr. Sérgio Luís Scombatti de Souza, pela minha iniciação à vida acadêmica durante o curso de mestrado, e pelos intensos momentos de convívio, que representaram um marco na minha vida.

A todos os colegas do Programa de Pós-Graduação em Periodontia da Faculdade de Odontologia de Araraquara, em especial aos queridos Andréa Márcia Marcaccini, Aline Cavalcante Viana, Alliny Souza Bastos, Cliciane Portela Silva, Débora Aline Silva Gomes, Denise Carleto Andia, Fábio Renato Manzolli Leite, Juliana Rico Pires, Yeon Jung Kim, Romeu Bellon Fernandez Filho, e Teresinha Costa de Santana, obrigada pela amizade e companheirismo em momentos de dificuldade e descontração.

Aos meus colegas de turma, Carla Raquel Fontana, Carlos Augusto Nassar, Joseane Maria Dias Bosco, e Sabrina Carvalho Gomes, pelo privilégio do convívio com pessoas tão capazes, e de formações tão variadas.

Às funcionárias da Faculdade de Odontologia de Araraquara, Ana Cláudia Gregolin Costa Miranda, Mara Cândida Munhoz do Amaral, Maria do Rosário Bento Clemente, Maria José da Silva Miquelon, Regina Lúcia da Silva, e Rosângela Aparecida Silva dos Santos, pela amizade, carinho, e eficiência.

Aos meus companheiros da Universidade "G. d'Annunzio" Chieti - Pescara, Itália, Alessandro Piccirelli, Prof. Dr. Antonio Scarano, Dra. Elisabetta Fiera, Dra. Giovanna Petrone, Dra. Vittoria Perrotti, e Dr. Tonino Traini, obrigada.

Ai miei compagni dell'Università "G. d'Annunzio" Chieti – Pescara, Italia, grazie.

Ao Dr. Alessandro Galhardo e Wellington Leão Favero, pela grande ajuda no manejo com os animais.

E finalmente, à FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo, Auxílio à Pesquisa, Processo 04/08141-3), ao CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Bolsa de Doutorado, Processo 141204/2004-4), e à CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Bolsa de Doutorado-Sanduíche, Processo 0989/05-3) agradeço pelo suporte financeiro a esta pesquisa; e à Conexão Sistema de Prótese Ltda., por prover os implantes dentários e demais componentes utilizados neste estudo.

SUMÁRIO

PREFÁCIO

Esta tese é constituída de quatro trabalhos:

Duas revisões de literatura desenvolvidas durante o estágio do Programa de Doutorado Sanduíche, na Universidade "G. d'Annunzio" Chieti – Pescara, Itália:

- **Capítulo 1**, artigo científico preparado para a *Consensus Conference* da Academia Européia de Osseointegração, publicado no periódico *Clinical Oral Implants Research* (autorização para publicação no Anexo 1); e

- **Capítulo 2**, capítulo do livro *"Estetica in Implantologia"*, encaminhado para publicação em sua versão em italiano.

Dois artigos científicos decorrentes do projeto de pesquisa desenvolvido durante o curso de doutorado nesta instituição:

- **Capítulo 3**, artigo científico com a análise dos dados clínicos e radiográficos, submetido para publicação no periódico *Journal of Periodontology*; e

- **Capítulo 4**, artigo científico com a análise dos dados histométricos, submetido para publicação no periódico *Clinical Oral Implants Research.*

LISTA DE ABREVIATURAS

ES: extensão do epitélio sulcular (análise histométrica)

EJ: extensão do epitélio juncional (análise histométrica)

IG: índice gengival (análise clínica)

ISS: índice de sangramento à sondagem (análise clínica)

JIC: junção implante-conector protético (análise radiográfica e histométrica)

JPC: junção prótese-conector protético (análise clínica)

NIR: nível de inserção relativo (análise clínica)

pCOI: primeiro contato osso-implante (análise radiográfica e histométrica)

POL: perda óssea lateral (análise radiográfica e histométrica)

PS: profundidade de sondagem (análise clínica)

PTM: posição do tecido marginal (análise clínica e histométrica)

TC: extensão do tecido conjuntivo (análise histométrica)

RESUMO

Pontes AEF. Avaliação das alterações dos tecidos ao redor de implantes inseridos em diferentes níveis em relação à crista óssea. Estudo clínico, radiográfico, e histométrico em cães [Tese de Doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2007.

O objetivo do presente estudo foi avaliar alterações ao redor de implantes inseridos em diferentes níveis em relação à crista óssea, e sob diferentes protocolos de restauração. Trinta e seis implantes foram inseridos nas mandíbulas edêntulas de 6 cães. Três implantes foram instalados por hemi-mandíbula, cada qual representativo de um grupo experimental. Estes foram determinados de acordo com a distância entre a junção implante-conector protético (JIC) e a crista óssea: *Ao Nível* (ao nível da crista óssea), *Menos 1* (1mm apical à crista óssea), ou *Menos 2* (2mm apical à crista óssea). Cada hemi-mandíbula foi submetida a um protocolo de restauração: convencional (prótese instalada 120 dias após a implantação) ou imediata (prótese instalada 24 horas após a implantação). Parâmetros clínicos, radiográficos, e histométricos foram avaliados após 90 dias de restauração, e os dados foram analisados estatisticamente (α=5%). A posição da margem do tecido mole (PTM) não foi influenciada pelo posicionamento apical da JIC; entretanto, sítios submetidos à restauração imediata tiveram a PTM significantemente mais coronal que os submetidos à restauração convencional ($p=0,02$, análise clínica). A Reabsorção do Rebordo não foi estatisticamente diferente entre os grupos ($p>0,05$, análise radiográfica). Menores quantidades de Perda Óssea Lateral (POL) foram observadas nos sítios restaurados imediatamente em comparação com os restaurados convencionalmente ($p=0,006$, análise histométrica). Os presentes achados sugerem que o posicionamento apical da JIC não põe em risco a altura de tecidos periimplantares moles ou duros. Além disto, a restauração imediata foi benéfica para manter a PTM, e minimizar a POL.

Palavras-chave: Implantes dentários; prótese dentária; estética; espaço biológico; radiografia; histologia; modelos animais.

ABSTRACT

Pontes AEF. Biologic width changes around loaded implants inserted in different levels in relation to crestal bone. Clinical, radiographic, and histometric study in dogs [Tese de Doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2007.

The aim of the present study was to evaluate changes that occur around dental implants inserted in different levels in relation to crestal bone, under different restoration conditions. Thirty-six implants were inserted in the edentulous mandible of six dogs. Three implants were inserted per hemimandible, each one representing an experimental group according to the distance from the implant-abutment junction (IAJ) to the crestal bone: *Bone Level* (at crestal bone level), *Minus 1* (1 mm below crestal bone), or *Minus 2* group (2 mm below crestal bone). Each hemimandible was submitted to a restoration protocol: conventional (prosthesis installed 120 days after implant placement) or immediate (prosthesis installed 24 hours after implant placement). Clinical, radiographic, and histometric parameters were evaluated after 90 days of restoration, and data was analyzed statistically (α=5%). The position of soft tissue margin (PSTM) was not influenced by the apical positioning of the IAJ; however, sites submitted to immediate restoration had the PSTM displayed significantly more coronally than those submitted to conventional restoration ($p=0.02$, clinical analysis). Ridge Loss was not statistically different among groups ($p>0.05$, radiographic analysis). Greater amounts of Lateral Bone Loss (LBL) were observed for conventionally than for immediately restored sites ($p=0.006$, histometric analysis). These findings suggest that the apical positioning of IAJ did not jeopardize the height of peri-implantar soft and hard tissues. Moreover, immediate restoration was beneficial to maintain the PSTM, and to minimize LBL.

Keywords: Dental implants; prosthesis; esthetics; biologic width; radiography; histology; models, animal.

1 INTRODUÇÃO

Um dos maiores desafios da Implantodontia é garantir resultados estéticos para os pacientes. Por isto, a manutenção da altura dos tecidos periimplantares em uma posição semelhante à do dente natural tem sido o foco de atenção de pesquisadores e clínicos.

Com base em um estudo histométrico em animais, Hermann et al. [18] concluíram que, em comparação com implantes de duas peças, os implantes de uma peça mantêm melhor a altura dos tecidos moles periimplantares. Entretanto, como próteses não foram usadas, a influência do carregamento não foi avaliada.

Por outro lado, Garber et al. [13] reportam que mesmo os proponentes do uso de implantes do sistema de estágio único, para obter melhora estética, consideram mais adequado o uso de protocolo de dois estágios em posição mais apical. A instalação de implantes deslocando a junção implante-conector protético (JIC) apicalmente permitiria o uso de cicatrizadores com perfil de emergência, contribuiria para a manutenção da textura e tonalidade da mucosa, e o restabelecimento da arquitetura dos tecidos marginais [20]. Saadoun et al. [24] e Wöhrle et al. [29] discutem a viabilidade de inserção de implantes 2 a 3 mm abaixo da junção cemento-esmalte dos dentes adjacentes, e sugerem a possibilidade de inserção em posição ainda mais apical.

A ocorrência de perda óssea significante tem sido relatada ao redor de implantes inseridos abaixo da crista óssea em comparação com implantes posicionados ao nível da crista ou acima [15]. No entanto, o posicionamento apical não é sempre relacionado à perda adicional da altura dos tecidos moles periimplantares [14]. É possível que, ao invés de migrar, estes tecidos sejam suportados pela crista óssea do dente ou implantes adjacente [26,27].

Por sua vez, estudos clínicos têm demonstrado que o protocolo de carregamento imediato tem impacto positivo na preservação de papilas [4,19,29]. Contudo, informações a respeito da resposta fisiológica à inserção de implantes em posição mais apical combinado à restauração imediata em comparação com restauração convencional não estão disponíveis na literatura, nem mesmo se tais modalidades de tratamento podem ser utilizadas com sucesso como alternativa válida em áreas estéticas.

Desta forma, o presente estudo foi desenvolvido para avaliar comparativamente as alterações ocorridas nos tecidos ao redor de implantes inseridos em diferentes níveis em relação à crista óssea, sob diferentes protocolos de restauração.

2 PROPOSIÇÃO

O objetivo do presente estudo foi avaliar comparativamente as alterações ocorridas nos tecidos ao redor de implantes inseridos em diferentes níveis em relação à crista óssea, e sob diferentes protocolos de restauração.

Objetivos específicos:

- Revisar a literatura referente ao contato dos tecidos moles com implantes e conectores protéticos (Capítulos 1 e 2);
- Avaliar clinicamente e radiograficamente as alterações ocorridas ao redor dos implantes e conectores protéticos (Capítulo 3)
- Avaliar histometricamente as alterações ocorridas ao redor dos implantes e conectores protéticos (Capítulo 4).

3 CAPÍTULO 1

Este capítulo é constituído pelo seguinte artigo de revisão de literatura:

Rompen E, Domken O, Degidi M, Pontes AEF, Piattelli A. The effect of material characteristics, of surface topography and of implant components and connections on soft tissue integration: a literature review. *Clin Oral Implants Res*. 2006; 17 (supplement 2); 55-67.

The effect of material characteristics, of surface topography and of implant components and connections on soft tissue integration: A literature review.

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INTRODUCTION

Soft tissue interface

To be functionally useful, oral implants have to pierce the gingiva or oral mucosa and enter the oral cavity, thus establishing a transmucosal connection between the external environment and the inner parts of the body.

In order to avoid bacterial penetration that could jeopardize either initial healing or long term behaviour of implants, the formation of an early and long-standing effective barrier capable of biologically protecting the peri-implant structures is mandatory. The establishment of this soft tissue barrier is a critical part of tissue integration and is fundamentally the result of wound healing that has to establish an effective interface between living tissues and a foreign body.

The soft tissue interface has been histologically assessed in animals and has a dimension of 3 to 4 mm in the apico-coronal direction called “biological width”. The interface consists of two zones, one of epithelium, which covers about 2 mm of the surface, while the rest is devoted to connective tissue adhesion.

Both these tissues contribute to the establishment of the so-called biological width, which may prevent oral bacteria and their products from penetrating into the body (Abrahamsson et al. 1996, 1997, 1998, Berglundh et al. 1991, 1996, Buser et al. 1992, Cochran et al. 1997).

Junctional epithelium

Owing to its capacity to proliferate and to move on surfaces, the epithelium found at the border of the incision crosses over the bridge of the fibrin clot / granulation tissue that rapidly starts forming after implant / abutment installation.

Upon reaching the surface of the implanted component, it moves in corono-apical direction, giving rise to a junctional epithelium about 2 mm long (Listgarten 1996, Lindhe & Berglundh 1998).

It must be emphasized that the epithelium found on the border of the wound is oral epithelium; sulcular and junctional epithelium have different morphology, structure and phenotypic expressions. This means that the epithelium, during its apical

proliferation along the implant surface, is subjected to many influences, undergoing major morphological and functional modifications.

Once the epithelial cells have reached the implant surface, their attachment occurs directly via a basal lamina (< 200 nm) and the formation of hemidesmosomes (James & Schultz 1973, Listgarten & Lai 1975, Hansson et al. 1983, Gould et al 1984, McKinney et al 1985, Steflik et al 1993, Kawahara et al 1998). Hemidesmosomes can be formed already at 2-3 days of healing (Swope & James 1981). A study was conducted (Gould et al, 1984) to determine if the behavior of epithelium in vitro is similar to its attachment behavior in vivo by the use of small sections of titanium-coated implants that could be inserted in human gingiva. The size of the implants allowed their insertion in a limited region and enabled the fixation and embedding procedures that are necessary for electron microscopy to be effective. Examination of the thin sections obtained from this material demonstrated that the epithelial cells attached to titanium in a manner similar to that observed in vitro and similar to the way that epithelium attaches to the tooth in vivo that is, there was a formation of hemidesmosomes and basal lamina.

Another possible attachment modality, which has been hypothesized, is an indirect epithelium/implant contact (Kawahara et al. 1998).

It is generally recognized that the epithelium lining the peri-implant sulcus shares many structural, ultrastructural and functional characteristics with the corresponding gingival tissue. Studies conducted in humans (Carmichael et al. 1991, McKenzie & Tonetti 1995, Liljenberg et al 1997) indicate that the epithelium surrounding oral implants possesses patterns of differentiation and function similar to gingival epithelium.

The presence of granulation tissue adhering to the surface of transmucosal implant components is considered the principal factor which stops the epithelium from moving further apically (Listgarten 1996). The role of the connective tissue in preventing epithelium downgrowth has been clearly demonstrated in animal models (Squier & Collins 1981, Chehroudi et al. 1992). Berglundh et al. (1991) also speculated that the reason why the epithelium stops migrating in an apical direction could be the interaction between the soft tissue and the layer of titanium oxide. It seems that mature connective tissue interferes more effectively than granulation tissue with epithelial downgrowth (Chehroudi et al. 1992). At initial phases of the healing phase, the quality and stability of the fibrin clot adhesion to the surface of the transmucosal components

most probably plays a role in the formation and positioning of the junctional epithelium (Lowenguth et al, 1993).

Connective tissue adhesion

After installation of the transmucosal component, the healing of the connective tissue wound involves distinct processes: formation and (hopefully) adhesion of a fibrin clot to the implant surface, adsorption of ECM proteins and subsequently of connective tissue cells to the implant surface, transformation of the clot into granulation tissue, migration of epithelial cells on top of the fibrin clot / granulation tissue (Descouts & Aronsson 1999, Meyle 1999).

After maturation, the connective tissue portion, located between the barrier epithelium and the marginal bone, has been found to be poor in cells and in vascular structures, but rich in collagen fibers.

It is now known that the connective tissue can be divided into two zones. The inner zone is in direct contact with the implant/abutment surface and is 50-100 μm thick. It is rich in fibers, with few scattered fibroblasts that appear to be in close contact with the transmucosal component. This thin fibroblast-poor barrier next to the titanium surface probably plays a role in the maintenance of a proper seal between the oral environment and the peri-implant bone. This layer of connective tissue resembles a scar tissue (Buser et al. 1992, Berglundh et al. 1994, Abrahamsson et al. 1996, Cochran et al. 1997, Chavrier & Couble 1999, Schierano et al. 2002).

The rest of the connective tissue, the outer zone, is formed of fibers running in different directions, richer in cells and blood vessels (Buser et al. 1992).

Hansson et al. (1983) furthermore reported that connective tissue cells and collagen fiber bundles were consistently separated from the titanium dioxide surface by a 20-nm-wide proteoglycan layer.

For most authors, these fibers run a course more or less parallel to the implant surface. This observation was made both in human subjects (Akagawa et al. 1989, Chavrier et al. 1994, Liljenberg et al. 1996, 1997) and in animal models: monkeys (Listgarten & Lai 1975, Gotfredsen et al. 1991) and dogs (Berglundh et al. 1991, Ericsson et al. 1995, Abrahamsson et al. 1996, Cochran et al. 1997, Çomut et al. 2001).

For other authors, the fibers are not parallel to the implant surface but either run in various directions (Arvidson et al. 1996, Fartash et al. 1990); a perpendicular

orientation was also found, with implants harboring a porous surface (Schroeder et al. 1981, Deporter et al. 1988), their orientation being potentially influenced by the quality of the mucosa: the fibers tend to be parallel in alveolar mucosa, while they seem to be organized more perpendicularly in keratinized mucosa.

Apart from the orientation of the fibers, the major difference between the connective tissue around teeth and around artificial abutments is related to their connection to the natural or artificial root surface:

At a natural tooth, the dento-gingival collagen fibers are firmly inserted into the cementum and the bone, and oriented perpendicular or oblique to the tooth surface, serving as a barrier to epithelial migration, and thus impeding bacterial invasion (Gargiulo et al. 1961, Stern 1981).

In contrast, implants lack cementum: the orientation of the "attachment" fibers in the supracrestal soft tissue compartment is parallel to the implant surface and, more importantly, they are not inserted in the implant surface (Berglundh et al. 1991, Buser et al. 1992, Listgarten et al. 1992, Chavrier et al. 1994).

Consequently, the connective tissue adhesion at implant has a poor mechanical resistance as compared to that of natural teeth (Hermann et al. 2001). In other words, the gingiva at implants can hardly be qualified of "attached".

As the connective tissue interface is considered of paramount importance to support the epithelium and block its apical migration, this lack of mechanical resistance can potentially endanger the prognosis of oral implants: tearing at the connective tissue/implant interface could occur due to a lack of soft tissue stability, which could induce the apical migration of the junctional epithelium, accompanied by gingival recessions or pocket formation and by bone resorption.

Soft tissue interface

Comparison between implants and teeth

From a comparative study in dogs (Berglundh et al., 1991), it is known that the soft tissue interface is slightly longer at (two-piece) implants than at teeth: if the junctional epithelium has comparable dimensions at teeth and implants (2.05 mm versus 2.14 mm), the connective tissue is 1.12 mm long around teeth versus 1.66 mm around implants. Comparable results were described by Ericsson & Lindhe in 1993.

These results were obtained with transmucosal implant components made of machined titanium, and cannot be extrapolated to other materials.

Clinical evaluation of the soft tissue interface

Animal studies

Despite comparable histological dimensions of the soft tissue compartments at teeth and implants, it has been shown that, when a probe pressure of 0.5 N is used in dogs, the probe tip penetrates on average 0.7 mm deeper at implant sites (Ericsson & Lindhe, 1993).

The histological sections with probes in situ evidenced that, around implants, the tip of the probe ended apically to the junctional epithelium, close to the bone crest, explaining why the clinical probing depth is higher. This is in accordance with the results of Gray et al. (2005) in baboons.

Lang et al. (1994) showed that at low pressure (0.2 N), clinical probing was able to identify the connective tissue adhesion level. In contrast, the probe penetration exceeded the connective tissue level in inflamed sites.

Human studies

Some human studies have compared periodontal and peri-implant probing, and confirmed that 0.5 to 1.4 mm deeper measurements are generally found at implants (Quirynen et al 1991, Brägger et al., 1997, Mombelli et al, 1997, Chang et al., 1999), illustrating that at implants the probe tip ends somewhere in the connective tissue and that the significance of probing at implants and at teeth is different.

Soft tissues' stability over time

Animal data are available to indicate a stability of the soft tissue dimensions over a 12 months period in loaded or unloaded conditions at one- or two-piece implants (Cochran et al. 1997, Hermann et al. 2000 b, Assenza et al. 2003).

Using clinical indices, several studies have gathered data that strongly suggest a longstanding stability of the soft tissue interface at one- and two-piece titanium implants in human patients.

Data supporting this stability are for instance available at 12 (Cune et al. 2004), 24 (Bengazi et al. 1996), 36 months (Quirynen et al. 1991), and even 10 years

(Hultin et al. 2000, Karoussis et al. 2004a). In the Karoussis et al. study, probing pocket depth increased from 1 to 10 years of 0.24 mm at implants versus 0.27 mm at teeth, while the probing attachment level varied of 0.37 mm at implant versus 0.30 mm at teeth.

Aims of the paper

To improve the quality and stability of the soft tissue/implant interface is of paramount importance for the short and long-term prognosis of oral implants.

This goal can be reached through the combination of different approaches: the first approach is to use surgical techniques focused at preserving or re-creating a soft tissue environment made of fibrous, keratinized stable gingiva, combined with conservative prosthetic techniques in order to avoid damaging the so-called biological width.

In addition, some characteristics of the transmucosal components are also of a crucial importance to obtain an effective interface: we will here focus on the impact of material characteristics, of surface topography and of implant components and connections on the adhesion of epithelium and connective tissue.

Note: A high percentage of papers looking at the implant-soft tissue interface are in vitro studies using cell cultures. The findings made in this type of study can never be fully extrapolated to the clinical situation.

Meanwhile, it must be noted that, even in vivo, the epithelial tissue is composed of cells in direct contact with each other without an extra cellular matrix; they will also be in direct contact with the implant components through hemi-desmosomes and a basal lamina. This is very close from what will be reproduced in vitro.

On the other hand, connective tissue cells are dispersed in a dense extra cellular matrix. These cells do not normally get in direct touch with each other, but are rather connected to their proteic environment. They can get in direct contact with implant components, but the adhesion of the tissue is more dependent on collagen fibers.

In addition, in vivo, the formation of a fibrin network through which fibroblasts will have to secondarily migrate is the first step of connective tissue formation after implant / abutment surgery. In vitro experiments of fibroblasts adhesion to implant materials never reproduce fibrin polymerization before cell seeding, meaning that in vitro conditions are more artificial and distant from the in vivo situation for fibroblasts than for

epithelial cells. The presence of 5 to 10% of serum in the culture medium does not allow to properly mimicking the in vivo conditions.

Influence of material's characteristics on soft tissue integration

Chemical composition

The reaction of cells and tissues to implanted foreign bodies depends on the material's properties and its behaviour upon contact with the body fluids. It must be noted that the chemical composition of the bulk material is sometimes significantly different from that of the surface that is at the interface with the living tissues: some materials demonstrate a surface oxidation (such as titanium that exhibits a surface layer of titanium oxide), while the mode of preparation or of sterilization of others will result in chemical contamination of the surface.

As it came to be realized that the interaction of a biomaterial with its environment was governed largely by surface properties, the chemical characterization of the surfaces took on greater importance and increasingly sophisticate means of analysis have been brought into play. Currently it is not uncommon for surfaces to be characterized by their X-ray Photoelectron Spectroscopy (XPS) that enables specific elements and their chemical state to be assessed. For example, the thickness of the titanium oxide layer may be determined from the intensity ratio of the metal to oxide signal. Moreover, the presence of organic and other contaminants can be determined using XPS. This information is important because some sterilization techniques, such as autoclaving, can introduce significant amount of contaminants to the surface and mask the properties of the underlying titanium. Further chemical analysis of contaminants can be obtained by such methods as mass spectroscopy. The surface, used in some of the older literature reviewed here was not characterized by such sophisticated methods, but it appears that higher standards on surface characterization are now being applied by biomaterials journals.

In vitro studies

Ti, gold, Al2O3 and dental ceramic. Räisänen et al. (2000) studied, in vitro, how epithelial cells attach to 5 different dental material surfaces (titanium, Ti6Al4V titanium alloy, dental gold alloy, dental porcelain and aluminum oxide).

The efficacy of adhesion was evaluated by SEM and immunofluorescence microscopy with antibodies to vinculin and α6β4 integrin.

Epithelial cells adhered and spread more avidly on metallic surfaces (c.p. titanium, Ti6Al4V titanium alloy, dental gold alloy) than on ceramic surfaces (dental porcelain and aluminum oxide). Well-organized focal contacts and pre-hemidesmosomes were found on metallic surfaces, but not on porcelain and aluminum oxide.

Previously, Jansen et al. (1985) had found focal contacts, hemidesmosome-like structures and extracellular matrix contacts between epithelial cells and titanium, gold, hydroxyapatite and carbon apatite.

Simion et al. (1991) examined human gingival fibroblasts / implant materials interface in vitro using a specific but not elsewhere validated model. Their results show an effective cell growth on acid-etched titanium and titanium alloy, on gold and gold porcelain, a "tenacious" cell adherence being found only on etched titanium.

Säuberlich et al. (1999) found an effective cell adhesion to c.p. titanium, and non-significant improvement by surface treatment by sulphur dioxide plasma etching, by plasma nitration, by silane coating. Coating titanium with a poly-vinyl-chloride polymer had a deleterious effect.

When Ti6Al4V was compared to c.p. titanium (Eisenbarth et al. 1996), gingival fibroblasts demonstrated a rounded cell shape and a reduced area of spreading on the alloy, presumably because of a minor toxicity to vanadium or aluminum.

Ti nitrite also proved to be suitable for fibroblasts adhesion and growth (Groessner-Schreiber et al., 2003).

Modified dental ceramics. Kokoti et al. (2001) modified chemical composition and surface morphology of dental ceramics and evaluated them, in vitro, for their ability to support fibroblasts attachment and proliferation. Four modified ceramics were constructed from body or shoulder porcelain after treatment with CaO, or CaO and P2O5. These oxides were selected because they had proved to improve cell attachment in bioactive ceramics (bioglasses) (Häkkinen et al. 1988). All modified ceramics promoted cell proliferation as compared to controls, shoulder modified ceramics proving to be the most effective.

HA surfaces. Kasten et al. (1990) found higher epithelial cell adhesion on HA compared to c.p. titanium, but the extremely low number of samples limits the significance of their results.

Human gingival fibroblasts attachment to c.p. titanium proved to be significantly higher than to non-porous and porous hydroxyapatite (Guy et al. 1993).

Metal Oxides. Photolithographic techniques have been used (Scotchford et al, 2003) to apply strips of metal oxides to glass surfaces in such a manner that comparisons can be made on a side-by-side basis. Titanium, Aluminum and Vanadium have been produced in this way and the adsorption of cells and proteins on these surfaces studied. Titanium oxide provided the best substratum overall for cell adhesion.

Animal studies

Ti, gold, Al2O3, dental ceramic. Abrahamsson et al. (1998 b) observed, in a dog model, that abutments made of c.p. titanium or highly sintered aluminum based ceramic (Al2O3) allowed the formation of a mucosal attachment that included one epithelial and one connective tissue portion of about 2 mm and 1.5 mm respectively. At gold alloy or dental porcelain abutments, no proper attachment formed at the abutment level, but the soft tissue margin receded and bone resorption occurred. Nevertheless, images of epithelium adhesion, but not of connective tissue, to gold are shown in the paper. The mucosal barrier was thus partially established to the fixture portion of the implant. The observed differences may be the result of varying adhesive properties of the materials studied or of variations in their resistance to corrosion.

McKinney et al. (1985) had already evidenced the presence of hemidesmosomal adhesion of epithelial cells to aluminum oxide implants in dogs.

HA surfaces. Çomut et al. (2001) observed in a dog model an effective formation of a mucosal attachment on c.p. titanium and on HA coated titanium, with a parallel fibers orientation on all samples.

Other studies indicate a favorable soft tissue response to dense HA (Kurashina et al. 1984, Jansen et al. 1991). In an investigation of the gingival reaction to permucosal dense hydroxyapatite implants in dogs (Kurashina et al. 1984), bundles of collagen fibers are reported to terminate perpendicularly to the interface of the implants.

Single-crystal sapphire implants. Soft tissues surrounding titanium implants and single crystal sapphire implants present no qualitative structural differences (Arvidson et al. 1996).

The epithelial cells adjacent to the sapphire implant surface have a well-ordered basal lamina with cell membrane hemidesmosomes (Hashimoto et al. 1989).

Zirconia. Kohal et al. (2004) compared bone and soft tissue integration of rough titanium versus zirconia implants in a monkey model. They found an effective formation of a mucosal attachment at both implant materials, the mean length of connective tissue being 1.5 mm on zirconia versus 2.4 mm on titanium, without evidence of perpendicular fibers. These differences did not reach the level of statistical significance.

Human studies

Zirconia. Degidi et al. (2006) conducted a comparative immunohistochemical evaluation of peri-implant soft tissues of titanium and zirconium oxide healing caps in five patients. Statistically significant differences were observed, with an overall lower inflammatory level in tissues surrounding zirconium oxide healing caps than at titanium caps.

Otherwise, only case reports are available those show a satisfactory clinical outcome in humans of the soft tissues, but these reports are not conclusive.

Surface free energy (wettability)

The wettability of the surface can play an important role not only regarding protein adsorption but also regarding cell attachment and spreading.

This physicochemical property of the substratum may influence cellular adhesion through:

(1) Effects on the adsorption of proteins on non-wettable surfaces lead to a reduced amount of proteins on the material surface, and the strength of adhesion of the molecules is reduced as well;

(2) Alteration of the conformation of adsorbed proteins can result from differences in the molecular sites contacting the material surface. The conformational changes can lead to differences in the expression of ligand sites interacting with cellular receptors (Colvin 1983).

Increasing wettability influences fibroblast attachment (Altankov et al. 1996, Lampin et al. 1997) and spreading (Ruardy et al. 1995).

Improvements in fibroblasts' adhesion in relation to surface cleanliness and wettability were shown with germanium and Co-Cr-Mo implants (Baier et al. 1984). No data were found concerning materials currently used for oral implants.

Surface contamination

A clean surface has a high surface energy, while a contaminated one has a lower surface energy (Kasemo & Lausmaa 1988).

Chemical contamination by cleaning, disinfection or sterilization procedures

The ultimate goal of cleaning procedures should be to remove the contaminants and restore the elemental composition of the surface oxide without changing the surface topography, either after the fabrication process, after handling in the dental laboratory, or when transgingival components are re-used.

Although specific protocols have been developed, it proves to be rather difficult to effectively clean a contaminated titanium surface, most probably because of the strong binding of proteins and amino-acids (Rowland et al. 1995, Zoller & Zentner 1996, Steinemann 1998).

Krozer et al. (1999) investigated in vitro the adsorption of amino-alcohol to machined titanium surfaces, and the possibilities to chemically remove the adsorbed alcohols in order to recover a pristine titanium surface. It was shown that rinsing in water, saline solution, or 5% H2O2 did not remove the amino-alcohol from the surface, while exposure to ozone resulted in complete removal of the adsorbed amino-alcohol. The results show that the amino-alcohol used forms a stable and dense film at the implant surface in vitro. Presence of such a film most likely prevents re-integration to occur at the implant-tissue interface in vivo.

Vezeau (1996) evaluated the surface changes and effects on in vitro cell attachment and spreading brought about on prepared commercially pure titanium by multiple exposures to common sterilization methods. In vitro analysis of cell attachment and spreading using gingival fibroblasts were performed. Results indicated that steam autoclave sterilization contaminated and altered the titanium surface, resulting in decreased levels of cell attachment and spreading in vitro.

Keller (1990) had also observed that sterilization of cp titanium surfaces by steam autoclaving caused a surface alteration and contamination, and a reduction of fibroblast cell attachment and spreading, in vitro.

Contamination by blood, saliva or plaque

Zöller & Zentner (1996) studied in vitro the influence of contaminations of titanium by saliva or serum on initial attachment of fibroblasts. Pre-treatment with serum showed consistent enhancing effect on cell adhesion. In contrast, pre-treatment with saliva diminished significantly cell adhesion. These results suggest that exposure of transgingival components to saliva at placement might inhibit adhesion of gingival fibroblasts and thus indirectly induce epithelial downgrowth.

Kawahara et al. (1998) investigated in vitro cell contact to titanium surfaces and adhesive strength of epithelial cells and fibroblasts under the influence of dental plaque extracts. Epithelial cells exhibited higher adhesive strength values than fibroblasts. The plaque extracts had a greater effect in decreasing the growth rate of fibroblasts than that of epithelial cells. This study suggests that the difference in growth, contact, and adhesive strength of the epithelial and fibroblastic cells to titanium surfaces may promote apical epithelialization under exposure to dental plaque.

Mouhyi et al. (1998) tested the surface composition of failed and retrieved machined titanium implants after various cleaning and disinfection techniques. Cleaning in citric acid followed by rinsing with deionized water for 5 min followed by cleaning in ultrasonic baths with trichloroethylene and absolute ethanol gave the best results with regard to macroscopical appearance and surface composition.

Sennerby et al. (1989) retrieved titanium cover screws and either rinsed them in saline or subjected them to ultrasonic cleaning and sterilization. After implantation in the abdominal wall of rats, cover screws induced the formation of a thick fibrous capsule, when unused screws did not. None of the decontamination procedures was effective.

Sennerby & Lekholm (1993) implanted titanium abutments in rats, after intra-oral contamination in humans for 1 min or 2 weeks and either rinsing in saline or ultrasonic treatment in amino-alcohols. All pre-contaminated abutments induced an altered tissue response as compared to pristine abutments, irrespective of the cleaning procedure.

In contrast, Ericsson et al. (1996) failed to show differences in soft tissue reaction between pristine titanium abutments with various surface roughness and corresponding contaminated abutments.

Mouhyi et al. (2000) evaluated the soft tissue response to clinically retrieved and decontaminated cover screws. The cover screws were cleaned by using citric acid, sterile water, hydrogen peroxide or CO2 laser alone or combined. After cleaning, the cover screws were implanted in the abdominal wall of the rat for 6 weeks. It was concluded that only CO2 laser used alone or in combination with hydrogen peroxide may be used clinically for sufficient decontamination of titanium surfaces.

Coating with bioactive molecules

Epithelial cells and fibroblasts have different affinities for adhesive proteins of the extracellular matrix.

Dean et al. (1995) observed in vitro that a fibronectin coating enhanced gingival fibroblast attachment to smooth (machined), plasma-sprayed, and hydroxyapatite-coated titanium surfaces two-to threefold, but it was less effective on epithelial cell attachment. In contrast, coating surfaces with laminin-1, a component of epithelial cell basement membranes, resulted in three- to fourfold enhancement of gingival epithelial cell binding but has less effect on fibroblast attachment.

Tamura et al.(1997) and El-Ghannam et al.(1998) observed in vitro the enhancement of epithelial cell attachment, spreading and hemidesmosomes assembly on laminin-5 coated titanium alloy.

Type IV collagen has also been shown to provide an excellent substratum for epithelial cell attachment on titanium surfaces whereas vitronectin restrains attachment of epithelial cells, compared with non-coated titanium surfaces (Park et al. 1998).

Influence of surfaces' topography on soft tissue integration

Definitions

A large number of surface treatment processes are available to alter surface topography of titanium implants, including machining/micromachining, particle blasting, Ti plasma spraying, HA plasma spraying, chemical/electrochemical etching, and anodization.

The topographic features that are obtained on the implant surface can range from nanometers to millimeter, that is from below the cell-size scale to the tissue scale.

One approach to characterizing the topography of implant surfaces is that of Wennerberg & Albrektsson (2000) who use a confocal laser scanning profilometer. The topography of the surface is defined in terms of form, waviness, and roughness (fig. 1), with the waviness and roughness often presented together under the term texture (Thomas 1999). The form relates to the largest structure (profile) while the roughness describes the smallest irregularities in the surface. Typical surface roughness is described by 3 parameters: Sa, Scx and Sdr.

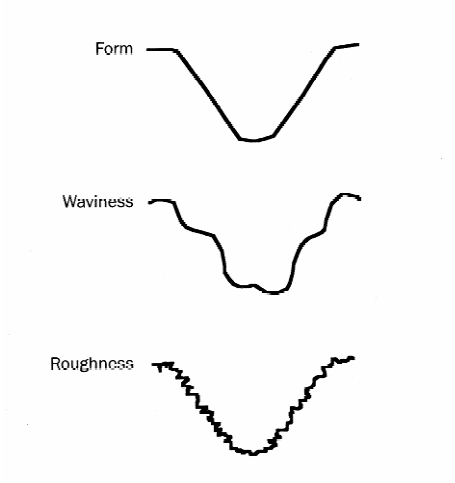

Figure 1. From Wennerberg & Albrektsson (2000).

A problem with the use of parameters based on averages, such as those listed above, is that surfaces with markedly different distributions of feature size may yield similar Ra values. Moreover fine roughness features that may be important for performance in a given application may not contribute significantly to the calculated overall roughness value if much larger features are also present. This discrepancy can occur when complex surfaces are prepared using different processes. For example, the Ra value of sand-blasted and etched surface will be largely determined by the contribution of the large surface features produced by grit blasting. A more sophisticated, albeit computationally intensive approach to this problem is the use of Fourier transforms to fit observed profiles of surfaces and enable roughness in different size ranges, termed

windows, to be determined and correlated with biological responses (Wieland et al. 2001).

Surface roughness can occur in two principal planes: one perpendicular to the surface and one in the plane of the surface (Thomas 1999). The orientation of the irregularities may be either isotropic or anisotropic. Surface structures without a dominating direction are called isotropic. Techniques to produce such surfaces include abrasive blasting, plasma-spraying, etching and oxidizing.

Other processes such as turning or milling result in a surface that has a distinct and regular pattern. Such a surface structure is denoted anisotropic.

Surface texture

Impact on protein adsorption

The composition of the protein film and the orientation of the molecules that are adsorbed on the implant surface may be affected by the surface roughness. Di Iorio et al. (2005) evaluated the fibrin clot extension in vitro on 3 different textures of c.p. titanium, and found that the surface microtexture complexity determines the formation of a more extensive and three-dimensionally complex fibrin scaffold.

This could be of crucial importance both for osseointegration and for the early formation of an effective connective tissue seal that would impair epithelial cells downgrowth.

Walivaara et al (1994) also showed that if the wettability of smooth titanium surfaces is correlated to fibrin adsorption, this correlation no longer exists on rough titanium.

François et al. (1997) showed a 50% decrease of fibronectin adsorption on acid-etched and on sandblasted and acid-etched (SLA) surfaces as compared to polished titanium.

Impact on cell and tissue adhesion

In vitro experiments. Hormia et al. (1991) compared the attachment and spreading of human gingival epithelial cells on three differently processed titanium surfaces (electropolished, acid-etched and sandblasted) by means of immunostaining. The results showed that epithelial cells attached and spread more readily on polished and etched titanium than on rougher surfaces (sandblasted titanium).

Könönen et al. (1992) and Hormia and Könönen (1994) showed the same results with human gingival fibroblasts.

Based on their model, smooth or finely grooved titanium surfaces could be optimal in maintaining the adhesion and specialized phenotype of gingival epithelial cells and fibroblasts. The authors also showed that the surface roughness of the substratum can affect the expression of integrin subunits.

Cochran et al. (1994) compared in vitro attachment and proliferation of human gingival or periodontal fibroblasts and epithelial cells grown on titanium surfaces with varying roughness (electropolished vs. fine or coarse sandblasted/acid-etched). Initial adhesion of fibroblasts was higher on smooth titanium, but their growth was good on all surfaces. Epithelial cells proliferation only happened on electropolished titanium.

Meyle (1999) showed that a sandblasted titanium surface delayed the adhesion and spreading of epithelial cells, while the corresponding features of fibroblasts and osteoblasts were enhanced.

Di Carmine et al. (2003) observed that a rough surface (sandblasted, Ra 2,14 μm) promoted the formation of multiple filopodia at the periphery of immortalized epithelial cells, while the cells were round and in direct contact with each other on smooth titanium (machined, Ra 0,8 μm) and on tissue culture plastic. They assume that the presence of filopodia suggests a higher level of adhesion. This assumption is dubious. It is not a normal behaviour for epithelial cells to display filopodia: in vivo and in vitro situations, these “cell-cup like” cells normally have a polygonal shape and are in close contact with each other. In addition, the SEM images in the paper clearly suggest that the epithelial cells are not in direct contact with the valleys of the roughened titanium, but rather bridge over the valleys.

Lauer et al. (2001) studied the adhesion, orientation and proliferation of human gingival epithelial cells (1) on glossy polished, (2) sandblasted and (3) plasma-sprayed titanium surfaces. Epithelial cells attached, spread and proliferated on all titanium surfaces with the greatest extension on the polished rather than on plasma-sprayed surfaces. Cells on polished surfaces developed an extremely flat cell shape, but on sandblasted and plasma-sprayed surfaces a more cuboidal shape.

Mustafa et al. (1998) observed that human gingival fibroblasts initially attach more to polished aluminum oxide abutments, but display a higher rate of proliferation on rougher Al2O3.

A recent paper from Baharloo et al. (2005) compared the adhesion, spreading and growth of epithelial cells on polished, rough grit-blasted, acid-etched and grit-blasted and acid-etched titanium (SLA). They evidenced a negative effect of titanium roughness on epithelial cells growth and spreading. As assessed by immunofluorescence staining for vinculin, they showed that epithelium formed less and smaller focal adhesions on rough titanium, suggesting that epithelial cells on rough surfaces are more susceptible to mechanical removal.

They also demonstrated that focal adhesions were primarily located on the ridges rather than the valleys on rough surfaces, with a tendency to bridge over the valleys, which confirms the images of Di Carmine et al. (2003). TEM measurements demonstrated this phenomenon: the average cell to titanium distance increased as the surface roughness increased.

Animal experiments

In a dog model, Abrahamsson et al. (2002) compared the soft tissue integration of turned (Sa 0.22 µm, Sdr 3.26%) versus acid-etched (Sa 0.45 µm, Sdr 8.57%) titanium abutments. They demonstrated that the soft tissue adhesion was not influenced by this kind of roughness of the transmucosal titanium components. The connective tissue fibers were found parallel both at smooth and at rough abutments.

In the past, a perpendicular orientation of the connective fibers had been found by some authors, particularly with implants harboring a porous surface (Schroeder et al. 1981, Deporter et al. 1988, Buser et al. 1992).

Their orientation appeared to be influenced by the quality of the mucosa: the fibers tended to be parallel in alveolar mucosa, while they seemed to be organized more perpendicularly in keratinized mucosa.

Human studies

Glauser et al. (2005) studied histometrically, in human biopsies, the soft tissue formed around one-piece micro-implants with different surface topographies (turned, oxidized or acid etched). The overall height of the soft tissue seal was approximately the same for all surfaces. However, the length of the junctional epithelium was higher on smooth titanium (2.9 mm) than for rough surfaces (1.4 - 1.6 mm), with an inverse relationship for the length of the connective tissue. The limited number of samples unfortunately limits the impact of their findings.

Contact guidance

Impact on cell and tissue adhesion

An isotropic surface texture may influence growth and proliferation of cells, leading to contact guidance, which depends upon the micropattern and size of the different geometrical elements. Contact guidance refers to the tendency of cell locomotion to be guided or directed by the dominating direction of the surface topography of the substratum to which the cells are adhering.

Brunette et al. (1983) reported that cells outgrowing from gingival explants are guided by grooves of a titanium-coated silicon wafer. Grooved surfaces were also found to orient fibroblasts and epithelium (Brunette 1986, 1987). Similar observations were made by Inoue et al. (1987), who found that circumferential grooves on Ti surfaces guide fibroblasts to form oriented capsule-like structures, whereas cells grown on porous surface showed no preferred orientation. Subsequent work demonstrated that there was a hierarchy in cell response to features, with larger features dominating smaller ones (Brunette, 1986).

The effects of grooved topography are considerable: Dunn & Brown (1986) showed the relationship between surface textural configuration and the shape that cells assume when cultured on it: they determined that 90% of cell shape, specifically elongation, was determined by the surface texture.

Moreover, cells can be exquisitely sensitive to features, features as small as 0.2 μm, having been observed to produce a cell response (Clark, 1987). Meyle et al. (1993) suggested that focal adhesions are mostly seen on ridges instead of contacting the surface in the groove, depending upon the groove's width and depth.

A considerable body of literature has now developed and reviews are available from some of the most active laboratories in this field (Brunette, 2001; Curtis, 1998).

Surface's form

Impact on cell and tissue adhesion

In vitro experiments. Chehroudi et al. (1988) and Chehroudi et al. (1989) studied in vivo and in vitro the effects of a grooved (V-shaped grooves, 10 μm deep) titanium-coated

substratum on epithelial cell behaviour. More epithelial cells were found attached to the grooved titanium surfaces than to adjacent flat surfaces. Clusters of epithelial cells were markedly oriented along the long axis of the grooves. In the grooved portion of the implant, epithelial cells interdigitated into the grooves and had rounded nuclei.

Histomorphometric measurements indicated that there was a shorter length of epithelial attachment, a longer length of connective tissue attachment, and less recession in the grooved, compared to the smooth portion of implants after 7 and 10 days.

These results indicate that horizontal grooves produced by micromachining can significantly impede epithelial downgrowth on titanium-coated epoxy implants.

The same authors (1990-1991) studied the effect of varying groove parameters such as depth, spacing, and vertical/horizontal orientation on epithelial downgrowth and attachment of epithelial cells and fibroblasts to percutaneous implants in vivo.

Close attachment of epithelial cells was found on the smooth, 10 µm and 3 µm deep, horizontally or vertically aligned grooved titanium surfaces; in contrast, epithelial cells bridged over the 22-microns-deep, horizontally oriented grooves. Although epithelium was in contact with the flat ridges between the 22-µm grooved surfaces, the cell nuclei were rarely found inside the 22-µm grooves.

Fibroblasts formed a capsule on the smooth surface as well as the 10 µm and 3 µm deep horizontally oriented grooves, but they inserted obliquely into the 22 µm deep, horizontally aligned grooved surface, with nuclei located within the grooves.

Epithelial downgrowth was accelerated on the vertically oriented grooved surfaces and inhibited on the horizontally oriented grooved surfaces. Moreover, the mechanism of inhibition of the epithelial downgrowth may differ among these surfaces. Epithelial cells bridged over the 22-µm deep grooves and their migration appeared to be inhibited by the fibroblasts that inserted into the implant surface. Thus, the optimal surface topography for cell attachment to implants may differ for different cell types.

However, in those studies, connective tissue and epithelium interacted with the same surface so that the effects of the surfaces on each population could not be determined separately.

In 1992, the same authors examined cell behaviour on implants in which connective tissue contacted grooved topographies and epithelium encountered only a smooth surface: at grooved surfaces, the orientation of fibroblasts changed from an

oblique to a more complex pattern, which included cells having round nuclei within the grooves, as well as cells oriented oblique or perpendicular to the grooves.

The apical migration of the epithelium was significantly inhibited by those micromachined surfaces due to an improved connective tissue anchorage.

Influence of implant's components and connections on soft tissue integration

Definitions

In a one-piece implant, the transmucosal component facing the soft tissues makes part of the implant.

In a two-piece implant, the transmucosal component (the abutment) dedicated at soft tissue integration is a separate part from the implant body. The interface between the transmucosal component and the implant is generally located in the neighbourhood of the alveolar bone level.

A one-piece implant is, in general, placed according to a one-stage surgery where the implant immediately pierces the soft tissue's barrier (non submerged fashion), when a two-piece implant system can either be submerged under the soft tissues for a waiting period (two-stage surgery) or be placed according to a one-stage surgery like one-piece implants.

Influence of surgical procedure on soft tissue integration

Animal studies

Several studies have looked at the potential impact of a submerged or non-submerged placement of implants on the localization, the type and the dimensions of the soft tissues.

Weber et al. (1996) found no difference in neither the global dimensions of the soft tissue interface nor in the bone level and length of connective tissue between submerged and non-submerged implants, but a longer junctional epithelium with two-stage surgery. These results were obtained using experimental implants.

With Brånemark two-piece implants (Ericsson et al. 1996, Abrahamsson et al. 1999), the dimensions and position of the soft tissues were found similar in both types of surgical approach.

Human studies

No clinical experiment has specifically compared the soft tissue integration after one- or two-stage surgery, but a number of clinical studies have looked at the marginal bone levels, which allow us to draw some conclusions, since a stable bone level implies that the soft tissue integration has not migrated apically. It has been demonstrated that there is no difference in marginal bone resorption, even in the long-term perspective, between one- and two-step surgical approaches with two-piece Brånemark implants (Petersson et al. 2001, Ericsson et al. 1994, 1997).

Soft tissue integration at one- or two-piece implants

Animal studies

Comparative studies were performed in dogs to determine the influence of implant design on soft tissue integration. Abrahamsson et al. (1996) demonstrated that the dimensions of the junctional epithelium and of the connective tissue are similar on one-piece implants (Straumann) and on two-piece implants (Brånemark system® and Astra Tech®). In addition, their position relative to the bone crest was also comparable, with the soft tissue integration located on the smooth implant's neck on one-piece implants and at the abutment level on two-piece implants.

Using the same experimental conditions, but after 6 months of undisturbed plaque accumulation, it was shown (Abrahamsson et al. 1998 a) that the extent of the plaque-related inflammatory infiltrate was comparable around one- and two-piece implants.

Using experimental implants with either a one-piece or a two-piece design, Hermann et al. (2000a, 2001) showed significantly higher apical migration of the soft tissues and marginal bone resorption with two-piece implants, suggesting a role of the subgingival position of the abutment/implant interface (so-called microgap) on tissue remodeling. It must be noted that in this experiment, all two-piece implants were clinically and histologically surrounded by an intense inflammatory process. This is in strong opposition with several animal studies (Abrahamsson et al. 1996,1997, 1998 a, b, 1999, 2001, 2002, Berglundh et al. 1991,1994,1996, Ericsson et al. 1995, Hermann et al. 2001, Lindhe & Berglundh, 1998) in which a soft tissue integration occurs at the abutment level.

In another experiment of the same group (Hermann et al. 2001), it was demonstrated that the size of the microgap between implants and abutments has little

influence on marginal bone remodeling, whereas micromovements of the abutments induce a significant bone loss, independent of the microgap's size. This strongly suggests that the mechanical disruption of the soft tissue interface is of importance.

An inflammatory cell infiltrate has been demonstrated at two-piece implants, in the close vicinity of the abutment/implant interface (Ericsson et al. 1995). This infiltrate does not impair the formation of effective soft tissue integration, and seems to be present at implants systems with an external implant/abutment connection as well as at systems with an internal morse taper connection, but not at one-piece implants (Abrahamsson et al. 1996, 1998).

In some experiments using commercially available implants, the infiltrate proved to be very limited in size (< 0.5 mm) and was not linked to a higher bone loss as compared to one-piece implants (Abrahamsson et al. 1996, 1998), while Broggini et al. (2003), with experimental implants, linked the 0.5 mm inflammatory infiltrate seen in their samples to a higher bone loss than at one-piece implants.

It has been shown that the seal provided by a locking taper connection at the implant/abutment interface effectively impairs bacterial leakage (Dibart et al. 2005). But it has not been clearly evidenced if the bacterial contamination of the internal components of some two-piece implant systems (Persson et al. 1996) is responsible for the inflammatory cell infiltrate seen at the abutment/implant interface.

Clinical studies

Several studies have demonstrated long-standing stability of the soft tissue interface and comparable marginal bone remodeling at both one-piece and two-piece implant systems (Cune et al. 2004, Bengazi et al. 1996, Quirynen et al. 1991, Hultin et al. 2000, Karoussis et al. 2004a).

Influence of abutment disconnection

The presence of a transmucosal component at two-piece implant systems can lead to intentional or unintentional disconnections of this abutment. Based on Hermann et al. (2001) results, an unintentional abutment loosening will lead to a disruption of the soft tissue integration and to increased bone remodeling.

It has also been shown that repeated intentional abutment disconnections and reconnections after alcoholic disinfection induces an apical repositioning of the soft tissues and marginal bone resorption (Abrahamsson et al. 1997). In contrast, a single

shift of a healing abutment and replacement by a final abutment proved to induce no marginal bone remodeling (Abrahamsson et al. 2003).

Conclusions

To be functionally useful, oral implants have to pierce the oral mucosa and enter the oral cavity, thus establishing a transmucosal connection between the external environment and the inner parts of the body.

In order to avoid bacterial penetration through this transmucosal piercing, the early formation of a long-standing effective barrier capable of biologically protecting the peri-implant structures is of paramount importance. It is a critical part of tissue integration, and may in part depend on:

Material chemistry

It is mandatory to place at the transmucosal level a material tissues can adhere to:

- c.p. titanium is the only material that has proven his biocompatibility towards the soft tissues in long-term clinical studies.
- Some favourable clinical data become available for zirconium and aluminum oxide
- Animal studies have shown that dental porcelain or gold are less biocompatible and should be avoided. Materials such as resins and composites should not be recommended up to now.
- The surface of the core material can be contaminated, altering the composition of the interface. Saliva has shown deleterious and hardly reversible effects in vivo. Other contaminations, such as handling in the dental laboratory , could also be detrimental.

It should be noted that, with one-piece implants, it is most unlikely to alter the composition of the transmucosal part, which will therefore always be biocompatible with currently commercially available one-piece systems.

Surface topography

No clinical studies are currently available on the effect of altered surface topographies on implant prognosis.

Results from in vitro and in vivo studies indicate that surface roughness and surface texture in the micrometer range may have an impact on the early events of healing by influencing attachment, orientation, proliferation and metabolism of epithelial and connective tissue cells.

- Some roughened titanium surfaces seem to improve the formation of a superficial fibrin network, which could hypothetically be positive for the initial stability of the interface and impair epithelial cells downgrowth.
- In vitro and in vivo studies tend to indicate that epithelial cells adhesion is lower on rough titanium surfaces than on machined titanium.
- Animal studies show that micromachined grooved surfaces of appropriate dimensions can improve connective-tissue ingrowth and inhibit epithelial downgrowth.

Implant components and connections

Comparative animal studies have shown equivalent soft tissue integration at one-piece implants and at abutments of two-piece implant systems.

These data are confirmed by long-term clinical studies demonstrating the stability of soft tissue integration and comparable marginal bone remodeling at both concepts.

It is meanwhile noteworthy that:

- At two-piece implants systems, animal studies have noticed a discrete inflammatory cell infiltrate at the abutment/implant interface, the effect of which on marginal bone level being limited and controversial.
- Unintentional or repeated intentional disconnections of the abutment at two-piece implant systems have been shown to disrupt the soft tissue integration and to induce an increased marginal bone remodeling.

As it is also more likely to place transmucosal components with an altered biocompatibility on two-piece implant systems (cf supra), effective soft tissue integration at one-piece implants seems easier to reproducibly obtain.

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4 CAPÍTULO 2

Este capítulo é constituído pelo seguinte capítulo de livro, aceito para publicação em sua versão em italiano:

Pontes AEF, Piattelli A. Contato dei tessuti moli alla superficie implantare. In: Assenza B, Leghissa G, Piattelli A. *Estetica in Implantologia*.

SOFT TISSUE CONTACT TO IMPLANT COMPONENTS

Titanium surfaces

Soft tissues around teeth and implant are very similar, and in both cases, there is an oral epithelium, continuous with a junctional epithelium. However, a relevant difference concerns the absence of cementum layer in peri-implantar structure. Consequently, while collagen fiber bundles in teeth are inserted in the cementum, and perpendicularly to the surface, in implant sites, a dense network of collagen fibers is observed, extending from the alveolar bone crest to the gingival margin, arranged parallelly in relation to the implant surface. There is no evidence of fiber insertion into the implant surface. (Berglundh et al. 1991, Listgarten 1992)

The epithelium formed after implant placement, consists of an internal basal lamina, composed by a lamina densa and lamina lucida, which is also observed on teeth. The contact to implant surface is reinforced by the presence of hemidesmosomes, and the secretion of laminins and fibronectin. In implant sites, hemidesmosomes are observed mostly in the lower region and rarely in the middle region; while in teeth sites, they are found throughout the interface (Dean et al. 1995, Ikeda et al. 2000). Laminins are a component of the basement membrane that contribute to epithelial cell migration and adhesion, and fibronectins are extracellular matrix proteins present in serum, which mediate cell attachment to subtract; both are detected at periodontal and peri-implant sites. (Degasne et al. 1999, Atsuta et al. 2005a, Atsuta et al. 2005b)

Connective tissue in contact to implant can be divided into 2 parts. The upper part, located under the JE, presents collagen fibers associated to type III collagen, and is relatively rich in fibroblasts (with a great number of secretory elements, which reflects an important turnover in this area). The lower part, closely bound to the implant, is poor in cells, and the extracellular matrix is represented by large and dense bundles of thick type I collagen fibers, which contributes to mechanical resistance and stability of the tissues (Chavrier & Couble 1999, Schierano et al. 2002).

Histological analysis in dogs revealed that periimplantar tissues present an inflammatory cell infiltrate at the level of implant/abutment junction, even if the animal is submitted to plaque control. It is suggested that this infiltrate represents an efforts by the host to limit the bacteria invasion, and this may contribute to the crestal bone loss observed after implant placement (Ericsson et al. 1995). In histological analysis of human tissues, absence of this inflammatory infiltrate was observed in the oral

epithelium and in its underlying connective tissue, nevertheless lymphocytes and macrophages could be found in adjacent connective tissue (Piattelli et al. 1997, Romanos & Johansson 2005).

The response of the host cells to different implant materials and topographies have been evaluated *in vitro* and *in vivo*. Laboratorial studies are performed using cell culture, in order to evaluate, among other characteristics, the morphology, orientation, proliferation and adhesion of them; while histological evaluation are performed in animals or humans to describe the physiological response of these cells to different surfaces and implant systems.

Considering specifically the **epithelial** cells, their phenotype, and attachment and spreading varies according to the surface (Lagneau et al. 1998). The initial attachment of cells on titanium (R_a = 0.05 μm), for example, is inferior to polystyrene (R_a = 0.03 μm) and glass surfaces (R_a = 0.03 μm) used as control (Shiraiwa et al. 2002), and higher than ceramic surfaces as alumina and dental porcelain (Raisanen et al. 2000).

Oral epithelial cells growth was evaluated in titanium sandblasted (R_a = 2.14 μm) and turned (R_a = 0.8 μm) surfaces. In sandblasted surfaces cells presented varied morphology with numerous, long and branched or dendritic filopodia closely adapted to the surface; while in turned surfaces they were display in a flat morphology (Di Carmine et al. 2003). A comparison among epithelial behavior in sandblasted (250 μm Al_2O_3 particles), plasma-sprayed, and polished titanium surfaces was performed. The authors concluded that those cells attached, spread, and proliferated with the greatest extension on the polished surface and with the lower extension on plasma-sprayed surfaces. Additionally, cells on polished surfaces presented a flap, and on the roughed surfaces developed a more cuboidal shape (Lauer et al. 2001).

The attachment and proliferation of oral **fibroblast** on titanium surfaces blasted with TiO_2 particles (mean particle sizes: 45μm, 45-63μm, or 63-90μm) were compared with turned surface, used as control. Human oral fibroblast culture was used, and the highest percentage of cell attachment was observed on the turned, and on surface blasted by 45μm-sized particles. The authors reported that an increase in diameter of the blasting particles inhibited cellular attachment, but no significant difference in the percentage of fibroblast cell attachment was observed among groups. (Mustafa et al. 1998)

The influence of titanium surface characteristic on gingival fibroblast morphology was demonstrated by using sand-blasted and acid-etched (R_a = 4.14 μm) and turned titanium surfaces (R_a = 0.54 μm). Sand-blasted and acid-etched surfaces showed cells orienting themselves along surface irregularities, and smooth surface exhibited a flat monolayer with cells oriented in a parallel manner. (Oates et al. 2005)

Currently, specific modifications have been proposed in the surfaces in order to create an ideal surface that could "modulate" the cellular behavior, for example by using laser (Khadra et al. 2005a, Khadra et al. 2005b); however, further studies are necessary.

The influence of the titanium surface topography has also been evaluated *in vivo*, and brief descriptions of the studies are presented in Table 1. In a general manner, no differences are observed among groups, and it is suggested that the roughness of the titanium surface has limited influence on the soft tissue attachment (Buser et al. 1992, Abrahamsson et al. 1996, Abrahamsson et al. 2001, Roccuzzo et al. 2001, Abrahamsson et al. 2002, Glauser et al. 2005).

Non-titanium surfaces

Ceramic materials as hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], alumina (Al_2O_3), and zirconia (ZrO_2) have been widely used in Implantology. Hydroxyapatite is frequently used as a coating material because of its property of "chemical bonding" to bone. However, a study that used this material to recover the lower part of the neck of the implant (the authors used the term "gingival fiber attachment zone" to describe this area) was presented. Dental implants with that zone with turned titanium surface (R_a = 0.2 μm), hydroxyapatite-coated by plasma-spraying (R_a = 1.8 μm), or hydroxyapatite coated by ion beam assisted deposition ('IBAD') (R_a = 0.2 μm) were inserted in dogs. Four months after implants placement, no histological signs of fragmentation or detachment of the hydroxyapatite coating was observed. Collagen fibers orientation was not different in the surfaces evaluated (in most sections, "vertical collagen fibers ran from bone to the epithelium, and horizontal fibers ran perpendicularly towards the implant surface and then, when close to the surface, became vertical"). No statistically significant differences were detected among groups concerning the percentage of the extension of tissue attachment (Çomut et al. 2001).

Table 1. Findings of comparative studies developed *in vivo* to compare differences among titanium surfaces.

Authors	Surface treatment	Findings	Details of the study
Buser et al. (1992)	(1) Sandblasted (2) "Fine" sandblasted (3) Turned	Differences were not observed among the surfaces, concerning the healing pattern of the soft tissues and the length of direct connective tissue contact	Histological analysis in dogs
Abrahamsson et al. (1996)	(1) Astra Tech Implants® Dental System (2) Brånemark System® (3) Bonefit® –ITI System	Mucosal barrier had similar composition among groups.	Histological analysis in dogs
Abrahamsson et al. (2001)	(1) Acid-etched (2) Turned	No significant differences were observed regarding soft tissues structure between the surfaces.	Histological analysis in dogs
Abrahamsson et al. (2002)	(1) Acid-etched (2) Turned	Soft tissue attachment was not influenced by the roughness of the titanium surface.	Histological analysis in dogs
Roccuzzo et al. (2001)	(1) Sandblasted and acid-etched (2) Plasma-sprayed	No significant differences were observed concerning mean probing depth average or marginal bone loss between the two treatment modalities.	Clinical analysis in humans
Glauser et al. (2005)	(1) Oxidized (2) Acid-etched (3) Turned	Oxidized and acid-etched implants presented less epithelial downgrowth and longer connective tissue seal than machined implants.	Histological analysis in humans

Even though resorption and degradability of the hydroxyapatite was not reported in this study, it is a frequent phenomenon, which takes place when the material gets in contact to biologic environment (Collier et al. 1993). Thus, the viability of the use of hydroxyapatite as a coating material on abutments (two-piece implants) or in the neck portion of implants (one-piece implants) still should be confirmed.

On the other hand, alumina (Al_2O_3) and zirconia are biocompatible but also stable, with a color similar to the teeth. Alumina has been employed for sandblasting implant surface, and to produce abutments, resulting in satisfactory function and esthetics, however, clinical studies are required to confirm the long-term performance of this type of restoration (Heydecke et al. 2002). The development of an entire implant was proposed by using the so-called "single crystal sapphire" (α-Al_2O_3), which revealed high success rates in long-term evaluation (Fartash et al. 1996). A comparison, between the soft tissues formed surrounding single crystal sapphire and titanium implants, revealed no

qualitative structural differences between these surfaces (Arvidson et al. 1996). Additionally, epithelial cells and fibroblasts develop more avidly on this material and on alumina in comparison with plastic dishes used as control in cell culture experiments (Arvidson et al. 1991, Mustafa et al. 2005).

The use of zirconia has been studied in sandblasting procedure, and in the production of entire abutments and implants (Kim et al. 2000, Akagawa et al. 1993). The main difference between alumina and zirconia concerns on the mechanical properties, which are better for zirconia (Piconi et al. 1999). This material is reported to present a contact with soft tissue similar to that observed in titanium implants (Dubruille et al. 1999, Kohal et al. 2004). Ceramic copings, constituted by a combination of zirconia (30%) and alumina (70%) were tested, and the results revealed clinical success with esthetical, functional, and harmonious replacement of missing teeth, even after a long follow-up period. (Hurzeler et al. 2002, Kohal & Klaus 2004, Nuzzolese 2005, Schiroli et al. 2004, Doring et al. 2004).

In a monkey model, a comparison between transmucosal implants custom-made of zirconia or titanium was performed. Zirconia surfaces were sandblasted (Al_2O_3 particles), and titanium surfaces were sandblasted (Al_2O_3 particles) and acid-etched (H_2O_2 and HF). Qualitative and quantitative analysis concerning the periimplantar soft tissues were not able to detect differences between the groups (Kohal et al. 2004). In humans, a comparative evaluation of soft tissue formed around titanium and zirconia healing caps was performed. The inflammatory infiltrate was mostly present, and the extension of infiltrate was much larger in the titanium specimens. Titanium sites resulted in a higher rate of inflammation-associated processes represented for example higher values of microvessels density, in comparison to zirconia sites (Degidi et al. 2006).

The use of gold was evaluated in comparative studies. Cell culture study was performed to compare epithelial adhesion and spreading of the following surfaces: titanium, titanium alloy (Ti6Al4V), dental gold alloy (Au 74.5%, Ag 12.0%, Cu 9.0%, Pb 3.5%, Zn 1.0%, Ru <1.0%), alumina, dental porcelain, and glass (used as control). Therefore, epithelial cells adhered more avidly to metallic surfaces than to ceramic surfaces (Raisanen et al. 2000). In dogs, titanium, alumina, and gold alloy (Au 60%, Pt 19%, Pd 20%, Ir 1%) were evaluated as abutment material. Six months after the abutment connection, those ones made of gold or alumina presented no proper attachment at the abutment level, moreover, the soft tissue margin receded and bone resorption occurred. The authors suggest that the material used influences the location and quality of

attachment between soft tissues and implant (Abrahamsson et al. 1998). Brief description of the studies is presented in Table 2.

Table 2. Findings of comparative studies developed using different methodologies to compare differences among surfaces.

Authors	Surface treatment	Findings	Details of the study
Abrahamsson et al. (1998)	(1) Titanium (2) Alumina (3) Gold	Sites in which abutments were made of gold alloy or dental porcelain, no proper attachment was formed at the abutment level, but the soft tissue margin receded and bone resorption occurred.	Histological analysis in dogs
Raisanen et al. (2000)	(1) Titanium (2) Titanium alloy (3) Dental gold alloy (4) Dental porcelain (5) Alumina (6) Glass (control)	Epithelial cells adhere more avidly to all metallic surfaces evaluated than to ceramic surfaces (dental porcelain and alumina).	Cell culture
Çomut et al. (2001)	(1) HA-coated (plasma-spraying) (2) HA-coated (IBAD deposition) (3) Turned	No statistically significant differences were detected among groups concerning the percentage of the extension of tissue attachment, and on fibers orientation.	Histological analysis in dogs
Kohal et al. (2004	(1) Zirconia (2) Sandblasted and acid etched titanium	Qualitative and quantitative analysis of periimplantar soft tissues were not able to detect differences between the surfaces.	Histological analysis in monkeys
Degidi et al. (2006)	(1) Zirconia (2) Titanium	Titanium sites resulted in a higher rate of inflammation-associated processes than zirconia.	Histological analysis in humans

HA= Hydroxyapatite
IBAD = Ion Beam Assisted Deposition

Dimension of soft tissues around implants

Biologic width is a physiologically formed complex (Berglundh & Lindhe 1996) that represents the dimension of transitional tissues not only around teeth, but also around implants. It is composed by sulcus depth, junctional epithelium and connective tissue attachment (Figure 1) (Gargiulo et al. 1961). The present section focus on the evaluation of the soft tissues dimension around implants with different surfaces or submitted to different treatment plans.

Histology seems to be the best way to evaluate peri-implantar, but this analysis frequently is not viable. For this reason, in some studies, clinical measurement are performed by probing, which is also considered a reliable way to access the

dimension of soft-tissues and crestal bone around implants. The reader should consider that the resistance offered by soft tissues around teeth is greater than that around implants, and consequently the probe penetration tends to be more advanced in implant sites, in which the tip of the probe extends near by the alveolar bone (Ericsson & Lindhe 1993, Lang et al. 1994, Gray et al. 2005). Brief description of studies, which accessed the dimension of soft tissues around teeth and implants, are presented in Tables 3 and 4.

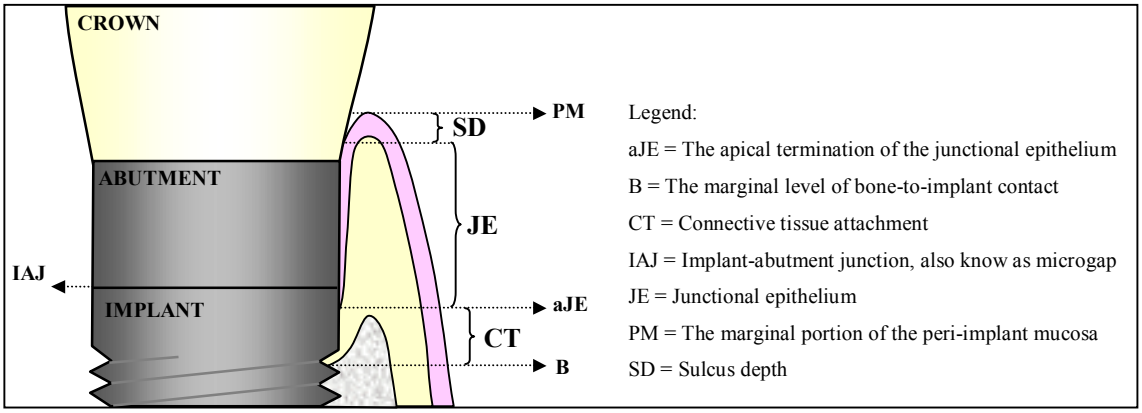

Figure 1. Critical parameters for the evaluation of biologic dimension around dental implants (Jalbout & Tabourian, 2004).

The influence of implant topography and design

The dimension of biologic width does not seem to be influenced by the topographic characteristic of the implant (as can be observed in Table 5), and is limitedly influenced by the implant design. (Abrahamsson et al. 2001, Abrahamsson et al. 2002, Roccuzzo et al. 2001, Glauser et al. 2005)

Table 3. Dimension of the soft tissues, around teeth in comparison to implants.

Authors	Dimensions around teeth (mm)	Dimensions around implant (mm)	Details of the study
Berglundh et al. (1991)	BW = 3.17 JE = 2.05 CT = 1.12	BW = 3.80 JE = 2.14 CT = 1.66*	5 dogs (3 implants each). Unloaded conditions. 6 months of healing. Histological analysis.
Ericsson & Lindhe (1993)†	GM-B = 2.5 JE = 1.5 CT = 1.0 P-B = 1.2 GM-P = 0.7 aJE-P = 0.2	PM-B = 3.3* JE = 1.7 CT = 1.6* P-B = 0.2* PM-P = 2.0 aJE-P = -1.3*	5 dogs (4 implants each). Unloaded conditions. Histometric measurement with a probe splinted to the tooth or abutment. 4 months of healing. Histological analysis.
Chang et al. (1999)	KM = 4.6 PD (f) = 2.5 PD (l) = 2.1 PD (p) = 2.5	KM = 3.9 PD (f) = 2.9 PD (l) = 3.5 PD (p) = 3.5	20 patients (20 implants). Single-tooth implant-supported crown. 6 months follow-up. Clinical analysis.
Kan et al. (2003)	BW (m) = 4.20 BW (d) = 4.20	BW (m) = 6.17 BW (f) = 3.63 BW (d) = 5.93	45 patients (45 implants). 2-stage procedure. One-year follow-up. Probing to bone analysis.

* P<0.05
† For this table, one experimental group was omitted.
(d) = on distal surface
(f) = on facial surface
(l) = on lingual surface
(m) = on mesial surface
(p) = on proximal surface
aJE = apical termination of the junctional epithelium
B = marginal bone level of bone-to-implant contact
BW = biologic width
CT = connective tissue contact
GM = gingival margin
JE = junctional epithelium
P = apical portion of the probe
PD = probing depth
PM = marginal portion of the peri-implant mucosa
KM= distance from PM to the mucogingival junction

An important variation on implant system design concerns the presence or absence of the microgap, which characterizes a “two-piece” or “one-piece implant”; however, others modifications as the length of the neck portion of one-piece implants are also discussed and further information are provided on “The influence of microgap” section.

Table 4. Evaluation of soft tissue dimensions in human.

Authors	Dimensions around implant (mm)	Details of the study
Buser et al. (1990)	PD = 2.74 DIM = -0.12 AL = 2.62 KM = 3.26	70 patients (100 one-stage implants). 12 months follow-up. Clinical analysis.
Piattelli et al. (1997)*	PD = 1.5 CT = 1.9	Case report, 1 patient (2 implants). 10 months after loading. Histological analysis.

* For this table, one dental implant was omitted.
AL = attachment level = PD + DIM
CT = connective tissue width
DIM = distance implant top-mucosa margin
KM = width of keratinized mucosa
PD = probing depth

Using implant systems commercially available, two studies investigated the length of their peri-implantar tissues (Table 6). In the first one, two two-piece systems ('Astra Tech Implants Dental System®' and 'Brånemark System®') and one one-piece implants ('Bonefit® –ITI System') were compared, but no differences statistically significant were observed among the groups (Abrahamsson et al. 1996). On the second study, two two-piece systems were evaluated ('Astra Tech Implants® Dental System' and 'Brånemark System'). In 'Astra' sites, the dimensions of height of the peri-implant mucosa (PM-B), marginal bone loss, and barrier epithelium (PM-aJE) were comparatively lower, however, the reader should consider the implants from the other groups were inserted in a more apical position (1.4 mm apical of the adjacent bone crest) (Berglundh et al. 2005).

The relevance of the length of the neck of one-piece implants was investigated as following. Twelve patients received bilaterally implants with different smooth neck portion lengths (2.8 mm or 1.8 mm). Twelve months later, the authors observed a greater relative attachment level dimension in longer neck sites (3.50mm) than in short neck sites (3.40mm). However, probing depth (3.30mm, for longer neck sites; and 3.37mm, for short neck sites) and bone level loss (0.86mm for longer neck sites; and 0.99mm for short neck sites) were not different between groups (Joly et al. 2003). In another study, the crestal bone level changes around two types of implants (2.8 mm or 1.8 mm collar) was investigated based on radiographic images. Sixty-eight patients (201 non-submerged titanium implants) were followed-up for up to 3 years after implant placement. No statistically significant differences were observed between both groups, thus, the authors concluded that implants with shorter smooth collar had no additional

bone loss, and that it may be useful to prevent the risk of metal exposed in aesthetic areas. (Hanggi et al. 2005)

Table 5. Dimension of the soft tissues, in difference surface topographies.

Authors	Dimensions around surfaces (mm)			Details of the study
Abrahamsson et al. (2001)	**Acid-etched surface**	**Turned surface**		Implant surfaces. 5 dogs (8 implants each dog). 6-months of healing period. Histological analysis.
	PM-B = 4.09 JE = 2.57 CT = 1.52	PM-B = 3.78 JE = 2.14 CT = 1.64		
Abrahamsson et al. (2002)	**Acid-etched surface**	**Turned titanium**		Abutment surfaces. 5 dogs (8 implants each dog). 6-months of healing period. Histological analysis.
	PM-B = 4.2 JE = 2.6 IAJ-B = 1.3 Size of ICI = 1.5 B-(c)abutment ICT = 1.0 B-(a)abutment ICT = 0.08	PM-B = 3.7 JE = 2.1 IAJ-B= 1.0 Size of ICI = 1.2 B-(c)abutment ICT = 0.8 B-(a)abutment ICT = 0.07		
Roccuzzo et al. (2001)	**Sandblasted and acid-etched surface**	**Plasma-sprayed surface**		Clinical trial. Randomized Controlled Trial. 32 patients (68 implants each group). 12-month follow-up.
	PD = 3.3 Bone loss = 0.65	PD = 2.9 Bone loss = 0.77		
Glauser et al. (2005)	**Oxidized Surface**	**Acid-etched surface**	**Machined surface**	Clinical Trial. 5 patients (12 implants). 8 weeks follow-up. Histological analysis.
	SD = 0.2 JE = 1.6 CT = 2.2	SD = 0.5 JE = 1.4 CT = 2.6	SD = 0.5 JE = 2.9 CT = 0.7	

(a) abutment ICT = apical level of the infiltrate at IAJ
(c) abutment ICT = coronal level of the infiltrate at IAJ
aJE = apical termination of junctional epithelium
AL = attachment level
B = marginal level of bone-to-implant contact
CT = connective tissue attachment
DIB = distance implant top-first bone-implant contact
DIM = distance implant top-mucosa margin
ED = extent of epithelial downgrowth
ICI= inflammatory cell infiltrate
IAJ = implant-abutment junction
PD = probing depth
PM = marginal portion of peri-implant mucosa
SD = sulcus depth

Table 6. Dimension of the soft tissues, in difference implant systems.

Authors	Dimensions around surfaces (mm)			Details of the study
Abrahamsson et al. (1996)	**'Astra' system** (two-piece implant, positioned at crestal bone level)	**'Branemark' system** (two-piece implant, positioned at crestal bone level)	**'Bonefit' system** (One-piece implant, the border of the neck was positioned at bone level)	5 dogs (6 implants each). Unloaded condition. 3 months of healing period. Histological analysis.
	PM-B = 3.11 JE = 1.64 IAJ-B = 0.57	PM-B = 3.42 JE = 2.14 IAJ-B = 0.62	PM-B = 3.50 JE = 2.35 IAJ-B = 0.50	
Berglundh et al. (2005)*	**'Astra' system** (two-piece implant, positioned at crestal bone level)	**'Branemark' system** (two-piece implant, positioned 1.4 mm below the crestal bone)		6 dogs (2 implants each). Unloaded conditions. 13 months of healing. Histological analysis.
	PM-B = 3.62 PM-aJE = 2.07 IAJ-B = 0.52	PM-B = 4.28 PM-aJE = 2.35 IAJ-B = 0.75		

* For this table, loaded implants were omitted.
aJE = apical termination of junctional epithelium
B = marginal level of bone-to-implant contact
IAJ = implant-abutment junction
PM = marginal portion of peri-implant mucosa

The influence of microgap

The microbiologic analysis of the internal surface of dental implant components in function for 1 to 8 years was evaluated, and no relation was observed between the number and type of microorganisms found in the samples the type and length of abutment, abutment stability, and bone loss. (Persson et al. 1996)

The bacteria present around the implant-abutment junction, however can lead to inflammation and bone loss around the implant-abutment junction (Dibart et al. 2005). In a histological analysis in dogs, a persistent acute inflammation was observed and investigated at the implant-abutment junction. Two-piece implants were placed at the alveolar crest and abutments connected either at initial surgery (non-submerged) or three months later (submerged), and the third implant was one-piece. The tissues surrounding two-piece implants resulted in a peak of inflammatory cells approximately 0.50 mm coronal to the microgap, consisted primarily of neutrophilic polymorphonuclear leukocytes. Around one-piece implants, however, no such peak was observed. Moreover,

significantly greater bone loss was observed for both two-piece implants compared with one-piece implants. The authors concluded that the absence of an implant-abutment interface, represented by the microgap was associated with "reduced peri-implant inflammatory cell accumulation and minimal bone loss." (Broggini et al. 2003) Moreover, the biologic width more similar to natural teeth was observed in one-piece non-submerged implants compared to either two-piece non-submerged or two-piece submerged implants (Hermann et al. 2001b).

However, in humans, comparisons of one-piece and two-pieces implants, inserted in one-stage or two-stage procedure did not reveal differences statistically significant among groups (Heydenrijk et al. 2002). Thus, the placement of the microgap at the crestal level does not appear to have an adverse effect on the amount of peri-implant bone loss. In a study, 60 patients were submitted to one of the following procedures: two-piece implants placed in a single-stage procedure; two-piece implants placed in the traditional two-stage procedure; and one-stage implants placed in a one-stage procedure. Clinical and radiographic evaluation was performed immediately after prosthesis placement and after 12 and 24 months. The results were not statistically different among groups. (Heydenrijk et al. 2003)

The influence of the vertical positioning of microgap was then investigated. In the first study, in dogs, dental implants were inserted, with the abutment/fixture junction positioned (a) 1 mm above, (b) at bone crest level or (c) 1 mm bellow bone crest. After 3 months of healing period, no significant differences were observed concerning junctional epithelium extension or connective tissue extension (Todescan et al. 2002). Furthermore, in monkeys, dental implants were positioned 1 to 2 mm above the alveolar crest; at the level of the alveolar crest; or 1 to 1.5 mm below the alveolar crest. These implants had been early loaded, immediately loaded, and inserted immediately postextraction. In the first group, a 0.13 mm bone increase was seen in the coronal direction. The authors reported that, "if the microgap was moved coronally away from the alveolar crest, less bone loss would occur and if the microgap was moved apical to the alveolar crest, greater amounts of bone resorption were present. This remodeling is not dependent on early and immediate loading of the implants or on immediate postextraction insertion" (Piattelli et al. 2003).

The influence of the size of microgap on crestal bone changes was also evaluated, in unloaded conditions. Dental implants were inserted in dogs, distributed into groups with different microgap sizes, welded or not to the abutment (to evaluate the

influence of micro-movements). Three months after implant placement, the authors observed that that crestal bone changes were significantly influenced by possible movements between implants and abutments, but not by the size of the microgap. (Hermann et al. 2001a, King et al. 2002)

The influence of the treatment plan

A histological evaluation of submerged (two-stage procedure) and non-submerged (one-stage procedure) implants was performed in dogs in all three studies described above (Table 7). Firstly, using a two-piece implant system, the following groups were considered: one-step group, in which implant and abutment were placed at the same section; or two-step group, in which the implant was inserted, and 3 months later, the abutment was placed. Six months later, no differences were observed between groups, considering the soft tissues dimensions evaluated (Ericsson et al. 1996). On the second study, the authors did not observe statistically significant differences between implants concerning distance implant top-mucosa margin, connective tissue contact. However, significant differences were observed for extent of epithelial downgrowth and attachment level. Thus, the authors concluded that based on these results, the apical extension of the epithelium is significantly greater and the attachment level significantly greather in submerged sites than in non-submerged one-stage implant (Weber et al. 1996). Finally, on the third study, the authors observed that the height of the mucosa, the length of the junctional epithelium and the height and quality of the zone of connective tissue integration were not statistically different between the submerged and non-submerged groups (Abrahamsson et al. 1999).

Comparisons about immediate and delayed implant placement were also performed. In a histological evaluation in dogs, according to the results obtained eight months after implant placement, the authors reported that immediate implants, due to bone resorption, presented a longer soft tissue-implant interface, but values concerning soft tissue-implant contact were not statistically different between groups (2.71 mm at immediate placement sites, and 2.14 mm at delayed placement sites) (Schultes & Gaggl 2001). The evaluation of the interproximal papilla levels after early or delayed placement of single-tooth implants revealed that the early placement of single-tooth implants may be preferable “in terms of early generation of interproximal papillae and the achievement of an appropriate clinical crown height, but no difference in papilla dimensions was seen at 1.5 years after seating of the implant crown” (Schropp et al. 2005).

Table 7. Dimension of the soft tissues, in submerged vs. non-submerged.

Authors	Dimensions around submerged implants (mm)	Dimensions around non-submerged implant (mm)	Details of the study
Ericsson et al. (1996)	PM-B = 3.9 JE = 2.4 PM-IAJ = 2.6 IAJ-B = 1.3 PM-aPICT = 1.6 B-aPICT = 2.3 B-(c)abutment ICT = 1.9 B-(a)abutment ICT = 0.8 aPICT-(c)abutment ICT = 0.5	PM-B = 3.5 JE = 2.1 PM-IAJ = 2.4 IAJ-BC = 1.1 PM-aPICT = 1.4 B-aPICT = 2.1 - - -	5 dogs (12 implants each). 6 months of healing. Histologic analysis. (Abutments were inserted 3 months after implantation.)
Weber et al. (1996)	DIM = 0.43 ED = 1.71 AL = 2.14 CT = 0.79 DIB = 2.92	DIM = 0.42 ED = 1.18* AL = 1.60* CT = 1.35 DIB = 2.95	6 dogs (38 implants). 6 weeks of healing. Histologic analysis. (Abutments were inserted 3 months after implantation.)
Abrahamsson et al. (1999)	PM-B = 3.00 JE = 1.85 CT = 1.16 IAJ-B = 0.85	PM-B = 3.15 JE = 1.97 CT = 1.18 IAJ-B = 0.68	6 dogs (6 implants each). Healing period varied from 3 to 6 months. Histologic analysis. (Abutments were inserted 3 months after implantation.)

* $P<0.05$
(a) abutment ICT = apical level of the infiltrate at IAJ
(c) abutment ICT = coronal level of the infiltrate at IAJ
IAJ = implant-abutment junction
AL = attachment level
aJE = apical termination of junctional epithelium
aPICT = apical level of plaque associated infiltrate
B = marginal level of bone-to-implant contact
CT = connective tissue attachment
DIB = distance implant top-first bone-implant contact
DIM = distance implant top-mucosa margin
ED = extent of epithelial downgrowth
PM = margin of peri-implant mucosa

The investigations concerning the dimensions around immediately loaded and delayed loaded implants are presented in Table 8. On the first study, in which patients were followed-up for at least two years, difference between groups were not found concerning probing depth (Romeo et al. 2002). Additionally, in a study developed in

monkeys, three months after loading, no significant differences were detected between groups, concerning sulcus depth, junctional epithelium, connective tissue contact, distance between the implant top and coronal gingiva, and distance between the implant top and first implant-to-bone contact (Siar et al. 2003).

Table 8. Dimension of the soft tissues, in immediately vs delayed loaded implants.

Authors	Dimensions around immediately loaded implants (mm)	Dimensions around delayed loaded implants (mm)	Details of the study
Romeo et al. (2002)	PD = 2.33	PD = 2.28	Clinical Trial, Randomized Controlled Trial. 20 patients. Follow-up: 2 year after loading. Clinical analysis.
Siar et al. (2003)	SD = 0.68 JE = 1.71 CT = 1.51 DIM = 2.27 DIB = 1.32	SD = 0.88 JE = 1.66 CT = 1.24 DIM = 2.38 DIB = 1.19	6 monkeys. 3 months of healing. Histologic analysis.

CT = connective tissue attachment
DIB = distance from implant top to first implant-to-bone contact
DIM = distance from implant top to coronal gingiva
JE = junctional epithelium
PD = Probing depth
SD = sulcus depth

Soft tissue stability overtime

In a four-year retrospective study, the soft tissue aspect of osseointegrated fixtures supporting overdenture was investigated. Eighty-six consecutive patients (196 'Branemark System®' implants) were included. Correlations were not detected with regard to marginal bone height and, plaque index, gingivitis index, presence or absence of gingiva around the abutment, or implant length. (Quirynen et al. 1991) Data concerning soft tissue dimension values is presented in Table 9.

A longitudinal evaluation of the position of the periimplant soft tissue margin was performed. Forty-one patients (163 'Branemark System®' implants) were evaluated during two years. All patients had partial or full-arch implant supported fixed prostheses. Re-examinations were performed after 6 months, 1 and 2 years concerning plaque acumulation, mucositis, probing depth, bleeding on probing, marginal soft tissue level, width of masticatory mucosa and marginal soft tissue mobility. On the final follow-

up, a slight decrease in probing depth (0.2 mm) and width of masticatory mucosa (0.3mm) was observed. The authors suggested that the recession of the peri-implant soft tissue margin mainly may be the result of a remodeling of the soft tissue in order to establish "appropriate biological dimensions". (Bengazi et al. 1996)

The clinical performance of the implants and abutments was evaluated. Ball-abutments were inserted in 18 patients who received overdentures and were followed-up for one year. The authors reported that probing depths hardly varied; the distance between the edge of the marginal peri-implant mucosa and the edge of the implant (recession) decreases mildly; and that marginal bone levels appeared stable. (Cune et al. 2004)

Table 9. Soft tissue stability overtime in human.

Authors	Dimensions around implant (mm)	Healing period	Details of the study
Quirynen et al. (1991)	REC = 1.8 PD = 2.7	6 months (n=70) 6 months (n=98)	86 patients (196 implants). Implants supporting overdentures. Clinical analysis.
	REC = 2.9 PD = 3.2	36 months (n=11) 36 months (n=17)	
Bengazi et al. (1996)	PD = 3.2 KM = 2.7	Baseline (n=163)	41 patients (163 implants). Implants supporting partial or full-arch fixed prostheses. Clinical analysis.
	ΔPD = -0.2 ΔKM = -0.3	24 months (n=158)	
Cune et al. (2004)	REC=3.0 PD=1.5	Baseline	Clinical trial. 18 patients. Implants supporting overdentures. Clinical analysis.
	REC=2.7 PD=1.4	12 months	

KM = width of keratinized mucosa
ΔKM = changes in width of keratinized mucosa
PD = probing depth
ΔPD = changes in probing depth
REC = gingival recession

An evaluation of the extension of biologic width was performed in dogs, in which non-submerged implants were submitted to unloaded and loaded conditions (Table 10). Values from histometric analysis revealed that the sum of the measurements was similar overtime (up to 12 months), and the authors concluded that biologic width around one-piece implants is a physiologically formed and stable dimension as around teeth (Cochran et al. 1997). Additionally, it was demonstrated that during healing period,

dynamic changes occurs, with a decrease on sulcus depth and connective tissue contact, and an increase on junctional epithelium dimension. However, a stability of biologic width dimension was observed (Hermann et al. 2000).

Crestal bone changes around implants in loaded and unloaded conditions were histologically evaluated in a dog model. Three months after the implantation of sandblasted and acid-etched implants, abutments or healing screws were placed. The implants were loaded or unloaded, evaluated with a 6 and 12 months healing period. No statistically significant differences were found concerning the amount of bone loss between different loading conditions, but statistically significant differences were found, in both groups, comparing the analysis performed at 6 months and at 12 months. The authors concluded that loading does not seem to be a relevant factor in the peri-implant bone loss observed during the first year of function, but the bone crest level changes could depend on the location of the microgap. (Assenza et al. 2003)

Response to plaque

Similarities between epithelium around teeth and implants are not restricted to morphological aspects (Berglundh et al. 1991, Listgarten et al. 1991), but extend to the homeostasis and defense mechanisms (Schmid et al. 1991, Schmid et al. 1992, Ingman et al. 1994, Schierano et al. 2003). However, some particularities are reported, and one of these concerns the vascularization. The supracrestal connective tissue lateral to the teeth is richly vascularized, with vasculature derived from supraperiosteal vessels and the vessels of the periodontal ligament. The corresponding site in the peri-implant tissue is almost devoid of vascular supply, and blood vessels are found to be terminal branches of larger vessels originating from the periosteum of the bone of the implant site. (Berglundh et al. 1994)

The supracrestal periimplantar soft tissues have been reported as a significant factor for long-term success of implant, since it works as a barrier against bacterial invasion (Berglundh et al. 1991, Ericsson et al. 1992), and the rupture of this barrier can lead to implant failure (Piattelli et al. 1998).

Table 10. Soft tissue stability overtime in animal model.

Authors	Dimensions around implant (mm)	Healing period	Details of the study
Cochran et al. (1997)*	SD = 0.50 JE = 1.44 CT = 1.01 IAJ-B = 2.91	3 months	Animal experiment. 6 dogs Loaded conditions. Histologic analysis.
	SD = 0.16 JE = 1.88 CT = 1.05 IAJ-B =2.95	12 months	
Hermann et al. (2000)*	SD = 0.50 JE = 1.44 CT = 1.01 BW = 2.94	3 months	Animal experiment. 6 dogs (24 implants). Loaded conditions. Histologic analysis.
	SD = 0.16 JE = 1.88 CT = 1.05 BW =3.08	12 months	
Assenza et al. (2003)	SD = 0.6 JE = 1.2 CT = 1.2 DIB = 1.24	6 months (unloaded conditions)	Animal experiment. 6 dogs (72 implants). Unloaded and loaded conditions. Histologic analysis.
	SD = 1.0 JE = 1.1 CT = 1.3 DIB = 2.9	12 months (unloaded conditions)	
	SD = 1.2 JE = 1.1 CT = 1.2 DIB = 1.32	6 months (loaded conditions)	
	SD = 1.1 JE = 0.9 CT = 1.2 DIB = 2.21	12 months (loaded conditions)	

* For this table, one experimental group was omitted.
BW = biologic width
CT = connective tissue attachment
DIB= distance implant top-first bone-implant contact
JE = junctional epithelium
SD = sulcus depth

The soft tissue around implant reacts to plaque forming an inflammatory lesion, which size and composition has many features in common with that formed around teeth (Berglundh et al. 1992, Sennerby & Lekholm, 1993). Also similarly to periodontium, under pathologic conditions periimplantar tissues may react with an apical

epithelialization (Kawahara et al. 1998a), and the osseointegration loss process is similar to that observed in aggressive periodontitis according to the number of T lymphocytes, but not to the vascular proliferation. (Bullon et al. 2004)

The interface between implant and soft tissue presents an epithelial cell attached zone, with a greater bond strength, that plays an important role in the prevention of bacterial invasion (Kawahara et al. 1998b). However, this mechanism seems to be more permeable around implants than around teeth (Ikeda et al. 2002). Connective tissue barrier was described in an experimental study, in which the area between the keratinized mucosa and dental implant was investigated in two distinct areas nearby the implant. The first one, close to the implant surface up to 40 μm apart, was characterized by abundant fibroblasts interposed between collagen fibers, and absence of blood vessels. The second area, continuous laterally to the first one, consequently further from the implant, contained fewer fibroblast but more collagen fibers and blood vessels. The authors suggest that this fibroblast rich barrier play a role in the maintenance a sealing between oral environment and peri-implant bone (Moon et al., 1999).

Influence of different topographies on plaque formation

The presence and density of periodontal pathogens subgingivally are more related to the patient's dental status than to the characteristics of the surface (Quirynen et al. 1993). However, factors related to the implant surface, as roughness and surface-free energy should also be considered, since they influence the plaque formation and maturation (Quirynen & Bollen 1995). Thus, these arguments justify the search for an ideal surface smoothness for reduction of bacterial colonization (Quirynen et al. 2002).

The influence of the surface roughness on plaque accumulation and gingivitis was studied in humans. In partially edentulous patients, four titanium abutments with different surface roughness were installed. After one month, only the two roughest abutments harbored spirochetes, and after 3 months, the composition subgingival microbiota showed little variation on the different abutment types, although spirochetes were only noticed around the roughest abutments. The analysis of anaerobic bacteria resulted in comparable values for all abutment types, supragingivally and subgingivally. Clinically, small differences in probing depth were observed among the sites, and in the roughest abutment, some attachment gain (0.2 mm) occurred during 3 months, whereas the other abutments had an attachment loss ranging from 0.8 to greater than 1 mm. These authors concluded that these observations indicate “the existence of a threshold roughness

below which no further impact on the bacterial adhesion and/or colonization should be expected. However, clinical evaluation seems to indicate that a certain surface roughness is necessary for increased resistance to clinical probing." (Quirynen et al. 1996)

Thus a reduction of the surface roughness does not seems to have major impact on the supra- and subgingival microbial composition. The influence of abutment surface roughness was evaluated on plaque accumulation and peri-implant mucositis. In the patients, abutments with two distinct surfaces were used: turned titanium (R_a = 0.2 µm), and highly polished ceramic material (Prozyr®, R_a = 0.6 µm). After 3 and 12 months, samples from supra- and subgingival plaque were analyzed, and clinical periodontal parameters were recorded. At 3 months, spirochetes and motile organisms were only detected subgingivally around the titanium abutments. After 12 months, microbiologic analysis failed to detect large inter-abutment differences, however, the aerobic culture data showed a higher proportion of Gram-negative organisms in the subgingival microbiota of the rougher abutments. Clinically, the smoothest abutment showed a slightly higher increase in probing depth between months 3 and 12, and more bleeding on probing. (Bollen et al. 1996)

In a dog model, soft tissue reactions to plaque formation with different surface topographies (acid-etched 'Osseotite' and turned abutments) was investigated. After 6 months, biopsies were obtained from surrounding tissues and the presence of an established inflammatory lesion in the connective tissue of the peri-implant mucosa was observed. The location, size and composition of the lesions were not different between groups, dominated by plasma cells and lymphocytes. Another inflammatory lesion was observed at abutment/implant junction, which contained a comparatively larger number of polymorphonuclear leukocytes. In this experiment, the different surface characteristics of abutment made of c.p. titanium did not influence plaque formation and the establishment of inflammatory cell lesions in the peri-implant mucosa. (Zitzmann et al. 2002)

In another study, each patient received one abutment of each group: turned (S_a = 0.259 µm), "additionally turned" (S_a = 0.402 µm), and sandblasted (Al_2O_3). The sandblasted surfaces were treated with particles of different sizes: 25 µm (S_a = 0.764 µm), 75 µm (S_a = 1.001 µm) or 250 µm (S_a = 1.870 µm). After four weeks, histological appearance of connective tissue was similar between different abutments, and no differences were observed between the surfaces in relation to plaque accumulation or the number of inflammatory cells. (Wennerberg et al. 2003)

The response of ceramic surfaces to plaque has been focus of varied researches. The response to plaque to different combinations of crowns and abutments was investigated. Each patient of the study received one of the following types of restorations with intracrevicular margins: (1) an all ceramic crown luted to a natural tooth; (2) an all-ceramic crown luted to a titanium implant-supported abutment; (3) a metal-ceramic crown (porcelain fused to high noble metal alloy) luted to a natural tooth; (4) a metal-ceramic crown (porcelain fused to high noble metal alloy) luted to a titanium implant-supported abutment; and (5) a titanium–ceramic crown luted to a natural tooth. All groups presented similar tissue response to gingival redness, swelling and bleeding scores. More plaque accumulation was observed in all-ceramic crown luted to a titanium implant-supported abutment in comparison to an all-ceramic crown luted to a natural tooth. (Kancyper & Koka 2001)

Bacterial colonization of zirconia surfaces (two different surfaces were evaluated, with R_a values ranging from 0.04 to 0.18 μm) was investigated in comparison to titanium (R_a = 0.22 μm) *in vivo* and *in vitro*. No one of the surfaces was able to inhibit bacterial colonization. *S. mutans* adhered significantly more in ceramic than in titanium surfaces, while *S. sanguis* seemed to adhere more easily to titanium. No differences were observed concerning the amount of *Actinomyces spp* and *P. gingivalis*. In vivo, early bacterial adhesion was evaluated in human, in whom the zirconia surface accumulated fewer bacteria than titanium in terms of the total number of bacteria and presence of potential putative pathogens. The authors concluded that ceramic material accumulates fewer bacteria than titanium. (Rimondini et al. 2002)

Zirconia (R_a = 0.73 μm) and titanium (R_a = 0.76 μm) disks were glued to a removable acrylic device adapted to the molar-premolar region of the volunteers of this study. After 24 hours in position, all disks were removed and processed. The area covered by bacteria in the zirconia specimens (12.1%) was statistically lower than that in titanium specimens (19.3%) (Scarano et al. 2004). These results demonstrate that zirconia may be a suitable material for manufacturing implant abutments with a low colonization potential. However, further studies should be performed to confirm these results.

Antibacterial characteristic of implant surfaces

The development of specific surfaces with a potential antibacterial property are been widely studied. Among others, the use of implants with a titanium nitride (TiN) layer, anodized surface, and treated by laser have been investigate

concerning the decrease on bacterial adhesion, notwithstanding long-term experiments are necessary to determine the clinical significance of their antibacterial effect. (Del Curto et al. 2005, Suketa et al. 2005)

In a preliminary study, ion implantation (Ca+, N+, F+), oxidation (anode oxidation, titanium spraying), ion plating (TiN, Al_2O_3), and ion beam mixing (Ag, Sn, Zn, Pt) with Ar+ on titanium plates were evaluated. These procedures are considered useful in controlling the adhesion of oral bacteria on titanium surfaces (Yoshinari et al. 2000). Then, the antibacterial effect of these surface modifications was investigated concerning their response to *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*. F+-implanted specimens inhibited significantly more the growth of both bacteria than the polished titanium. The other surface-modified specimens did not exhibit effective antibacterial activity. The authors suggest that these results were caused by the formation of a metal fluoride complex on the surfaces. Additionally, F+-implanted surfaces did not inhibit the proliferation of fibroblast. The authors concluded that surface modification is useful in providing antibacterial activity of oral bacteria to titanium. (Yoshinari et al. 2001)

A comparison among the following surfaces modification procedures was evaluated: TiN (R_a = 0.19 µm) zirconium nitride (ZrN) (R_a = 0.20 µm), thermal oxidation (R_a = 0.19 µm), laser radiation (R_a = 1.00 µm), and turned surface (R_a = 0.14 µm). Discs were incubated in bacterial cell suspension, and *Streptococcus mutans* and *Streptococcus sanguis* were counted. A significant reduction of the number of adherent bacteria was observed on TiN, ZrN and thermically oxidated titanium surfaces compared to turned titanium. Thus, the authors suggested that physical modification of titanium implant surfaces such as coating with TiN or ZrN may reduce bacterial adherence and hence improve clinical results. (Grossner-Schreiber et al. 2001)

The biocompatibility of osteoblasts and fibroblasts was observed on an anodized surface prepared by discharging in NaCl solution. These surface exhibited high antibacterial activity, and enhanced cell extension and cell growth compared with the pure titanium. The author concluded that the titanium chloride (TiCl) formed is a promising material for use in dental implant systems. (Shibata et al. 2004)

The bacterial adhesion to TiN-coated (R_a = 0.79 µm) and titanium (R_a = 0.76 µm) implants was evaluated in humans. With this aim, a removable acrylic device was adapted to the molar-premolars, and dental implants were glued in this device. After 24 hours, TiN-coated surfaces were covered by a significantly lower amount of bacteria

compared to that formed on control implants. Even though the surface roughness was similar in both groups, TiN surfaces showed a significant reduction of the presence of bacteria. (Scarano et al. 2003)

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5 METODOLOGIA

5.1 Animais e grupos experimentais

O presente estudo foi aprovado pelo Comitê de Ética em Experimentação Animal (CEEA) da Faculdade de Odontologia de Araraquara – UNESP (Protocolo nº 24/2003).

Seis cães de raça indefinida, com boa saúde geral, e de 23,0 ± 6,30 kg foram selecionados para este estudo. Os animais receberam dieta à base de ração e água, e doses de antiparasitários [*], e vacinas [**]. Quinze dias antes do procedimento cirúrgico inicial, os cães foram submetidos à raspagem supragengival manual para remoção de cálculo dentário, e moldagem com silicona de condensação para confecção de modelos de gesso [***].

Trinta e seis implantes [****] (cônicos de hexágono interno; dimensões de 4,3 x 10 mm; e superfície tratada por jateamento com óxido de titânio) foram utilizados neste estudo. Em cada cão, seis implantes foram inseridos, sendo três implantes por hemi-mandíbula, cada qual representativo de um grupo experimental. Os grupos experimentais foram criados de acordo com a distância da JIC à crista óssea (Figura 1):

Grupo *Ao Nível*: implante inserido ao nível da crista óssea;

Grupo *Menos 1*: implante inserido um milímetro apical à crista óssea; e

Grupo *Menos 2*: implante inserido dois milímetros apical à crista óssea.

Cada hemi-mandíbula foi submetida a um dos seguintes protocolos de restauração:

Restauração convencional: prótese instalada 120 dias após a implantação; e

Restauração imediata: prótese instalada 24 horas após a implantação.

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[***] Zhermack SPA, Badia Polesine, Itália.
[****] Conexão Sistema de Prótese Ltda, São Paulo, Brasil.

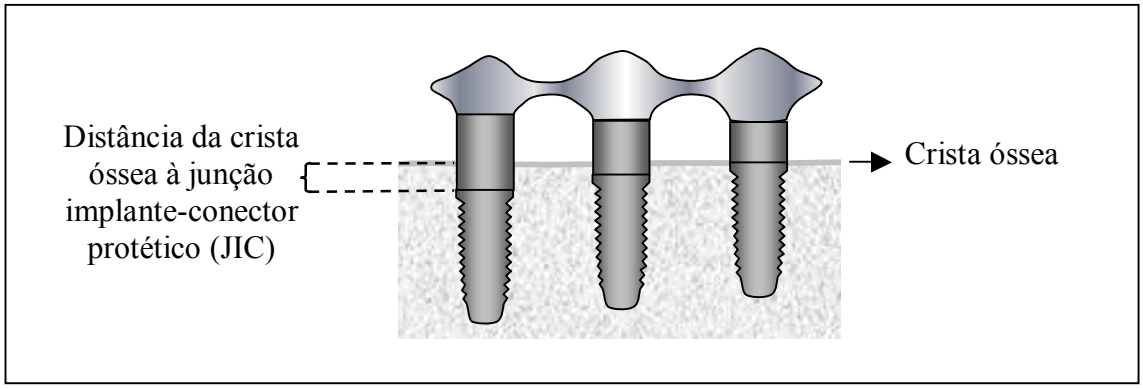

FIGURA 1 - Esquematização da distância entre o implante e a crista óssea. Neste caso, são representados implantes dos grupos *Menos 2*, *Menos 1* e *Ao Nível*, respectivamente.

Um revezamento foi realizado, com seis combinações de posição, de tal forma que um implante representativo de cada grupo foi inserido em um sítio diferente em cada cão (Figura 2 e Tabela 1).

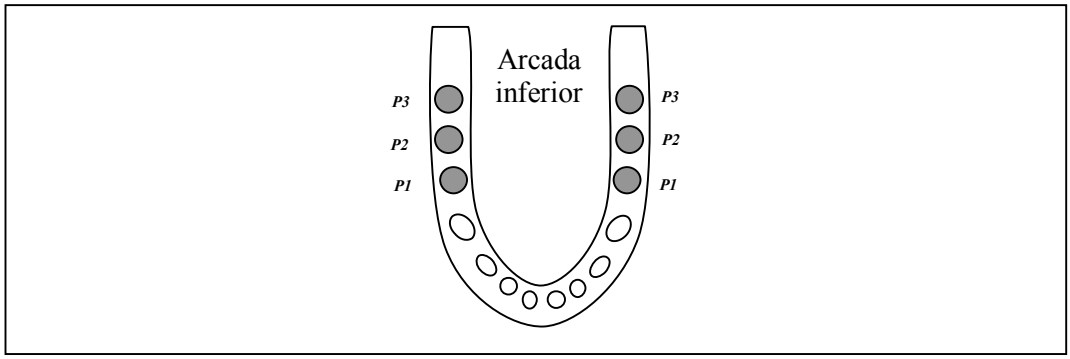

FIGURA 2 - Esquema de distribuição dos sítios na arcada inferior dos cães.

Tabela 1 - Esquema de revezamento de grupos e protocolo de restauração por cão.

Sítio	Cão 1	Cão 2	Cão 3	Cão 4	Cão 5	Cão 6
P1 Direito	*Ao Nível* Convencional	*Menos 2* Convencional	*Menos 1* Convencional	*Menos 2* Imediato	*Menos 1* Imediato	*Ao Nível* Imediato
P2 Direito	*Menos 1* Convencional	*Ao Nível* Convencional	*Menos 2* Convencional	*Menos 1* Imediato	*Ao Nível* Imediato	*Menos 2* Imediato
P3 Direito	*Menos 2* Convencional	*Menos 1* Convencional	*Ao Nível* Convencional	*Ao Nível* Imediato	*Menos 2* Imediato	*Menos 1* Imediato
P1 Esquerdo	*Ao Nível* Imediato	*Menos 2* Imediato	*Menos 1* Imediato	*Menos 2* Convencional	*Menos 1* Convencional	*Ao Nível* Convencional
P2 Esquerdo	*Menos 1* Imediato	*Ao Nível* Imediato	*Menos 2* Imediato	*Menos 1* Convencional	*Ao Nível* Convencional	*Menos 2* Convencional
P3 Esquerdo	*Menos 2* Imediato	*Menos 1* Imediato	*Ao Nível* Imediato	*Ao Nível* Convencional	*Menos 2* Convencional	*Menos 1* Convencional

5.2 Experimento

O cronograma do experimento é apresentado na Figura 3.

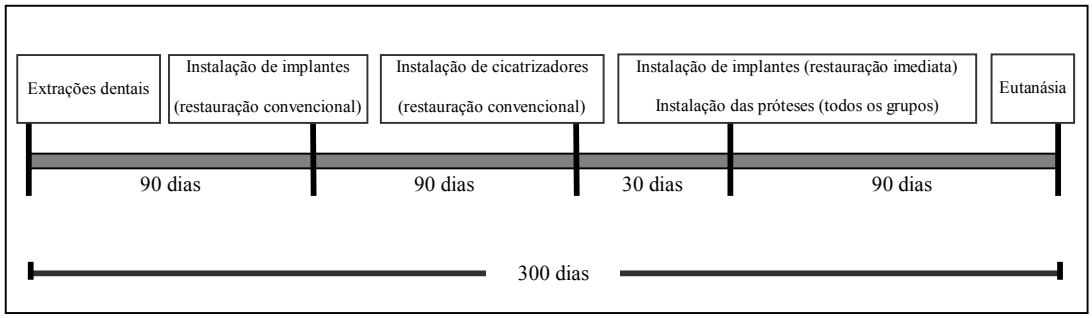

FIGURA 3 - Cronograma do experimento.

Cuidados relacionados a procedimentos cirúrgicos

Todas as cirurgias foram realizadas em ambiente asséptico. Inicialmente, os cães receberam injeção de acepromazina a 1% [*] como indutor pré-anestésico (na proporção de 0,02 mg / kg, 0,1 mL / kg, via intramuscular). Em seguida, foram submetidos à anestesia geral por injeção de tiopental sódico [**] (na concentração de 12,5

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mg / kg e na proporção de 0,5 mL / kg, via endovenosa), dividida em dose inicial e doses de manutenção.

Os animais foram mantidos com soro fisiológico endovenoso durante todo o ato cirúrgico. Solução de digluconato de clorexidina a 0,12% [*] foi utilizada para anti-sepsia da cavidade oral dos cães. A anestesia local foi realizada por infiltração de cloridrato de mepivacaína 2% com norepinefrina 1:100.000 [**].

Incisões foram realizadas com lâmina de bisturi nº 15 montada em cabo de bisturi nº 3. Nas incisões supracrestais foi tomado o cuidado de manter quantidades semelhantes de tecido queratinizado em cada lado da incisão. Retalhos mucoperiosteais foram rebatidos utilizando descolador tipo Molt, e ao final do procedimento, foram suturados com pontos tipo colchoeiro horizontal e fio de nylon 4.0 [***], de tal forma a buscar o fechamento do retalho por primeira intenção.

Em seguida, os animais receberam aplicação de protetor hepático [****] (10 mL por via endovenosa); injeções de uma associação dos antibióticos penicilina e estreptomicina [*****] (24.000 UI / kg, 0,1 mL / kg, intramuscular); e de analgésico cetoprofeno a 1% [******] (na proporção de 2 mg / kg, 0,2 mL / kg, intramuscular). Nos dois dias seguintes ao procedimento cirúrgico os animais receberam doses adicionais de analgésico (mesma dose inicial). Os cães foram mantidos com dieta líquida e pastosa por uma semana, depois da qual os animais eram alimentados com ração seca. As suturas foram removidas dez dias após as cirurgias. Os animais foram submetidos a um rigoroso controle de placa por meio de escovações com gel de digluconato de clorexidina a 0,12% [*******], 3 vezes por semana, desde a cirurgia de instalação dos cicatrizadores até o sacrifício dos animais.

Todos os cuidados pré e pós-operatórios descritos acima foram repetidos nos demais procedimentos cirúrgicos.

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[*] Periogard, Colgate-Palmolive Ltda, Osasco, Brasil.
[**] Spécialités Sptondont, Saint – Maur, França.
[***] Johnson & Johnson Company, São Bernardo do Campo, Brasil.
[****] Frutoplex LM, Marjan Indústria e Comércio Ltda, São Paulo, Brasil.
[*****] Pentabiótico Fort Dodge Saúde Animal Ltda, Campinas, Brasil.
[******] Ketofen, Merial, São Paulo, Brasil.
[*******] Farmácia da Faculdade de Farmácia da UNESP, Araraquara, Brasil.

Extrações dentárias

Inicialmente (Figura 4A), incisões intrasulculares foram feitas nas faces vestibulares e linguais, que foram unidas, estendendo-se da face distal do canino à face mesial do 1º molar inferior. O retalho foi rebatido, e as extrações foram realizadas com alavancas e fórceps infantis. No caso de dentes bi-radiculares, a secção foi realizada na área de bifurcação, com o auxílio de broca tronco-cônica carbide 701 [*], em alta velocidade, sob irrigação constante com soro fisiológico. Os bordos dos retalhos foram coaptados e suturados (Figura 4B).

Instalação de implantes (restauração convencional)

Noventa dias após as extrações dentárias (Figura 5A), na hemi-mandíbula designada à restauração convencional, uma incisão foi feita na crista óssea, e o retalho mucoperiosteal foi rebatido. Os implantes representativos de cada grupo foram inseridos usando a crista óssea mesial como ponto de referência. As seguintes distâncias horizontais foram respeitadas: de 6 mm entre as superfícies de implantes adjacentes, e de 4 mm entre a superfície mesial do 1º molar e o implante (Figura 5B). Os bordos dos retalhos foram coaptados e suturados.

Instalação de cicatrizadores (restauração convencional)

Noventa dias após a instalação dos implantes, uma incisão supracrestal foi realizada, e retalhos mucoperiosteais foram elevados. Em seguida, e conforme disponibilidade comercial, cicatrizadores de 3 mm, 4 mm e 5,5 mm de altura foram conectados respectivamente aos implantes dos grupos *Ao Nível*, *Menos 1* e *Menos2*. Por fim, os retalhos foram coaptados e suturados.

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[*] KG Sorensen, São Paulo, Brasil.

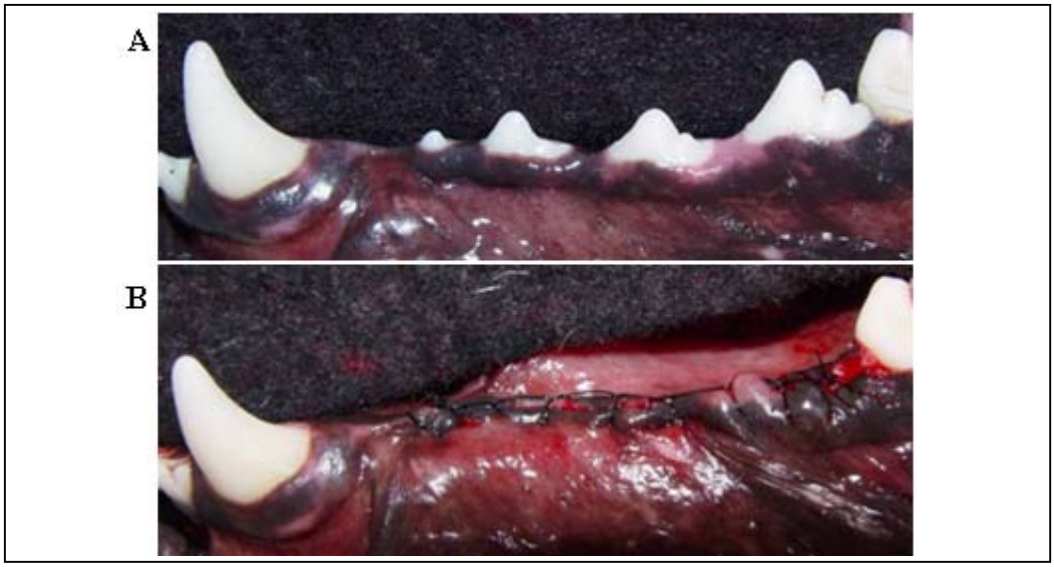

FIGURA 4 - Aspecto clínico (A) prévio e (B) após as extrações dentárias.

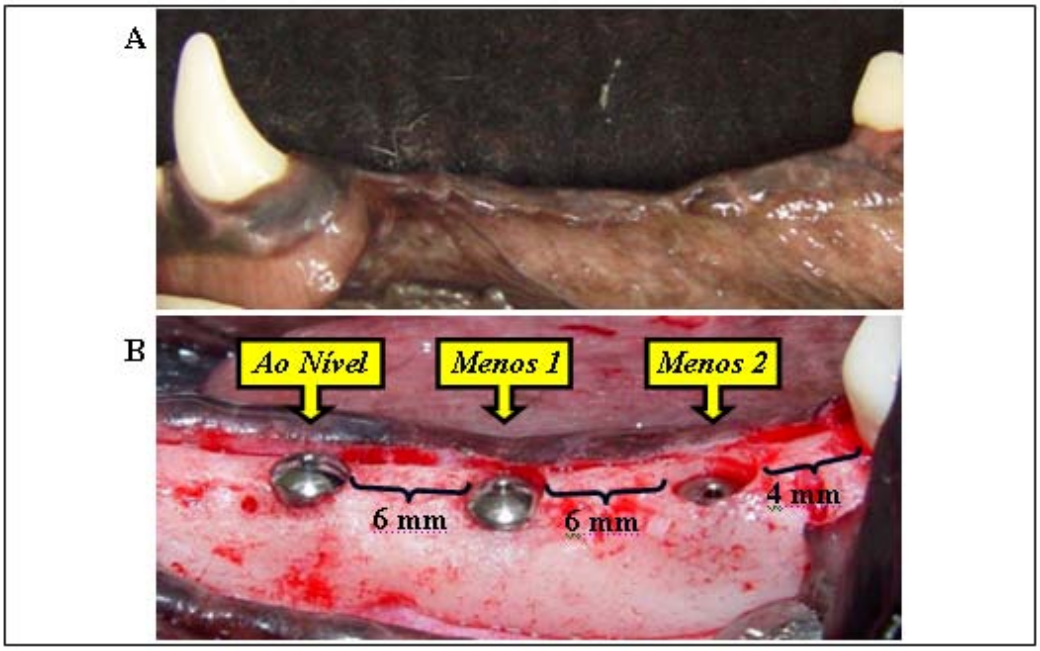

FIGURA 5 - Aspecto clínico (A) prévio e (B) após a instalação dos implantes. Neste caso, os implantes mesial, médio e distal fizeram parte dos grupos *Ao Nível*, *Menos 1* e *Menos 2*, respectivamente. Observam-se também as distâncias respeitadas entre implantes (6 mm) e entre implante e dente (4 mm).

Instalação de implantes (restauração imediata) e dos conectores protéticos (restauração convencional e imediata)

Trinta dias após a instalação dos cicatrizadores, os animais foram submetidos a novos procedimentos cirúrgicos (Figura 6). Na hemi-mandíbula a ser submetida à restauração convencional, os cicatrizadores foram removidos e os conectores foram instalados. No lado oposto, os implantes foram instalados como previamente descrito, e em seguida, os conectores protéticos foram parafusados. Estes tinham cintas de 3 mm, 4 mm e 5,5 mm de altura, e foram instalados respectivamente nos implantes dos grupos *Ao Nível*, *Menos 1* e *Menos2*. Por fim, a moldagem de arrasto foi realizada com moldeira aberta individualizada, utilizando material à base de elastômero de condensação para a confecção laboratorial das próteses.

Instalação das próteses

Vinte e quatro horas após a instalação dos conectores protéticos, a próteses metálicas foram passivamente parafusadas bilateralmente. Todas as coroas protéticas estavam livres de contatos oclusais.

Eutanásia

Noventa dias após a instalação das próteses (Figura 7), os animais foram submetidos à eutanásia com doses letais de tiopental sódico.

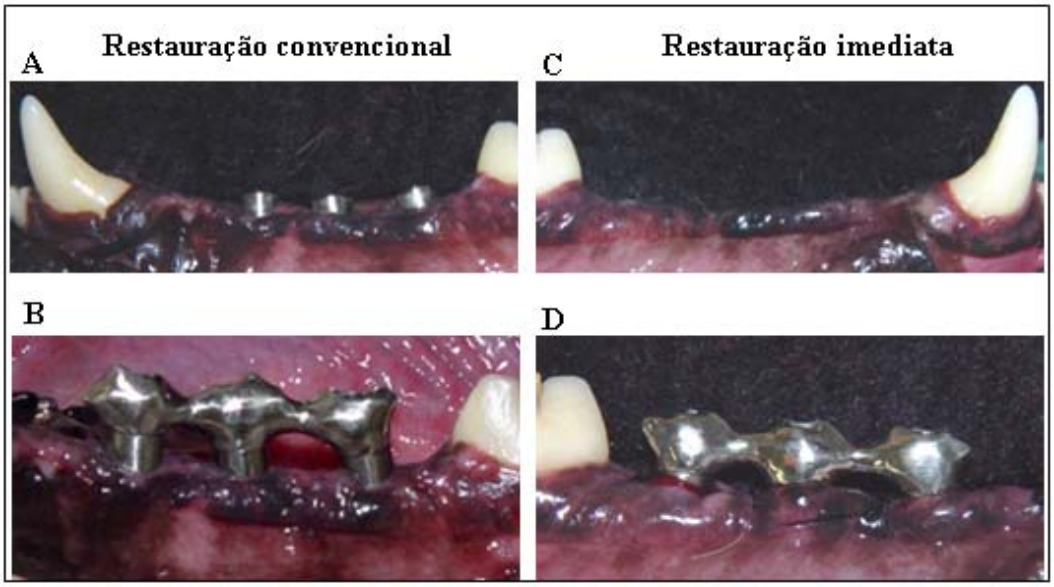

FIGURA 6 - Aspecto clínico (A) previamente à remoção dos cicatrizadores, e (B) após a instalação da prótese, no lado submetido à restauração convencional; e (C) previamente à instalação dos implantes e (D) após a instalação da prótese no lado submetido à restauração imediata.

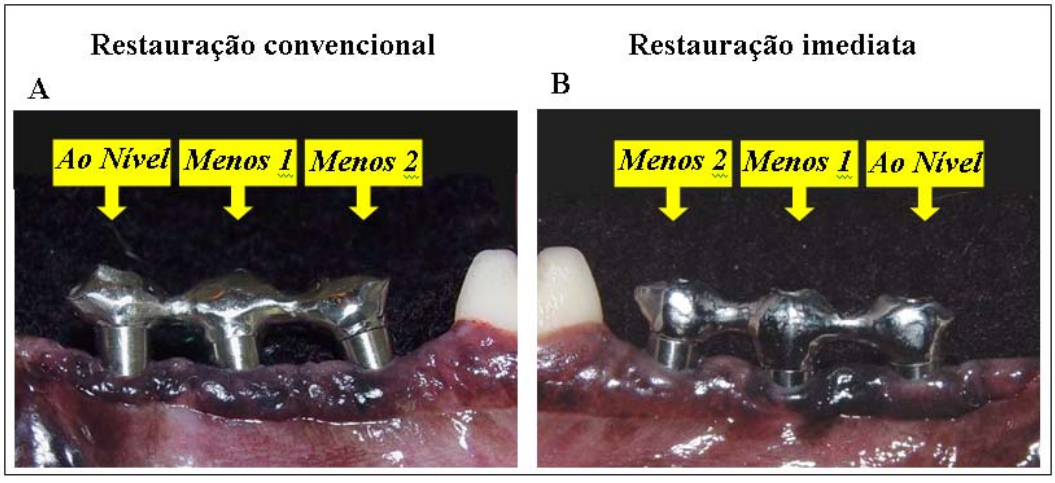

FIGURA 7 - Aspecto clínico no dia do sacrifício, no lado submetido a (A) restauração convencional e (B) restauração imediata.

5.3 Análise dos resultados

Análise clínica

As medidas foram feitas 90 dias após a colocação das próteses. As áreas ao redor dos implantes foram avaliadas usando uma sonda periodontal milimetrada Carolina do Norte [*]. Os valores não exatos foram aproximados para o 0,5 mm mais próximo. Os seguintes parâmetros foram considerados (Figura 8):

(1) PTM-JPC, distância da posição mais coronal do tecido mole marginal (PTM) à junção prótese-conector protético (JPC);

(2) Profundidade de sondagem (PS); e

(3) Nível de Inserção Relativo (NIR), correspondente à distância da profundidade de sondagem à JPC. Os valores foram aferidos nas superfícies mesio-vestibular e mesio-lingual. Adicionalmente, dados referentes ao Índice gengival (IG), dicotômico [2], e ao Índice de sangramento à sondagem (ISS), dicotômico [21] foram aferidos na superfície mesial.

Análise radiográfica

Após a eutanásia, as mandíbulas foram dissecadas e fixadas em formalina a 10% por pelo menos 48 horas. As hemi-mandíbulas foram radiografadas utilizando um sistema digital [**] posicionado 20 cm da unidade de raio-X (70 kV, 0,95 kVa, e 0,1 s de tempo de exposição). Todas as imagens foram obtidas na mesma sessão, e foram analisadas utilizando um programa de computação apropriado [***].

As seguintes medidas foram feitas no sítio mesial de cada implante (Figura 9):

(1) JIC-pCOI, medida vertical da JIC ao primeiro contato osso-implante (pCOI);

(2) Rebordo-pCOI, medida vertical do rebordo à pCOI;

(3) Rebordo-JIC, medida vertical do rebordo à JIC;

(4) Perda Óssea Lateral (POL), medida horizontal do rebordo ao corpo do implante.

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[*] Hu-friedy, Chicago, IL, EUA.
[**] Sens-A-ray, Regam Medical Systems International AB, Sundsvall, Suécia.
[***] ImageJ 1.34, National Institutes of Health, Bethesda, MA, EUA.

Além disto, foi calculada a Reabsorção do Rebordo, com base na Rebordo-JIC, seguida da adição de 1 mm aos sítios *Menos 1*, e 2 mm aos sítios *Menos 2*.

Processamento das peças

As lâminas histológicas foram preparadas de acordo com o método previamente descrito por Piattelli et al. [22]. Resumidamente, as peças fixadas foram desidratadas usando concentrações crescentes de álcool de 60% a 100%. Em seqüência, a embebição em resina foi realizada com banhos em concentrações decrescentes de álcool e crescentes de resina [*].

No presente estudo as peças foram polimerizadas e cortadas em secções de aproximadamente 150 μm usando o sistema *Precise 1 Automated System*[**], sendo as mesmas lixadas até uma espessura aproximada de 100 μm. Uma lâmina representativa de cada bloco foi criada, observando a porção mais central do implante e o pico de maior altura da mucosa. As lâminas foram coradas com Azul de Toluidina e Fucsina Ácida.

[*] Technovit 7200 VLC. Kulzer, Wehrheim, Alemanha.
[**] Assing, Rome, Itália.

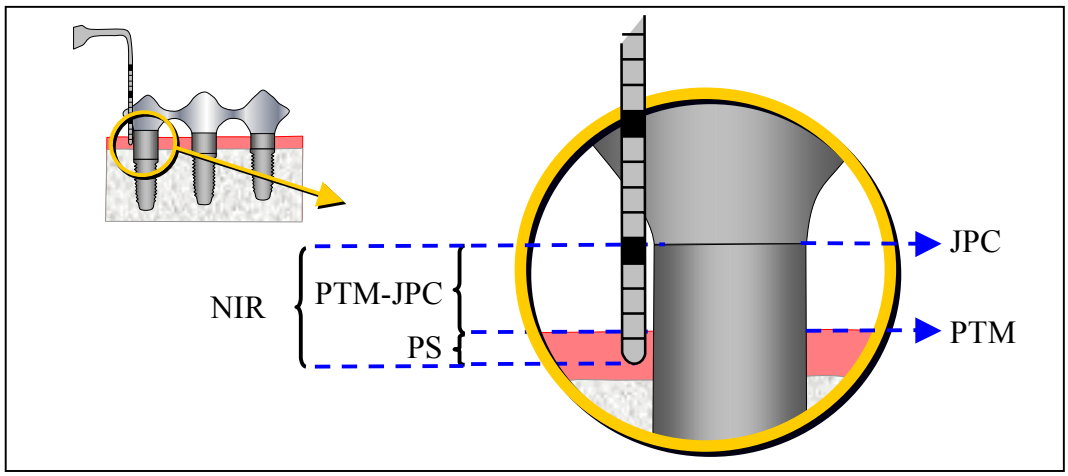

FIGURA 8 - Parâmetros considerados na análise clínica. JPC = junção prótese-conector protético; NIR = nível de inserção relativo; PS = profundidade de sondagem; PTM = posição do tecido marginal.

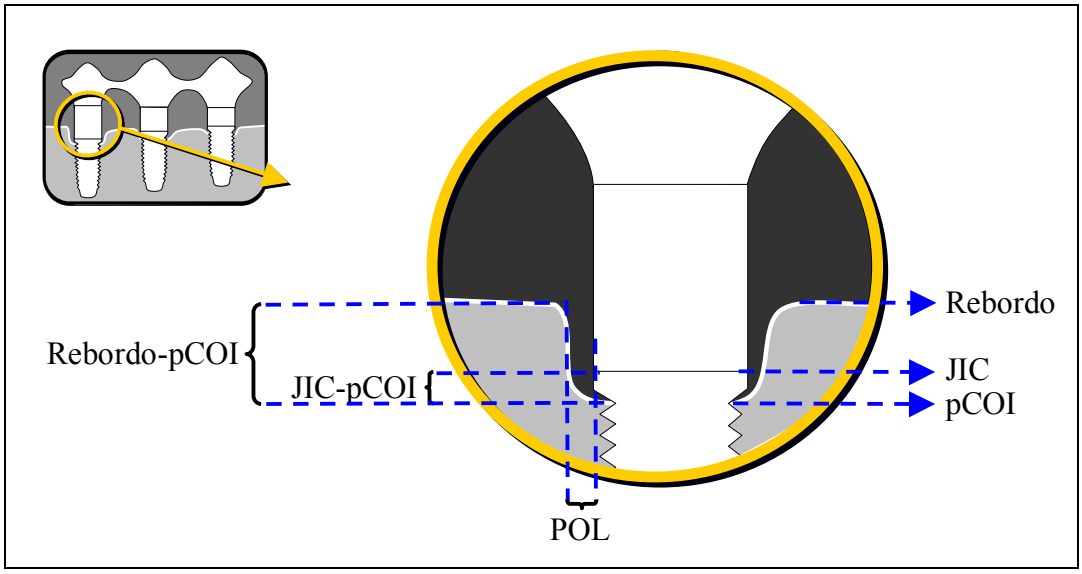

FIGURA 9 - Parâmetros considerados na análise radiográfica. JIC = junção implante-conector protético; pCOI = primeiro contato osso-implante; POL = perda óssea lateral.

Análise histométrica

As lâminas tiveram suas imagens enviadas a um microscópio conectado a uma câmera de vídeo ligada a um computador, para visualização dos pontos anatômicos de referência. As mensurações foram realizadas usando um programa de computador apropriado [*], com relação aos seguintes parâmetros (Figura 10):

(1) Extensão do Epitélio Sulcular (ES), medida da PTM à porção mais coronal do epitélio juncional;

(2) Extensão do Epitélio Juncional (EJ), medida da porção mais coronal à mais apical do EJ;

(3) Extensão do Tecido Conjuntivo (TC), medida da porção mais apical do EJ ao pCOI;

(4) PTM-pCOI, medida da PTM ao pCOI;

(5) PTM-JIC, medida da PTM à JIC;

(6) JIC-pCOI, medida da JIC ao pCOI;

(7) Rebordo-pCOI, medida do rebordo ao pCOI; e

(8) POL, medida da perda óssea lateral, medida do rebordo ao corpo do implante.

Adicionalmente, os valores PTM-JIC foram ajustados para avaliar a altura da PTM, adicionando 1 mm ao valor do grupo *Menos 1* e 2 mm ao valor do grupo *Menos 2*.

[*] ImageJ 1.34, National Institutes of Health, Bethesda, MA, EUA.

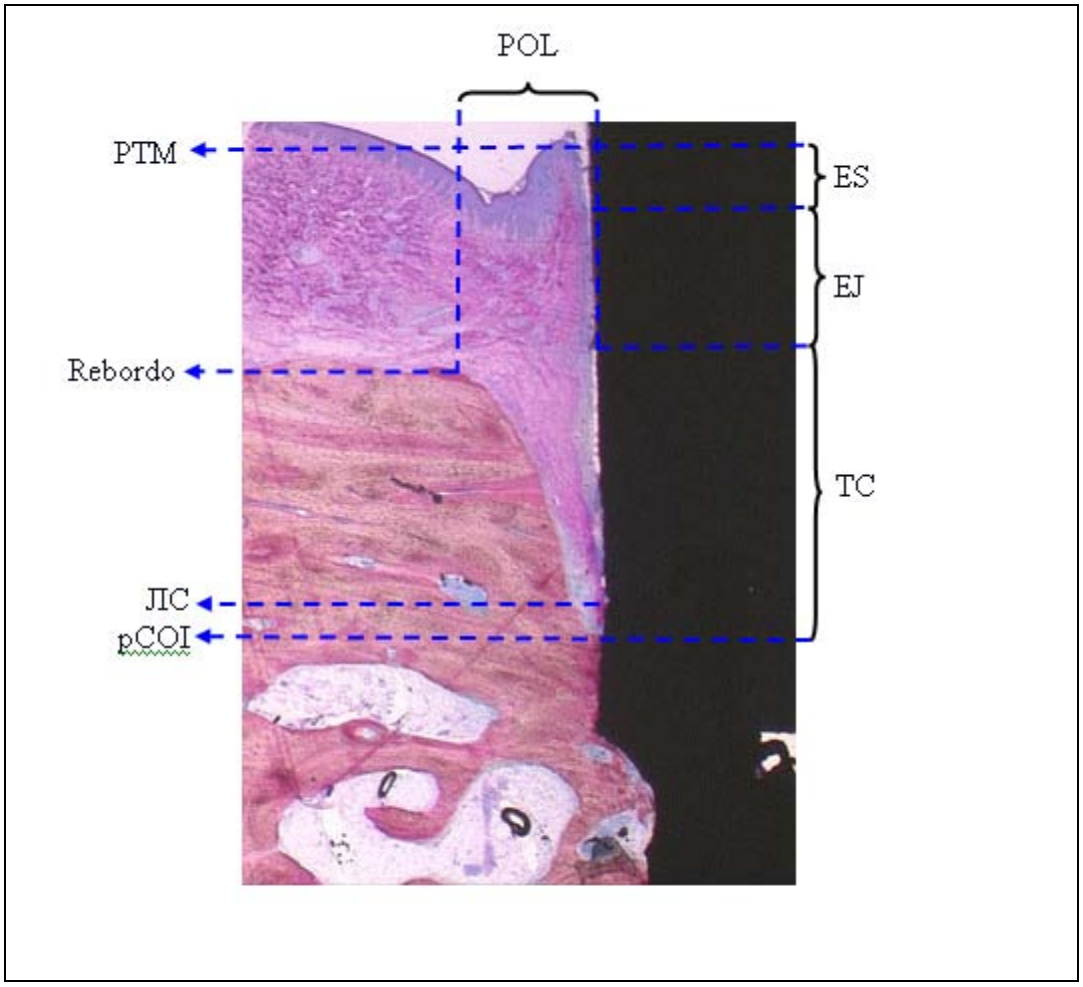

FIGURA 10 - Parâmetros considerados na análise histométrica. ES = extensão do epitélio sulcular; EJ = extensão do epitélio juncional; POL = perda óssea lateral; PTM = posição do tecido marginal; JIC = junção implante-conector protético; pCOI = primeiro contato osso-implante; TC = extensão do tecido conjuntivo.

Análise estatística

Sítios dos 36 implantes foram utilizados para avaliação dos dados. Os valores da PTM-JPC e NIR no grupo *Menos2* foram reduzidos em 0,5 mm para compensar o comprimento do componente protético, que era comparativamente 0,5 mm mais longo que a dos demais grupos.

Os valores foram expressos em médias e desvios-padrão, e a unidade de análise foi o cão. A análise estatística foi desenvolvida por meio de um programa específico [*], considerando a hipótese nula baseada na ausência de diferença entre as modalidades de tratamento ($\alpha = 5\%$).

As medidas foram realizadas pelo mesmo examinador, e a confiabilidade intra-examinador foi avaliada pelo cálculo do erro-padrão, conforme previamente descrito por Araújo et al. [5], e pelo cálculo do coeficiente de correlação de Spearman, com relação ao PTM-JPC (erro-padrão = 0,42 mm, coeficiente de correlação = 0,900), avaliado clinicamente; à JIC-pCOI (erro-padrão = 0,08 mm, coeficiente de correlação = 0,996), avaliada radiograficamente; e à PTM-JIC (erro-padrão = 0,21 mm, coeficiente de correlação = 0,987) e JCI-pCOI (erro-padrão = 0,11 mm, coeficiente de correlação = 0,977), avaliadas histometricamente.

Os dados experimentais foram submetidos a teste de normalidade (Shapiro-Wilk). Os valores de IG, ISS tiveram distribuição não-normal, então foram analisados usando o teste Friedman, e o teste Wilcoxon. Os demais dados foram analisados pelo teste ANOVA seguido de comparação múltipla de Bonferoni, e pelo teste "t" de Student.

A análise de variância testou o efeito do posicionamento do implante (*Ao Nível versus Menos 1 versus Menos2*) dentre os grupos submetidos ao mesmo protocolo de restauração. O efeito do protocolo de restauração (convencional *versus* imediata) foi testado comparando cada posição vertical separadamente (por exemplo, *Menos 1* sob restauração convencional *versus Menos 1* sob restauração imediata); e agrupando os dados de cada hemi-mandíbula (*Ao Nível + Menos 1 + Menos 2* sob restauração convencional *versus Ao Nível + Menos 1 + Menos 2* sob restauração imediata).

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[*] BioEstat 3.0, Sociedade Civil Mamirauá / MCT – CNPq, Belém, Brasil.

6 CAPÍTULO 3

Este capítulo é constituído pelo seguinte artigo, que aborda a análise clínica e radiográfica dos dados desta tese:

Pontes AEF, Ribeiro FS, Silva VC, Margonar R, Piattelli A, Cirelli JA, Marcantonio Jr E. Biologic width changes around loaded implants inserted in different levels in relation to crestal bone. Clinical and radiographic study in dogs. (Submetido ao periódico *Journal of Periodontology*)

Biologic width changes around loaded implants inserted in different levels in relation to crestal bone. Clinical and radiographic study in dogs.

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Sources of support:
CAPES (Government Agency for the Development of Higher Education), CNPq (Brazilian Council for Scientific and Technological Development, scholarship no. 141204/2004-4), and FAPESP (São Paulo Foundation for the Support of Research, grant no. 04/08141-3) provided financial support. Moreover, Conexão Sistema de Prótese Ltda provided the implants and related supplies used in the present study.

There are 8 figures and 2 tables in this manuscript.

Running title: Loaded implants inserted in different vertical positions.

ABSTRACT

Background. The aim of the present study was to evaluate clinical and radiographic changes that occur around dental implants inserted in different levels in relation to crestal bone, under different loading conditions.

Material and methods. Thirty-six implants were inserted in the edentulous mandible of 6 mongrel dogs. Each implant was assigned to an experimental group according to the distance from the implant-abutment junction (IAJ) to the crestal bone: *Bone Level* (at crestal bone level), *Minus 1* (1 mm below crestal bone), or *Minus 2* group (2 mm below crestal bone). Each hemimandible was submitted to a loading protocol: conventional (prosthesis installed 120 days after implant placement) or immediate restoration (prosthesis installed 24 hours after implant placement). Clinical and radiographic parameters were evaluated after 90 days of loading.

Results. The apical positioning of the implants did not influence the Ridge Loss and the Position of Soft Tissue Margin (PSTM) ($p>0.05$). However, sites submitted to immediate restoration had the PSTM positioned significantly more coronally than those submitted to conventional restoration ($p=0.02$).

Conclusions. These findings suggest that the apical positioning of IAJ did not jeopardize the height of peri-implant soft and hard tissues evaluated by clinical and radiographic analyses. Moreover, immediate restoration was beneficial to the maintenance of the PSTM. Further studies are suggested to evaluate the significance of these results in longer healing periods.

Key-words: Dental implants; Esthetics; Prosthesis; Radiography; Models, Animal; Soft tissue.

INTRODUCTION

One of the greatest challenges in Implantology is to guarantee aesthetic results for patients. Thus, the maintenance of the peri-implant tissues height in a position similar to that of natural tooth has been the focus of researchers and practitioners.

In a histometric study, Hermann et al.[1] concluded that the height of tissues is more similar to natural teeth in one-piece implants compared to two-piece implants. In this animal study, prostheses were, however, not used; therefore, the influence of loading was not evaluated.

However, according to Garber et al.[2], even proponents of one-stage implant systems consider the use of two-stage protocol with an implant placement deeper than usual for esthetics improvement. A more apical positioning of the implant-abutment junction (IAJ) would contribute to the maintenance of the mucosa texture and tonality; permit the use of healing caps with emergence profile; and reestablish the architecture of marginal tissues[3]. Saadoun et al.[4] and Berglundh & Lindhe[5] discussed the viability of inserting dental implants in a vertical position 2 or 3 mm below the cemento-enamel junction (CEJ) of the adjacent teeth, and moreover, the authors suggested the possibility of using the fixtures in an even deeper position.

Greater amounts of bone loss are reported to occur around implants positioned below the bone crest in comparison to implants positioned at the level of the crestal bone or above it[6]. However, the implant apical positioning is not always related to additional height loss of peri-implant soft tissues[7]. It is possible that these tissues, instead of migrating, are supported by the ridge of an adjacent tooth or implant[8,9].

Moreover, clinical studies demonstrated that immediate loading have a positive impact on the papilla preservation[10,11,12]. Nevertheless, information regarding the physiological response to the insertion of implants in deeper apical positioning under immediate and conventional restoration protocols is not reported in the literature. In addition, there are no studies on whether those modalities of treatment could be successfully used as an alternative approach valid for aesthetic situations.

The aim of the present study was to evaluate clinical and radiographic changes in tissues around implants inserted in different levels in relation to crestal bone, and under different loading conditions.

MATERIALS & METHODS

The present study was approved by the Ethical Committee in Animal Research from the State University of São Paulo. Six mongrel dogs, featuring good health, weighting 23.0 ± 6.30 kg were included in the present study. Previously to the first surgical intervention, the dogs were submitted to coronal scaling and were molded with condensation silicon[*].

Thirty-six dental implants (Conect, Conexão Sistema de Prótese Ltda, São Paulo, Brazil) were used in this study (4.3 x 10 mm, sandblasted with titanium oxide, root-form, and internal hexagon). In each dog, six dental implants were inserted, three per hemimandible, each one representing an experimental group. The experimental groups were designed according to the distance between the IAJ and the crestal bone: *Bone Level* group (inserted at crestal bone level), *Minus 1* group (one millimeter below crestal bone), and *Minus 2* group (two millimeters below crestal bone) (Fig. 1). Each hemimandible was submitted to a different loading protocol: conventional restoration (prostheses installation occurred 120 days after implant placement), or immediate restoration (prostheses installation occurred 24 hours after implant placement). Thus, six sets of arrangement were designed, so that an implant representing each group was inserted one time in any site.

In order to carry out surgical procedures, 1% acepromazine (0.02 mg / kg, 0.1 mL / kg, intramuscular) was administered, followed by thiopental (10 mg / kg, 0.5 mL / kg, intravenous). The oral cavity was disinfected with gauzes soaked in 0.12% chlorhexidine solution[†], and local anesthesia was performed with mepivacaine 2% HCl with Norepinephrine 1:100.000[‡]. An intrasulcular incision was performed, and after the mucoperiosteal flap was reflected, bicuspids were sectioned with high-speed bur under saline irrigation. All lower premolars were extracted with forceps, and flaps were closed with 4.0 nylon suture. After the surgical procedures, antibiotic association (penicillin and streptomycin, 24.000 UI / kg, 0.1 mL / kg, intramuscular) and analgesic ketoprofen (2 mg / kg, 0.4 mL / kg, intramuscular) were administered. In the following 2 days, the dogs received additional doses of analgesic.

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[*] Zhermack SPA, Badia Polesine, Italy.
[†] Periogard, Colgate-Palmolive Ltda, Osasco, Brazil.
[‡] Spécialités Septondont, Saint Maur, France.

During the first week post-surgery, the animals were fed a soft diet. Ten days after surgical procedures, sutures were removed. During the experimental period, animals were submitted to a rigorous plaque control with tooth brushing using 0.12% chlorhexidine gel, 3 times a week. These preoperative and postoperative cares were repeated on following surgical procedures.

After a 90-days period of healing, a crestal incision was performed on the hemimandible designed to be submitted to conventional restoration, maintaining similar quantities of keratinized tissue on each side of the incision, and a mucoperiosteal flap was reflected. Dental implants representing each group were inserted, using the mesial crestal bone as reference point. Horizontal distances were determined as following: 6 mm between the surfaces of adjacent implants, and 4 mm between the mesial surface of the first molar and the implant. In sequence, flaps were sutured.

Ninety days afterwards, a crestal incision was performed on the same side, the cover screws were removed, and healing caps were screwed. The heights of healing caps were selected according to commercial availability: 3 mm, 4 mm and 5.5 mm, and were used respectively in *Bone Level*, *Minus 1* and *Minus 2* sites. Then, flaps were closed.

Thirty days afterwards, on the conventional restoration side, the healing caps were removed, the abutments were placed, and impression was taken using custom-made trays with condensation silicone. On the other side, a crestal incision was performed, the dental implants were inserted, abutments were placed, impression was taken, and flaps were closed. The abutments heights corresponded to those from healing caps.

Twenty-four hours later, metallic fixed partial prostheses were passively screwed. Special attention was taken to avoid occlusal contact. The animals were followed-up for 90 days after prostheses installation.

Clinical evaluation. Clinical measurements were performed 90 days after prostheses installation. Dental implants were evaluated using a North Carolina periodontal probe* with regard to the following parameters: (1) PSTM-PAJ, distance between Position of the Soft Tissue Margin (PSTM) and the prosthesis-abutment junction (PAJ); (2) Probing Depth (PD); and (3) Relative Attachment Level (RAL), distance between PD and the

* Hu-friedy, Chicago, IL, USA.

prosthesis-abutment junction. Values were assessed at mesio-buccal, and mesio-lingual surfaces. Additionally, data related to Gingival Index (GI)[13], and Bleeding on Probing[14] (BOP) were assessed at mesial surfaces.

Radiographic evaluation. After the animals were killed, the mandible was dissected and fixed in 10% formalin for at least 48 hours. Dog hemimandibles were radiographed using a digital system* positioned 20 cm from the x-ray unit (70 kV, 0.95 kVa, and 0.1 second exposure time). Images of all specimens were obtained at the same session, and were analyzed by the same examiner using appropriate software†. The following measurements were performed on the mesial sites of each implant (Fig. 2): (1) IAJ-fBIC, vertical measurement from the IAJ to the first bone implant contact (fBIC); (2) Ridge-fBIC, vertical measurement from the ridge to fBIC; (3) Lateral Bone Loss, horizontal measurement from the ridge to the implant body. Moreover, (4) Ridge Loss, a vertical measurement, was calculated based on the distance from the ridge to IAJ, followed by the addition of 1 mm to *Minus 1*, and 2 mm to *Minus 2* values.

Statistical analysis. All the 36 implants were available for data collection. Values were expressed in means, and the unit of analysis was the dog. Intraexaminer reliability of the examiner was determined as described elsewhere[15] by calculating standard error of measurement (SE) and Spearman correlation coefficient (CC) for clinical (SE = 0.42 mm, CC = 0.900) and radiographic measurements (SE = 0.08 mm, CC =0.996).

Experimental data was submitted to a normality test (Shapiro-Wilk). Analysis of variance tested the effect of implant positioning (*Bone Level* versus *Minus* versus *Minus 2*) among groups submitted to the same loading protocol. The effect of loading protocol (conventional versus immediate restoration) was tested separately for each implant positioning, and by gathering data from the three implant positions in each hemimandible. Values from GI, and BOP were non-normally distributed; hence, they were analyzed using Friedman's test, and Wilcoxon's test. Remaining data were analyzed by ANOVA followed by multiple comparison, and Student *t* test. The null hypothesis was based on the absence of differences among the modalities of treatment ($\alpha = 5\%$).

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* Sens-A-ray, Regam Medical Systems International AB, Sundsvall, Sweden.
† ImageJ 1.34, National Institutes of Health, Bethesda, MA, USA.

PSTM-PAJ and RAL values of *Minus2* sites were reduced in 0.5 mm to compensate the length of the abutment, which was comparatively 0.5 mm longer in comparison to *Bone Level* and *Minus 1* sites.

RESULTS

Healing was uneventful in all animals, and no loss of either implants or prostheses was observed during the experimental period. Despite the absence of primary stability, mainly in *Minus 2* sites, no continuous peri-implant radiolucent areas were apparent on any radiographs. Overall signs of inflammation were discrete (GI = 2.8 ± 16.7%, BOP = 27.8 ± 34.7%), and no statistically significant differences were found for any of these parameters.

The clinical aspect of the groups at the end of experiment is presented in Figure 3, and clinical data is in Table 1.

PSTM-PAJ data is represented in Figures 4 and in a Box-Plot graphic (Fig. 5). Ninety days after implantation, sites submitted to immediate restoration (0.9 ± 0.8 mm) had significantly smaller PSTM-PAJ means than the conventionally restored ones (1.6 ± 0.7 mm) ($p = 0.02$). Comparing sites under the same vertical position and different loading protocols, statistically significant difference was observed when *Minus 1* sites under immediate restoration (0.6 ± 1.0 mm) were compared to *Minus 1* sites under conventional restoration (1.8 ± 0.6 mm) ($p = 0.04$).

At the end of the experiment, the smallest PD values, among conventional restored groups, were observed for *Bone Level* sites (2.6 ± 0.5 mm). These values were statistically different from *Minus 2* sites (3.5 ± 0.6 mm) ($p = 0.02$). On the other hand, among immediate restored sites, the smallest values were reported for both *Bone Level* (2.9 ± 0.4 mm) and *Minus 2* (3.0 ± 0.4 mm) sites, when compared to *Minus 1* (3.8 ± 0.6 mm) ($p = 0.01$).

In conventionally restored groups, mean RAL was smaller for *Bone Level* sites (4.1 ± 0.3 mm) than both *Minus 1* (4.9 ± 0.4 mm) ($p = 0.04$) and *Minus 2* sites (5.1 ± 0.9 mm) ($p = 0.04$). Among the immediate restored groups (*Bone Level* versus *Minus 1* versus *Minus 2*), differences were not statistically significant.

Data from radiographic analysis are presented in Table 2 and represented in Figure 6. In a general manner, the immediate restored groups (Ridge Loss = 0.5 ± 0.7 mm) maintained the height of the ridge more effectively than conventionally restored groups (Ridge Loss = 0.9 ± 0.6 mm); however this difference was not statistically significant. Additional data from Ridge Loss are represented in a Box-Plot graphic (Fig. 7).

The distance between IAJ and fBIC was shorter for *Minus 2*, followed by *Minus 1* and *Bone Level* groups; however, statistically significant differences were not observed for this parameter.

On the other hand, concerning the distance from Ridge to fBIC, the smallest values were observed in *Bone Level* followed by *Minus 1* and *Minus 2* sites; the same sequence was observed among conventionally ($p = 0.0005$) and immediately restored groups ($p = 0.0003$). In one of the *Bone Level* sites submitted to conventional restoration (Dog 4, Ridge to fBIC = 0.0 mm), and in another, submitted to immediate restoration (Dog 5, Ridge to fBIC = 0.0 mm), the bone defect had low values, because horizontal bone losses occurred.

Lateral Bone Loss corresponds to the horizontal component of the defect size (Fig. 8). The smallest values were observed in *Bone Level* sites; this tendency was statistically significant in conventionally restored groups ($p = 0.03$), but not in immediately restored groups. Similarly to the vertical component of bone size, in one of the *Bone Level* sites, submitted to conventional restoration (Dog 4, Lateral Bone Loss = 0.0 mm), as well as in another under immediate restoration (Dog 5, Lateral Bone Loss = 0.0 mm) the bone defect had low values, because horizontal bone loss occurred. Moreover, *Minus 2* immediately restored sites (1.0 ± 0.4 mm) had statistically less significant Lateral Bone Loss than its conventionally restored correspondent (1.3 ± 0.3 mm) ($p = 0.04$).

DISCUSSION

The present study evaluated changes that occurred around dental implants inserted in different vertical positions, while submitted to different loading protocols. This methodology was designed to clarify some contradictions found in the current literature.

First, the use of two-piece implants is discouraged in aesthetic zones, because the presence of the microgap has been reported to contribute to significant bone loss[1,6,16,17]. Secondly, the insertion of the IAJ apically to the crestal bone has been related to additional bone resorption[1,6,16]. On the other hand, the insertion of two-piece implants permits the insertion of the IAJ below the crestal bone, which has been suggested to optimize the emergence profile, to contribute to the maintenance of the height, texture, and tonality of peri-implant tissues, and to allow the substitution of the abutment in case of marginal tissue recession.[2,3,4]

It is important to mention that those studies, which evaluated implants inserted in different vertical positions, used different types of implants with varied distances from IAJ to the rough/smooth border, or were developed under unloaded conditions. Nevertheless, the proximity between IAJ and rough/smooth border has shown to interfere in the amount of bone loss.[17] In addition, mechanical load has shown to play an important role in bone remodeling and formation[18,19,20]. For this reason, in the present study, only one type of two-piece implant was used, and its surrounding tissues were analyzed with emphasis on the height maintenance. Thus, two vertical positions (1 and 2 mm below crestal bone) were tested in comparison with implants inserted at crestal bone level, and the effect of immediate restoration was compared to a conventional protocol.

Immediate and conventional restoration models were chosen, and prostheses were prepared to avoid direct occlusal contact with the opposing dentition[21]. However, load still could be transmitted during feeding, and due to muscle action. The avoidance of centric and eccentric contacts had been previously used by Ericsson et al.[22], Andersen et al.[11], and Lorenzoni et al.[12]. In this last study, occlusal splints were provided, which decreased the risk of overloading the implants, and improved biomechanical distribution. For the same reasons, splinted metallic crowns were used in the present investigation.

Ninety days after prosthesis installation, PSTM-PAJ values were smaller in the immediately restored groups ($p = 0.02$), which clinically suggests that the height of soft tissues was better maintained. This finding corroborates the clinical observation of papillary maintenance adjacent to immediately restored implants followed from nine to

36 months[10], one year[12], and five years[11]. However, it is not in accordance with the histological data by Siar et al.[23], where the difference between groups was not statistically significant ($p = 0.516$), and mucosal margin remained more coronal to the implant platform in delayed loaded (2.38 ± 0.81 mm) than in immediately loaded groups (2.27 ± 1.18 mm). This could be explained by differences in the type of study, a histometric study, as well as in implant design, a platform switching system with a 2-mm smooth transmucosal collar.

In the present investigation, PSTM may have been influenced by the Ridge Loss, which was smaller in the immediately restored groups ($p > 0.05$). Additionally, it should be considered that despite of equal loading periods (90 days), peri-implant tissues around conventionally restored sites had been submitted to a longer healing period (120 days) than immediately restored sites.

The distance from IAJ to fBIC was evaluated to provide information concerning the vertical component of bone defect. There was a tendency toward smaller amounts of bone resorption around implants inserted in deeper positions at baseline ($p > 0.05$). This trend was also documented by Todescan et al.[24] who evaluated for a 3-month healing period, implants placed 1 mm above, 1 mm below, or at crestal bone level under unloaded conditions. Furthermore, it is not in accordance with previous studies, which followed unloaded implant for a 6-months healing period, and observed the loss of approximately 2 mm below microgap to reestablish the biologic width[17,25]. It could be suggest that the healing period of the present investigation was not sufficient to rearrange the anatomy around implants inserted in the deepest positions, since great amounts of bone resorption should occur in these groups. However, according to Hermann et al.[1], the changes in alveolar crest location around two-piece implants occurred within the first 4 weeks after abutment connection, even for implants inserted 1 mm below crestal bone.

In conventionally restored groups, values from PD and RAL were greater as the implants were inserted in deeper positions. This finding corroborates the histometric study by Todescan et al.[24], in which longer epithelium and connective tissue were observed around implants placed 1 mm below crestal bone, in comparison to those placed at crestal bone level. Nevertheless, this situation was not observed among the immediately restored groups, since *Minus 1* sites had higher PD ($p = 0.01$) and RAL means ($p < 0.05$) than *Bone Level* and *Minus 2* sites. Nevertheless, loading protocol did not influence these parameters. This observation is similar to that by Romeo et al.[26] in

their clinical study, which compared PD of immediate and delayed loaded implant supporting overdentures for 2 years.

Nevertheless, concerning Lateral Bone Loss results, *Bone Level* groups had the smallest values. This finding may be explained by the occurrence of horizontal bone resorption in some sites of these groups. Consequently, the absence of cup-shaped bone defects, seemed to have brought the values of this measurement to a minimum. If these groups were not considered, *Minus 2* sites submitted to immediate restoration would present the lowest values. Interestingly, among the groups with immediate restoration, the type of bone defects clearly tended to be wider in *Minus 1*, and narrower in *Minus 2* sites. Among sites with conventional restoration, bone defects became wider as much as the implant was inserted in deeper positions.

The width of bone defect is an important parameter to be considered while choosing the ideal three-dimensional positioning of an implant. According to Tarnow et al.[8], lateral bone loss is estimated in 1.34 to 1.40 mm in humans. Hence, between adjacent implants, a distance of 3 mm should be maintained, to prevent lateral bone loss overlapping, crestal bone resorption, and the increase in the distance from the crestal bone to the contact point, which would result in apical migration of the soft tissue margin.

Since there is a clear relationship among apicocoronal, mesiodistal, and buccolingual positioning of an implant, it is important to mention that the deep position of an implant should be restricted to cases in which adequate mesiodistal and buccolingual space are available. Then, the crestal bone of adjacent tooth or implant will support the architecture of the soft tissue margin[7,9]. In non-aesthetic areas, the use of apically positioned implants is not justifiable, and the IAJ (for two-piece implant) or the rough-smooth border (for one-piece implants) should be positioned at the crestal bone level or even more coronally.

Finally, the development of this animal trial permitted the creation of controlled conditions, and allowed comparisons among different groups. Nevertheless, studies with longer healing periods and human clinical trials should be conducted to provide data to support these findings, and evaluate its clinical significance.

In conclusion, within the limits of the present study, the apical positioning of IAJ did not jeopardize the height of peri-implant soft and hard tissues evaluated by clinical and radiographic analyses. Moreover, immediate restoration was beneficial to the maintenance of the PSTM. Theses results suggest that apical positioning

of the implants can be successfully used, mainly in combination with an immediate restoration protocol. Further studies are suggested to evaluate the significance of these results in longer healing periods.

ACKNOWLEDGMENTS

The authors would like to thank CAPES (Government Agency for the Development of Higher Education), CNPq (Brazilian Council for Scientific and Technological Development, scholarship no. 141204/2004-4), and FAPESP (São Paulo Foundation for the Support of Research, grant no. 04/08141-3) for financial support. In addition, they would like to express their gratitude to Conexão Sistema de Prótese Ltda for providing the implants and related supplies used in the present study.

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TABLES

Table 1. Mean values (mm ± standard deviation) from the clinical analysis.

	Conventional restoration				**Immediate restoration**			
	Bone Level	***Minus 1***	***Minus 2***	P	***Bone Level***	***Minus 1***	***Minus 2***	P
PSTM -PAJ	1.5 ± 0.7	1.8 ± 0.6	1.6 ± 0.9	ns	1.1 ± 0.7	0.6 ± 1.0	0.9 ± 1.1	ns
PD	2.6 ± 0.5[a]	3.0 ± 0.4	3.5 ± 0.6[a]	0.02	2.9 ± 0.4[b]	3.8 ± 0.6[bc]	3.0 ± 0.4[c]	0.01
RAL	4.1 ± 0.3[de]	4.9 ± 0.4[d]	5.1 ± 0.9[e]	0.04	4.0 ± 0.4	4.4 ± 0.6	3.9 ± 1.2	ns

Identical letters indicate statistically significant differences ($p < 0.05$, ANOVA test).

ns Non-significant.

PAJ = Prosthesis-Abutment Junction.

PD = Probing Depth.

PSTM = Position of the Soft Tissue Margin.

RAL = Relative Attachment Level.

Table 2. Mean values (mm ± standard deviation) from the radiographic analysis.

	Conventional restoration				Immediate restoration			
	Bone Level	***Minus 1***	***Minus 2***	P	***Bone Level***	***Minus 1***	***Minus 2***	P
Ridge Loss*	0.8 ± 0.7	0.9 ± 0.6	0.8 ± 0.6	ns	0.7 ± 0.7	0.5 ± 0.7	0.4 ± 0.7	ns
IAJ-fBIC	1.5 ± 0.4	1.2 ± 0.3	0.9 ± 0.5	ns	1.4 ± 0.5	1.1 ± 0.6	0.7 ± 0.5	ns
Ridge-fBIC	0.6 ± 0.4^{ab}	1.2 ± 0.5^{ac}	2.0 ± 0.4^{bc}	0.0005	0.6 ± 0.4^{de}	1.7 ± 0.6^{df}	2.2 ± 0.5^{ef}	0.0003
LBL	0.8 ± 0.4^{gh}	1.2 ± 0.3^{g}	1.3 ± 0.3^{h}	0.03	0.8 ± 0.5	1.2 ± 0.3	1.0 ± 0.4	ns

Identical letters indicate statistically significant differences ($p < 0.05$, ANOVA test).

* Value obtained using hypothetical ridge level at baseline.

ns Non-significant.

IAJ = implant-abutment junction.

fBIC = first bone-implant contact.

LBL = lateral bone loss.

FIGURES

Figure 1. Buccal view of the sites after implants installations. In this case (Dog 1), *Bone Level*, *Minus 1*, *Minus 2* groups, respectively.

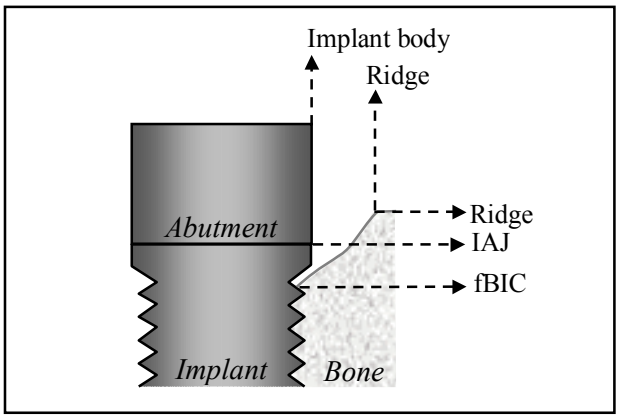

Figure 2. Schematic diagram representing the parameters used in radiographic evaluation. IAJ = Implant-abutment junction; fBIC = First bone-implant contact.

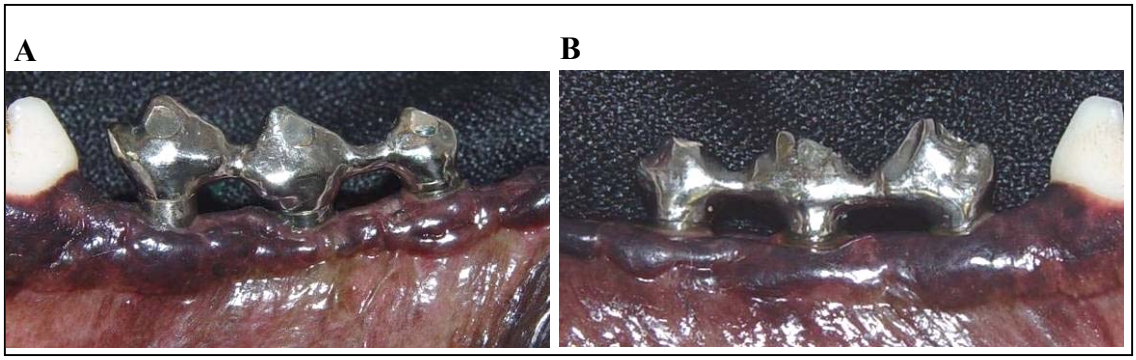

Figure 3. Clinical aspect of (A) conventionally (*Minus 2*, *Minus 1*, and *Bone Level* sites, respectively) and (B) immediately (*Bone Level*, *Minus 1*, and *Minus 2* sites, respectively) restored groups, 90 days after prostheses installation, in the same dog of Figure 1.

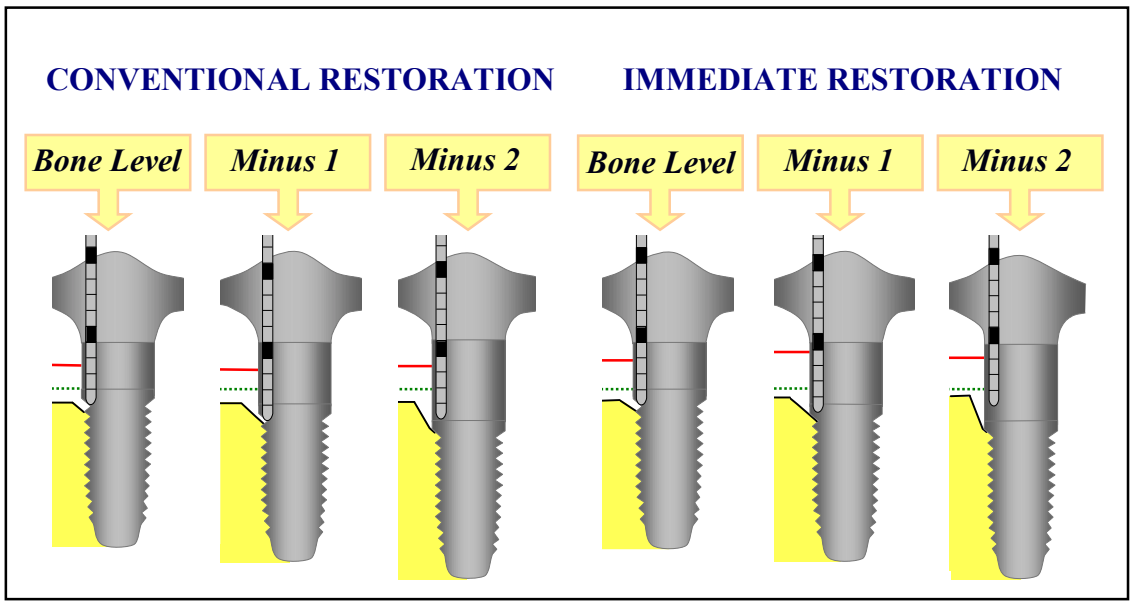

Figure 4. Schematic diagram of different groups, 90 days after prostheses installation. Green line represents a hypothetical ridge at baseline; red line represents the position of soft tissue margin at the end of the experiment. Periodontal probes simulate Probing Depth and Relative Attachment Level measurements.

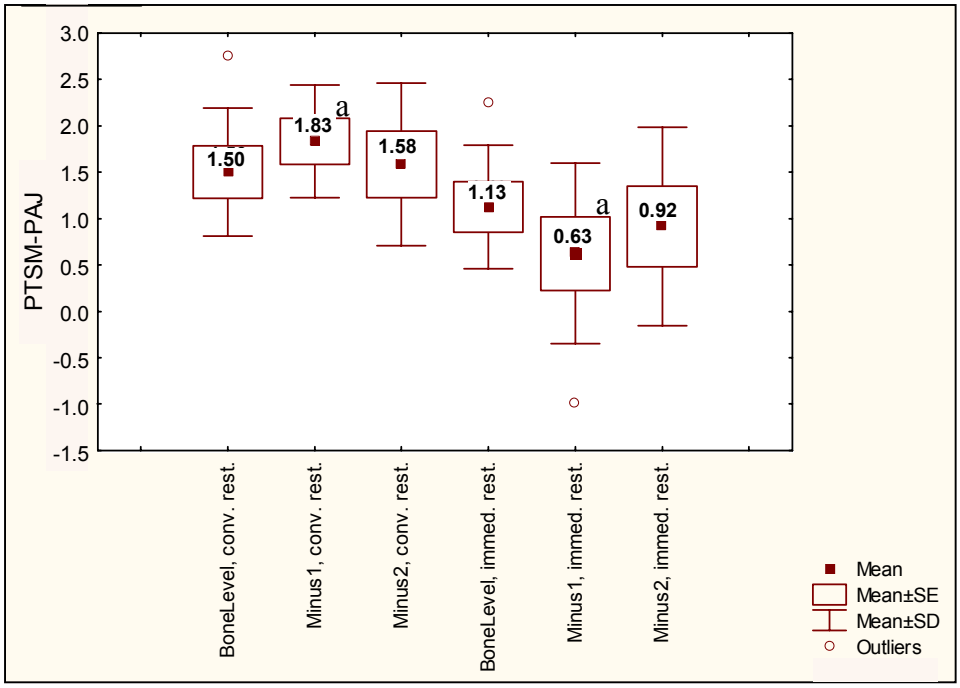

Figure 5. Mean values (mm) from the Position of the Soft Tissue Margin (PSTM) to prosthesis-abutment junction (PAJ), 90 days after loading. Conv. rest. = conventional restoration; immed. rest. = immediate restoration. Identical letters indicate statistically significant differences ($p < 0.05$, Student *t* test).

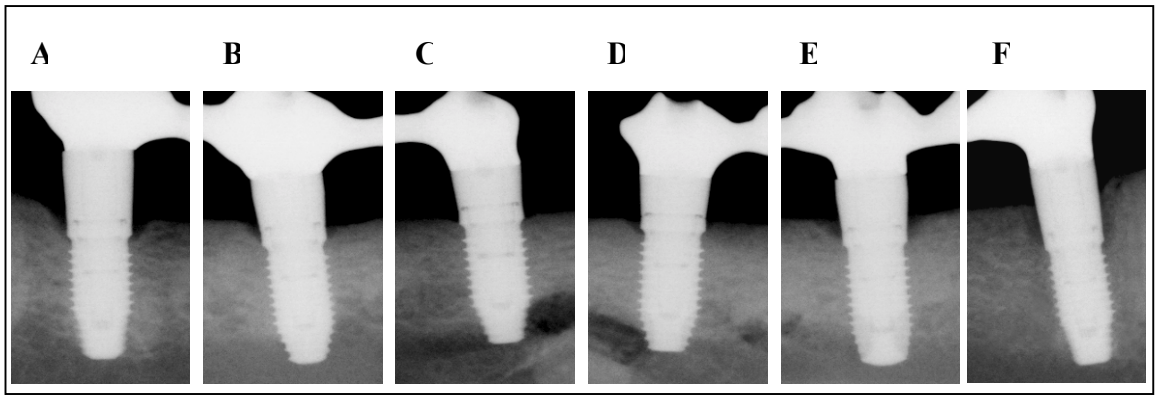

Figure 6. Radiographic aspect of conventionally (A = *Minus 2*, B = *Minus 1*, and C = *Bone Level*) and immediately (D = *Bone Level*, E = *Minus 1*, and F = *Minus 2*) restored groups 90 days after loading, in the same dog of Figure 1.

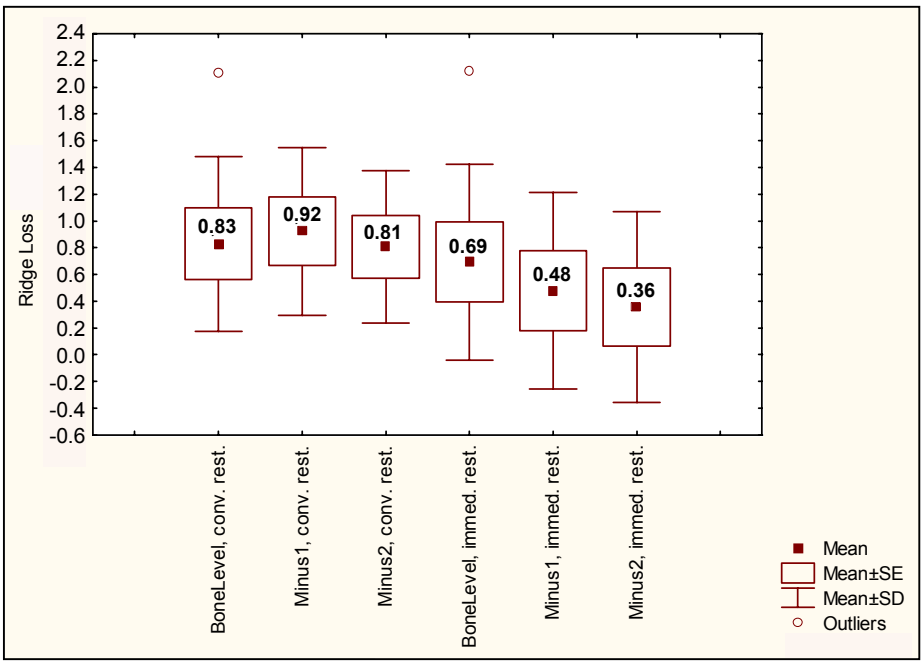

Figure 7. Mean values (mm) from the Ridge loss, obtained using hypothetical crestal bone level at baseline. Conv. rest. = conventional restoration; immed. rest. = immediate restoration.

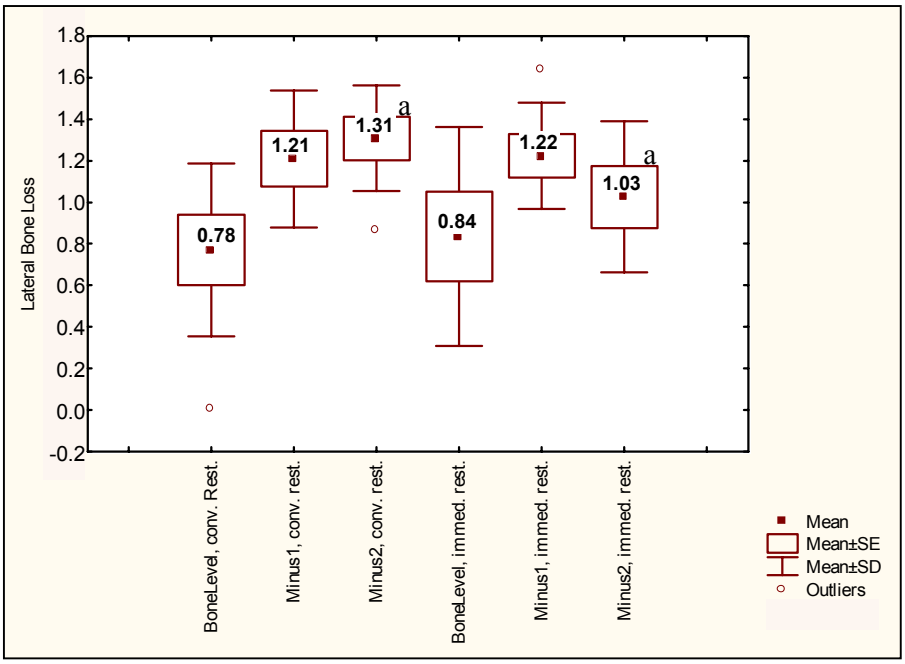

Figure 8. Mean values (mm) from the Lateral bone loss. Conv. rest. = conventional restoration; immed. rest. = immediate restoration. Identical letters indicate statistically significant differences ($p < 0.05$, Student t test).

7 CAPÍTULO 4

Este capítulo é constituído pelo seguinte artigo, que aborda a análise histométrica dos dados desta tese:

Pontes AEF, Ribeiro FS, Iezzi G, Piattelli A, Cirelli JA, Marcantonio Jr E. Biologic width changes around loaded implants inserted in different levels in relation to crestal bone. Histometric evaluation in canine mandible. Submetido ao periódico *Clinical Oral Implants Research.*

Biologic width changes around loaded implants inserted in different levels in relation to crestal bone. Histometric evaluation in canine mandible.

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Running title: Loaded implants inserted in different vertical positions.

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Key-words: Dental implants, Prosthesis, Esthetics, Histometry, Animal Study, Soft tissue, Biologic Width.

ABSTRACT

Pontes AEF, Ribeiro FS, Iezzi G, Piattelli A, Cirelli JA, Marcantonio Jr E. Biologic width changes around loaded implants inserted in different levels in relation to crestal bone. Histometric evaluation in canine mandible. Clin Oral Impl Res.

Objectives. The aim of the present study was to evaluate, histometric changes around dental implants inserted at different levels in relation to crestal bone, under different loading conditions.

Material and methods. Thirty-six implants were inserted in the edentulous mandible of 6 mongrel dogs. Each implant was assigned to an experimental group according to the distance from the implant-abutment junction (IAJ) to the crestal bone: *Bone Level* (at crestal bone level), *Minus 1* (1mm below crestal bone), or *Minus 2* group (2mm below crestal bone). Each hemimandible was submitted to a loading protocol: conventional or immediate restoration. After 90 days, the animals were killed. Specimens were processed, and measurements were performed concerning the length of soft and hard peri-implant tissues. Data were analyzed using ANOVA and Student's *t* test (α=5%).

Results. Among conventionally restored sites, the distance from the more coronal position of soft tissue (PSTM) and first bone-implant contact (fBIC) was greater for *Minus 2* than for *Bone Level* and *Minus 1* sites (p=0.03), but significant differences were not observed among immediately restored sites. Differences among groups were not observed concerning the PSTM, and the distance from IAJ to fBIC. Greater amounts of Lateral Bone Loss were observed for conventionally than for immediately restored sites (p=0.006).

Conclusions. These findings suggest that the apical positioning may not jeopardize the position of soft peri-implant tissues, and that immediate restoration can be beneficial to minimize lateral bone loss. Further studies are suggested to evaluate the clinical significance of these results in longer healing periods.

INTRODUCTION

Currently, plenty of research in dental implant has been focused on improving the aesthetic outcomes of peri-implant soft tissues. With this aim, one-piece and two-piece implants have been inserted under different loading conditions, and in different apicocoronal positions.

In 2001, Hermann et al. evaluated changes that occurred around implants with different designs, and observed that the use of two-piece implants resulted in significantly increase in crestal bone loss, in more apical position of the soft tissue margin, and higher degree of inflammation. The authors concluded that the use of one-piece implants leads to the formation of a biologic width more similar to that found around natural teeth, in comparison with two-piece implants.

On the other hand, even proponents of one-stage implant systems consider the use of two-stage protocol with an implant placement deeper than usual for aesthetic improvement (Garber et al. 2001). A more apical positioning of the implant-abutment junction (IAJ) would allow the use of healing caps with emergence profile, and the substitution of the prosthetic component in case of marginal tissue recession, contributing to the maintenance of the mucosa texture and tonality, as well as providing the reestablishment of the marginal tissues architecture (Louise & Borghetti 2002).

In 2002, Todescan et al. performed a study in which one type of two-piece implant was placed at different depths in canine mandible. The dimension and relationship of the peri-implant tissues were evaluated, and the mean values of vertical bone loss, represented by the distance from the IAJ to the first bone-implant contact (fBIC), were smaller as much as the implants were placed in deeper positions, and peri-implant mucosa showed no signs of inflammation.

It is important to mention that the implants in Hermann et al. (2001) and Todescan et al. (2002) studies were unloaded. On the contrary, mechanical loading, mainly the use of immediate restoration, has shown to play an important role in bone remodeling and formation (Frost et al. 1992, Degidi et al. 2003, Degidi et al. 2005).

Moreover, concerning apicocoronal position, a hypothesis is that the apical positioning of the implant is not always related to additional loss of peri-implant soft tissues height (Grunder 2000), because instead of apically migrating, these tissues would be supported by the ridge of an adjacent tooth or implant (Tarnow et al. 2000, Tarnow et al. 2003).

However, no information regarding the physiological response to the insertion of implants in deeper positioning under immediate and conventional restoration protocols is reported in the literature. In addition, there are scarce studies on whether those modalities of treatment could be successfully used as an alternative approach valid for aesthetic situations.

Thus, the aim of the present study was to evaluate comparatively the histometric changes in tissues around implants inserted at different levels in relation to the crestal bone, and under different loading conditions.

MATERIAL AND METHODS

The present study was approved by the Ethical Committee in Animal Research from the State University of São Paulo. Six mongrel dogs, featuring good health, weighing 23.0 ± 6.30 kg were included in the present study. Previously to the first surgical intervention, the dogs were submitted to coronal scaling and were molded with condensation silicon (Zhermack SPA, Badia Polesine, Italy).

Thirty-six dental implants (Conect, Conexão Sistema de Prótese Ltda, São Paulo, Brazil) were used in this study (4.3 x 10 mm, sandblasted with titanium oxide, root-form, internal hexagon). In each dog, six dental implants were inserted, three per hemimandible, each one representing an experimental group. The experimental groups were designed according to the distance between the IAJ and the crestal bone: *Bone Level* group (inserted at crestal bone level), *Minus 1* group (one millimeter below crestal bone), and *Minus 2* group (two millimeters below crestal bone). Each hemimandible was submitted to a different loading protocol: conventional restoration (prostheses installation occurred 120 days after implant placement), or immediate restoration (prostheses installation occurred 24 hours after implant placement). Thus, six sets of arrangement were designed, so that an implant representing each group was inserted one time in any site.

In order to carry out surgical procedures, 1% acepromazine (0.02 mg / kg, 0.1 mL / kg, intramuscular) was administered, followed by thiopental (10 mg / kg, 0.5 mL / kg, intravenous). The oral cavity was disinfected with gauzes soaked in 0.12% chlorhexidine solution, and local anesthesia was performed with 2% mepivacaine HCl with Norepinephrine 1:100.000 (Spécialités Septondont, Saint Maur, France). An intrasulcular incision was performed, and after the mucoperiosteal flap was reflected, bicuspids were sectioned with high-speed bur under saline irrigation. All mandibular premolars were extracted with forceps, and flaps were closed with 4.0 nylon suture. After the surgical procedures, antibiotic association (penicillin and streptomycin, 24.000 UI / kg, 0.1 mL / kg, intramuscularly) and analgesic ketoprofen (2 mg / kg, 0.4 mL / kg, intramuscular) were administered. In the following 2 days, the dogs received additional doses of analgesic. During the first week post-surgery, the animals were fed a soft diet. Ten days after surgical procedures, sutures were removed. During the experimental period, animals were submitted to a rigorous plaque control with tooth brushing using

0.12% chlorhexidine gel, 3 times a week. These preoperative and postoperative cares were repeated on following surgical procedures.

After a 90-days period of healing, a crestal incision was performed on the hemimandible designed to be submitted to conventional restoration, maintaining similar quantities of keratinized tissue on each side of the incision, and a mucoperiosteal flap was reflected. Dental implants representing each group were inserted, using the mesial crestal bone as reference point. Horizontal distances were determined as following: 6 mm between the surfaces of adjacent implants, and 4 mm between the mesial surface of the first molar and the implant. In sequence, flaps were sutured.

Ninety days afterwards, on the same side, a crestal incision was performed, the cover screws were removed, and healing caps were screwed. The heights of healing caps were selected according to commercial availability: 3 mm, 4 mm and 5.5 mm, and were used respectively in *Bone Level*, *Minus 1* and *Minus 2* sites. Then, flaps were closed.

Thirty days after, on the conventional restoration side, the healing caps were removed, the abutments were placed, and impression was taken using custom-made trays with condensation silicone. On the other side, a crestal incision was performed, the dental implants were inserted, abutments were placed, impression was taken, and flaps were closed. The abutments heights corresponded to those from healing caps.

Twenty-four hours later, metallic fixed partial prostheses were passively screwed. Special attention was taken to avoid occlusal contact. The animals were followed-up for 90 days after prostheses installation.

After the animals were killed, mandible and maxilla were dissected, and the specimens were prepared according to a method previously described by Piattelli et al. (1997). The fixation process was accomplished by using 10% neutral formalin for 48 hours. The specimens were dehydrated by using increasing alcohol concentrations, from 60 to 100%. Then, plastic infiltration was processed, with combinations of alcohol and resin (Technovit 7200 VLC. Kulzer, Wehrheim, Germany).

The specimens were polymerized, sectioned at about 150μm using a specific system (Precise 1 Automated System, Assing, Rome, Italy), and ground down to about 100 μm. Slides were stained with toluidine blue and acid fuchsine, and were analyzed using a microscope connected to a video camera interfaced to a computer, where specific processing software was used for measurements (ImageJ 1.34, National Institutes of Health, Bethesda, MA, USA).

Histometric analysis. The following parameters were evaluated (Fig. 1): (1) sulcus depth (SD), distance from the most coronal position of soft tissue margin (PSTM) to the most coronal point of the junctional epithelium; (2) junctional epithelium (JE), distance from the most apical to the most coronal point of the junctional epithelium; (3) connective tissue attachment (CT), distance from the most apical point of the junctional epithelium to the fBIC; (4) PSTM-fBIC, distance from PSTM to fBIC; (5) PSTM-IAJ, distance from PSTM to IAJ; (6) IAJ-fBIC, distance from IAJ to fBIC; (7)Ridge-fBIC, distance from the ridge to fBIC; and (8) lateral bone loss (LBL), from the implant body to the ridge.

Additionally, PSTM-IAJ values were adjusted to evaluate the height of PSTM adding one millimeter to *Minus 1*, and two millimeters to *Minus 2* values.

Statistical analysis. All the 36 implants were available for data collection. Values were expressed in means, and the unit of analysis was the dog. Intraexaminer reliability of the examiner was determined by calculating standard error of measurement (SE) and Spearman correlation coefficient (CC) for PSTM-IAJ (SE = 0.21 mm, CC = 0.987mm) and IAJ-fBIC (SE = 0.11 mm, CC = 0.977mm)

Experimental data was submitted to a normality test (Shapiro-Wilk). Analysis of variance tested the effect of implant positioning (*Bone Level versus Minus 1 versus Minus 2*) among groups submitted to the same loading protocol. The effect of loading protocol (conventional *versus* immediate restoration) was tested for each implant positioning separately, and by gathering data from the three implant positions in each hemimandible. Data was analyzed by ANOVA (followed by Bonferroni's method for multiple comparisons), and Student *t* test. The null hypothesis was based on the absence of differences among the modalities of treatment (α = 5%). The influence of implant position in the arch and among dogs was checked for possible confounding of the results, and was not significant (ANOVA, $p > 0.05$).

RESULTS

Healing was uneventful in all animals, no loss of either implants or prostheses was observed during the experimental period, and a direct contact was observed between living bone and all implants without interposed soft tissues at the light microscope level. Images representing each group are presented in Figures 2, 3, 4, 5, 6, and 7. A schematic diagram of soft and hard tissues remodeling is presented in Figure 8.

For all implants, keratinized oral epithelium was continuous with a junctional epithelium facing the implant and abutment surface. Subjacent connective tissue with a dense network of collagen fibers was observed, with few vascular structures and scattered inflammatory cells.

Data from histometric measurements are summarized in Tables 1 and 2. Differences among groups were not observed concerning SD, JE, CT ($p > 0.05$). The distance from PSTM and fBIC tended to be greater the deeper the implants were placed, however, statistically significant differences were observed only among conventionally restored groups, as *Minus 2* sites featured statistically greater values than *Bone Level* and *Minus 1* sites ($p=0.03$).

The distance from PSTM to IAJ revealed that the greater amounts of soft tissues were available over implants inserted in deeper positions (Fig. 9). For conventionally restored groups, this finding was statistically significant ($p = 0.005$). Instead, for immediately restored sites, statistically significance was observed when *Bone Level* sites were compared to *Minus 1* ($p = 0.01$), and *Minus 2* ($p = 0.01$), but not when *Minus 1* sites were compared to *Minus 2* ($p > 0.05$).

In another analysis, PSTM-IAJ values were adjusted to compare the height of PSTM among groups (Table 3). No differences were observed concerning this parameter, but *Minus 1* sites submitted to immediate loading presented the soft tissue margin were in the most coronal position (2.04 ± 0.56 mm), in comparison with the other groups (mean values ranged from 1.41 ± 0.79 mm to 1.56 ± 0.81 mm) ($p > 0.05$).

The vertical bone loss was evaluated by measuring the distance from IAJ to fBIC (Fig. 10). Statistically significant differences were not observed for this parameter ($p > 0.05$), but the values increased as the implants were inserted in deeper positions.

The distance from ridge to fBIC was representative of the vertical size of the bone defect. Thus, greater values were observed as the implants were inserted in

deeper positions, so that *Bone Level* presented statistically smaller bone defects than *Minus 1* and *Minus 2* sites, under either conventional ($p = 0.01$) or immediate restoration ($p = 0.003$).

Lateral bone loss was representative of horizontal component of bone defect (Fig. 11). The lowest values were observed for *Bone Level* and *Minus 2*, followed by *Minus 1* sites. Furthermore, *Minus 2* immediately restored sites (0.83 ± 0.28 mm) presented statistically less lateral bone loss than conventionally restored sites (1.31 ± 0.32 mm) ($p = 0.01$). In a general manner, bone defects were narrower for immediately restored implants in comparison with conventionally restored implants ($p = 0.006$). In fact, this was the only parameter in which differences between immediately and conventionally restored implants were statistically significant.

DISCUSSION

The methodology of the present study was designed to clarify the histological aspect of soft and hard tissues around dental implants inserted in different vertical positions, and submitted to different loading protocols. After completion of the healing period, the most significant findings were that the position of soft tissue margin was maintained in spite of the vertical position of the IAJ at baseline; and lateral bone loss was narrower for immediately restored sites in comparison with the conventionally restored ones. The clinical implication is that, at least under the conditions studied, submerging two-piece implants not necessarily jeopardize the location of the soft tissue margin for final restoration; also, immediate restoration could be considered in treatment planning as an alternative to control the magnitude of lateral bone loss.

The extension of SD featured values ranging from 0.43 mm (*Minus 1* site, immediately restored) to 0.83 mm (*Minus 2* sites, conventionally restored). These values are in accordance with the results of the animal studies by Hermann et al. (2001) and Siar et al. (2003). In the former, implants were inserted at crestal bone level (SD mean = 0.14 mm) and 1 mm below it (SD mean = 0.14 mm), and were followed-up for 3 months after abutment connection without loading. While in the latter, implants were inserted 1 mm below crestal bone, and remained under immediate loading (SD mean = 0.68 mm) or delayed loading (SD mean = 0.88 mm) conditions for 3 months.

Mean extension of JE ranged from 0.85 mm (*Bone Level* sites, immediately restored) to 1.04 mm (*Minus 2* sites, immediately restored) in the present study ($p > 0.05$). These low values may be explained by the location of the apical portion of the epithelium, which was not always found apical to the IAJ, similarly to previous studies (Berglundh et al. 1991, Berglundh & Lindhe 1996, Abrahamsson et al. 1996). In the study by Todescan et al. (2002), where the implants were unloaded, implants positioned at crestal bone level and 1 mm below crestal bone presented mean JE length of 1.936 mm and 2.781 mm, respectively ($p > 0.05$). Whereas, in the study by Siar et al. (2003), implants inserted 1 mm below crestal bone featured a mean JE of 1.66 mm (delayed loading) and 1.71 mm (immediate loading) ($p > 0.05$).

In the present investigation, mean CT ranged from 1.49 mm (*Bone Level*, immediately restored) to 2.76 mm (*Minus 2*, immediately restored), with higher values for implants in deeper positions; however this finding was not statistically significant. These values corroborated the study by Todescan et al. (2002), but presented higher values,

since implants positioned at crestal bone level and 1 mm below crestal bone had mean CT values of 0.927 mm and 1.636 mm, respectively ($p > 0.05$).

In the present investigation, the extension of soft tissue (PSTM-fBIC) included the SD, JE and CT lengths. Within these parameters, mean CT values varied the most (ranged from 1.49 mm to 2.76 mm), and its extension seemed to be responsible for filling the space created by the vertical bone loss. The mean JE length varied the least (ranged from 0.85 mm to 1.04 mm). This is not in accordance with Hermann et al. (2000), in whose study, the epithelium was found apical to the IAJ in every case. One possible explanation is that in the present study, under immediate restoration, the prostheses were installed without removing and subsequent reconnecting the abutments. According to Abrahamsson et al. (1997), the apical portion of the JE was found apically to the microgap, due to the disruption of the mucosal barrier causing epithelial proliferation and to the bone resorption, to allow the formation of a connective tissue contact of proper dimension. Nevertheless, in sites under conventional restoration, healing cap was removed and sterilized abutment was connected under aseptic condition, which probably caused the disruption of the mucosal barrier, but permitted the reestablishment of the direct contact of soft tissues to the abutment, instead of the epithelial migration to the IAJ level.

The extension of soft tissue coronally to the implant-abutment junctions was calculated by PSTM-IAJ measurement. It is important to mention that soft tissue heights of less than 2 mm are reported to be challenging for aesthetic restoration (Saadoun et al. 1999). In the present study, the use of implants inserted at crestal bone level (*Bone Level* groups) resulted mean in soft tissues heights of 1.47 ± 0.97 mm and 1.56 ± 0.81 mm, respectively for conventional and immediate restoration. On the other hand, soft tissue heights of more than 4 mm could result in the formation of an infra-osseous defect, peri-implant pocket, complications in the second phase, difficulty in abutment connection, and cement excess at the restoration fixation (Saadoun et al. 1999). In the present study, mean *Minus 1* values ranged from 2.41 ± 0.79 mm and 3.04 ± 0.56 mm, while *Minus 2* mean values reached 3.51 ± 0.89 mm and 3.43 ± 1.40 mm, respectively for conventional and immediate restoration. The stability of these results, and their clinical significance should be evaluated over a longer period.

The PSTM-IAJ data were also analyzed compensating the apicocoronal positioning, by adjusting it to a hypothetical crestal bone level at baseline. This measurement is an important parameter to provide the outer morphology of the soft

tissues margin. Statistical methods were not efficient in detecting differences among groups, probably due to the high standard deviation values.

The measurement of the distance from ridge to fBIC was used to clarify the vertical size of bone defect, while the distance from IAJ to fBIC was used to evaluate the vertical bone loss bellow IAJ level. The former increased ($p < 0.05$), while the latter one decreased as the implants were placed in deeper positions ($p > 0.05$). The reduction of IAJ-fBIC values was not statistically significant, and has been previously reported by Todescan et al. (2002). However, it is not in accordance with other studies, in which a bone loss of approximately 2 mm below the microgap was observed for a 6-months healing period under unloaded conditions, to reestablish biologic width (Alomrani et al. 2005) (Cochran et al. 1997). This difference may be related to the experimental design, implant design, and the presence of inflammatory infiltrate that differed among the studies.

Finally, Lateral Bone Loss was calculated. Although *Minus 1* groups presented the highest mean values, no differences were observed among groups concerning implant position. However, loading protocols influenced this parameter ($p = 0.006$), and *Minus 2* implants undergoing immediate restoration featured statistically narrower bone defects in comparison with *Minus 2* sites under conventional restoration ($p = 0.01$). This fact may be explained due to the stimulation caused by mechanical loading (Frost et al. 1992, Degidi et al. 2003, Degidi et al. 2005).

The width of bone defect should be considered while choosing the ideal three-dimensional positioning of an implant, because if a minimum lateral bone loss is not preserved, adjacent bone defects overlap, and crestal bone undergoes resorption, which could result in the apical migration of the soft tissue margin (Tarnow et al. 2000). Thus, the deep position of an implant should be restricted to cases in which adequate mesiodistal and buccolingual spaces are available. Then, the crestal bone of adjacent tooth or implant will support the architecture of the soft tissue margin (Grunder 2000) (Tarnow et al. 2003), as occurred in *Minus 1* and *Minus 2* sites under immediate restoration. However, in non-aesthetic areas, the use of apically positioned implants is not justified, and the implant-abutment junctions (if two-piece implants are chosen) should be positioned at crestal bone level or even more coronally.

This animal trial allowed the creation of controlled conditions, and clarified the histological results of different protocols use. Nevertheless, data from studies

with longer healing periods, and human clinical trials should be conducted to support these findings, and evaluate their clinical significance.

In conclusion, within the limits of the present study, the apical positioning may not jeopardize the position of soft peri-implantar tissues, and immediate restoration can be beneficial to minimize lateral bone loss. These findings suggest that apical positioning can be successfully used, mainly combined with immediate restoration protocol. In addition, further studies are suggested to evaluate the clinical significance of these results in longer healing periods.

ACKNOWLEDGMENT

The authors are extremely grateful for the assistance of Dr. Rogério Margonar in the prosthetic phase, and would like to express their gratitude to Conexão Sistema de Prótese Ltda for providing the implants and related supplies used in the present study. This research project was supported by CAPES (Government Agency for the Development of Higher Education, scholarship no. PDEE 0989/05-3), and FAPESP (São Paulo Foundation for the Support of Research, grant no. 04/08141-3).

TABLES

Table 1. Mean values (mm ± standard deviation) for sulcus depth (SD), junctional epithelium (JE), connective tissue (CT), and PSTM-fBIC.

	Conventional restoration				Immediate restoration			
	Bone Level	***Minus 1***	***Minus 2***	**P**	***Bone Level***	***Minus 1***	***Minus 2***	**P**
SD	0.46 ± 0.17	0.65 ± 0.37	0.83 ± 0.46	ns	0.51 ± 0.18	0.43 ± 0.15	0.52 ± 0.17	ns
JE	0.94 ± 0.68	0.95 ± 0.67	0.92 ± 1.09	ns	0.85 ± 0.37	1.04 ± 1.18	0.97 ± 0.50	ns
CT	1.59 ± 0.39	1.90 ± 0.45	2.47 ± 0.88	ns	1.49 ± 0.55	2.24 ± 1.21	2.76 ± 1.15	ns
PSTM -fBIC	3.00 ± 0.90[a]	3.50± 0.59[b]	4.48 ± 1.04[ab]	0.03	2.85 ± 0.60	3.71 ± 0.90	4.25 ± 1.41	ns

Identical letters indicate statistically significant intergroup differences ($p < 0.05$, ANOVA test).
ns = Non-significant.
SD = sulcus depth; JE = junctional epithelium; CT = connective tissue; PSTM = position of soft tissue margin; fBIC = first bone-implant contact.

Table 2. Mean values (mm ± standard deviation) for PSTM-IAJ, IAJ-fBIC, Ridge-fBIC, and LBL.

	Conventional restoration				Immediate restoration			
	Bone Level	***Minus 1***	***Minus 2***	**P**	***Bone Level***	***Minus 1***	***Minus 2***	**P**
PSTM-IAJ	1.47 ± 0.97[ab]	2.41 ± 0.79[ac]	3.51 ± 0.89[bc]	0.005	1.56 ± 0.81[de]	3.04 ± 0.56[d]	3.43 ± 1.40[e]	0.01
IAJ-fBIC	1.46 ± 0.31	1.26 ± 0.43	1.00 ± 0.32	ns	1.54 ± 0.57	1.07 ± 0.73	0.82 ± 0.51	ns
Ridge-fBIC	0.78 ± 0.37[fg]	1.64 ± 0.70[f]	2.02 ± 0.74[g]	0.01	0.77 ± 0.32[hi]	2.06 ± 0.55[h]	2.42 ± 1.06[i]	0.003
LBL	0.87 ± 0.42	1.33 ± 0.46	1.31 ± 0.32	ns	0.84 ± 0.23	1.08 ± 0.24	0.83 ± 0.28	ns

Identical letters indicate statistically significant intergroup differences ($p < 0.05$, ANOVA test).
ns = Non-significant
PSTM = position of soft tissue margin; IAJ = implant-abutment junction; fBIC = first bone-implant contact; LBL = lateral bone loss.

Table 3. Mean values (mm ± standard deviation) for PSTM-IAJ value adjusted by increasing 1 mm to *Minus 1* sites, and 2 mm to *Minus 2* sites.

	Conventional restoration				Immediate restoration			
	Bone Level	***Minus 1***	***Minus 2***	**P**	***Bone Level***	***Minus 1***	***Minus 2***	**P**
PSTM-IAJ (adjusted)	1.47 ± 0.97	1.41 ± 0.79	1.51 ± 0.89	ns	1.56 ± 0.81	2.04 ± 0.56	1.43 ± 1.40	ns

ns = Non-significant
PSTM = position of soft tissue margin; IAJ = implant-abutment junction.

FIGURES

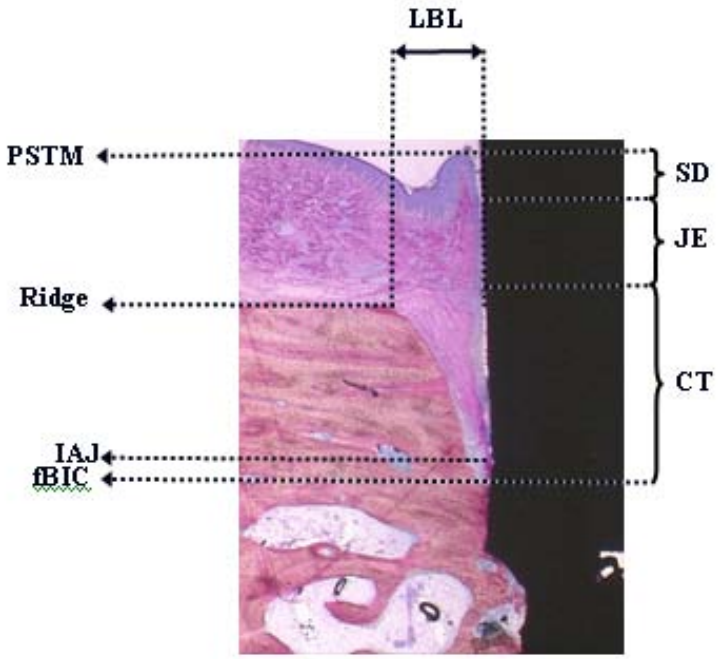

Figure 1. Schematic drawing illustrating the landmarks used for histometric analysis. CT = connective tissues; fBIC = first bone-implant contact; IAJ = implant-abutment junction; JE = junctional epithelium; LBL = lateral bone loss; PSTM = position of soft tissue margin; SD = sulcus depth.

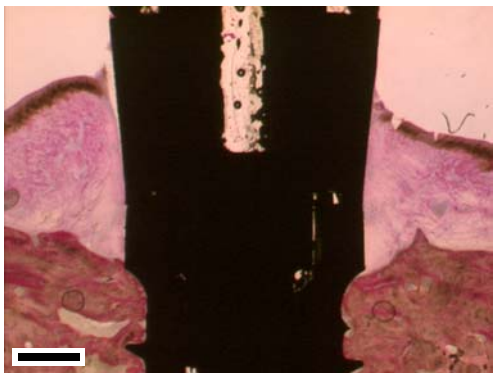

Figure 2. Mesio-distal section of a *Bone Level* implant submitted to conventional restoration protocol. Non-decalcified histological section; toluidine blue and acid fuchsine stain; original magnification X12; black bar = 1 mm.

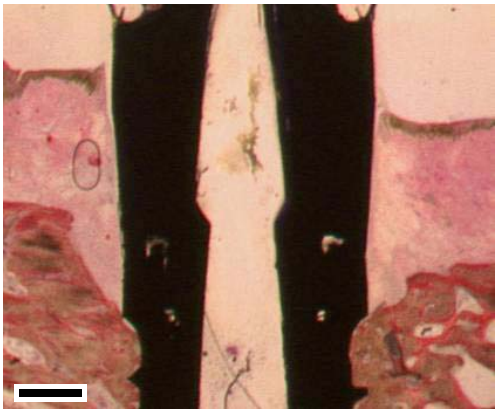

Figure 3. Mesio-distal section of a *Minus 1* implant submitted to conventional restoration protocol. Non-decalcified histological section; toluidine blue and acid fuchsine stain; original magnification X12; black bar = 1 mm.

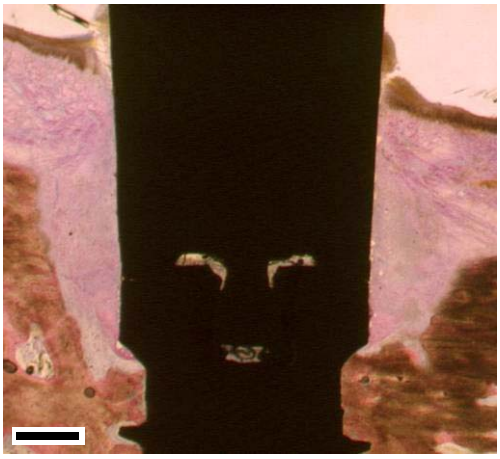

Figure 4. Mesio-distal section of a *Minus 2* implant submitted to conventional restoration protocol. Non-decalcified histological section; toluidine blue and acid fuchsine stain; original magnification X12; black bar = 1 mm.

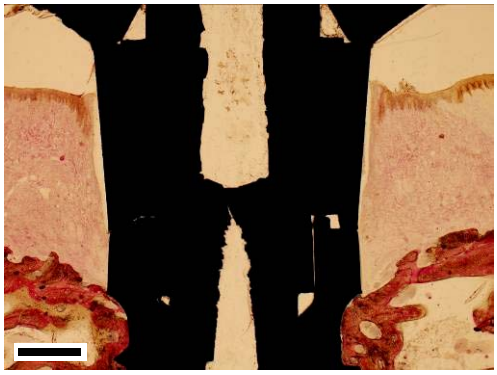

Figure 5. Mesio-distal section of a *Bone Level* implant submitted to immediate restoration protocol. Non-decalcified histological section; toluidine blue and acid fuchsine stain; original magnification X12; black bar = 1 mm.

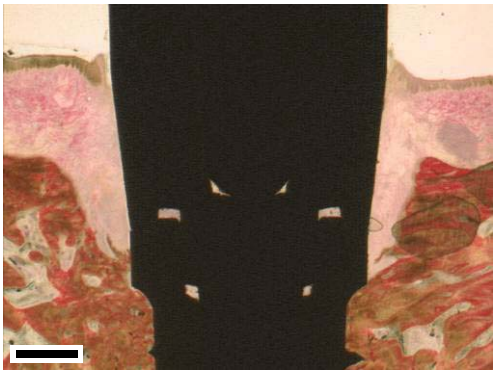

Figure 6. Mesio-distal section of a *Minus 1* implant submitted to immediate restoration protocol. Non-decalcified histological section; toluidine blue and acid fuchsine stain; original magnification X12; black bar = 1 mm.

Figure 7. Mesio-distal section of a *Minus 2* implant submitted to immediate restoration protocol. Non-decalcified histological section; toluidine blue and acid fuchsine stain; original magnification X12; black bar = 1 mm.

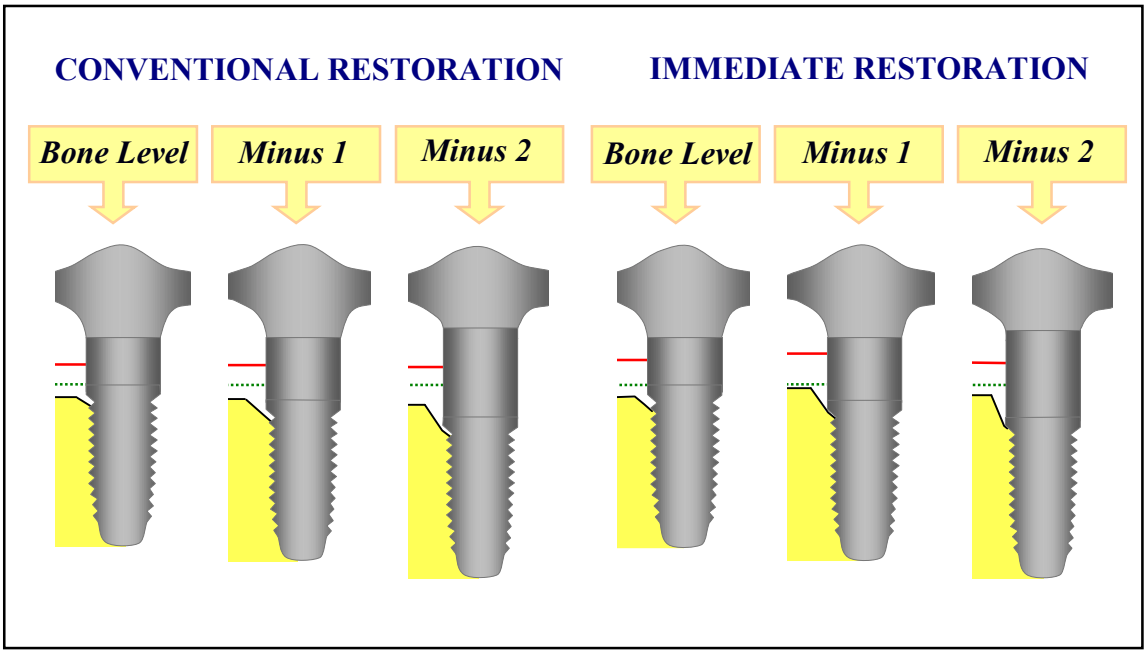

Figure 8. Schematic diagram of different groups, 90 days after prostheses placement. Green line represents a hypothetical ridge at baseline, and red line represents the position of soft tissue margin at the end of the experiment.

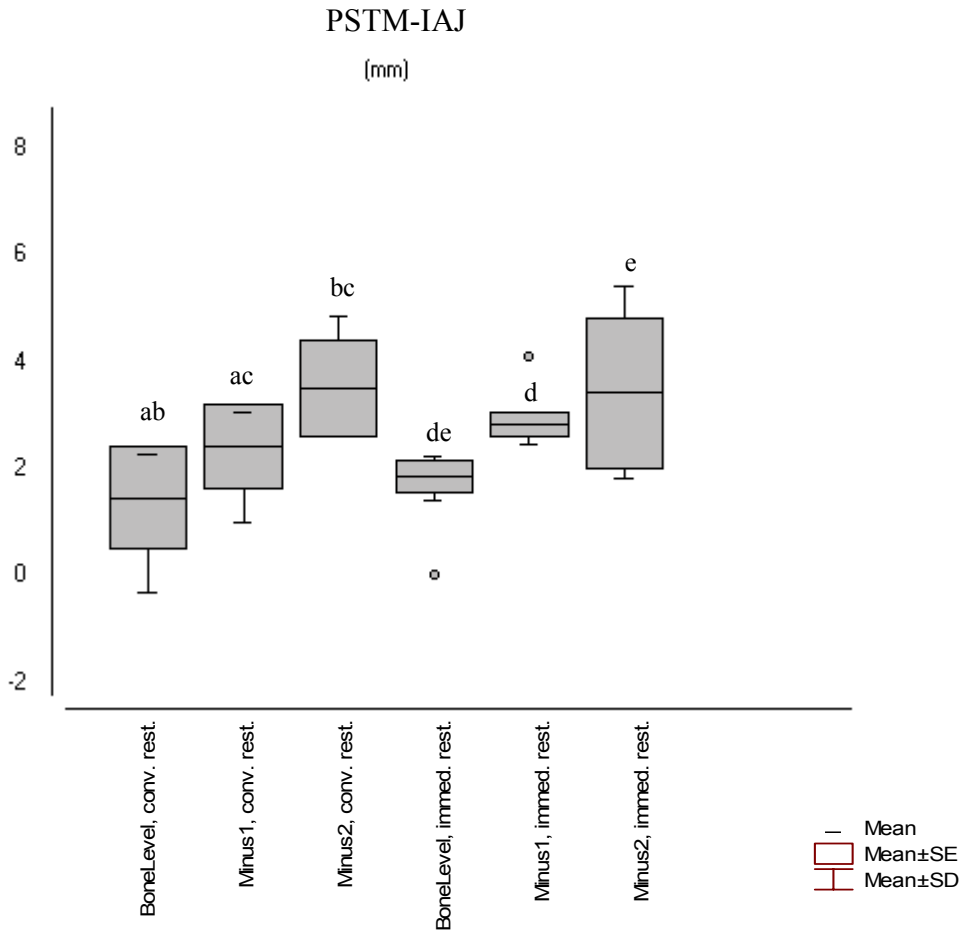

Figure 9. Box-plot from the distance from PSTM to IAJ. Conv. rest. = conventional restoration; immed. rest. = immediate restoration. Identical letters indicate statistically significant intergroup differences ($p < 0.05$, ANOVA test).

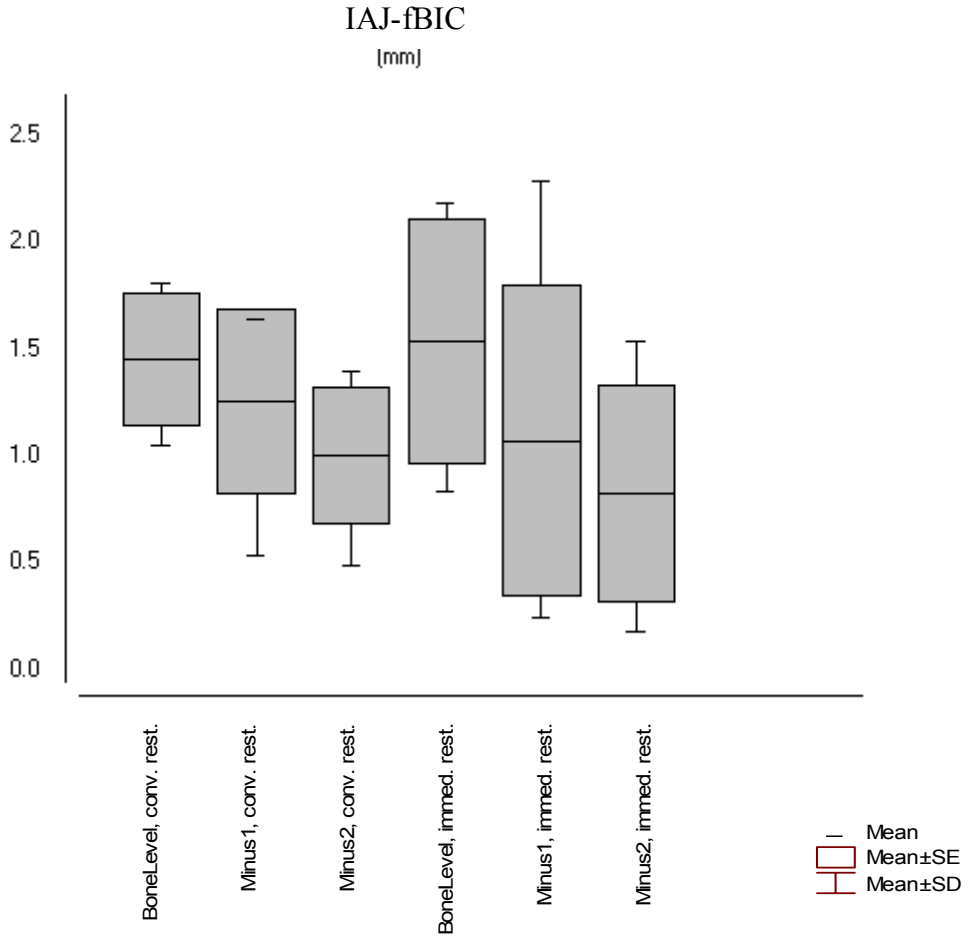

Figure 10. Box-plot from the distance from IAJ to fBIC. Conv. rest. = conventional restoration; immed. rest. = immediate restoration.

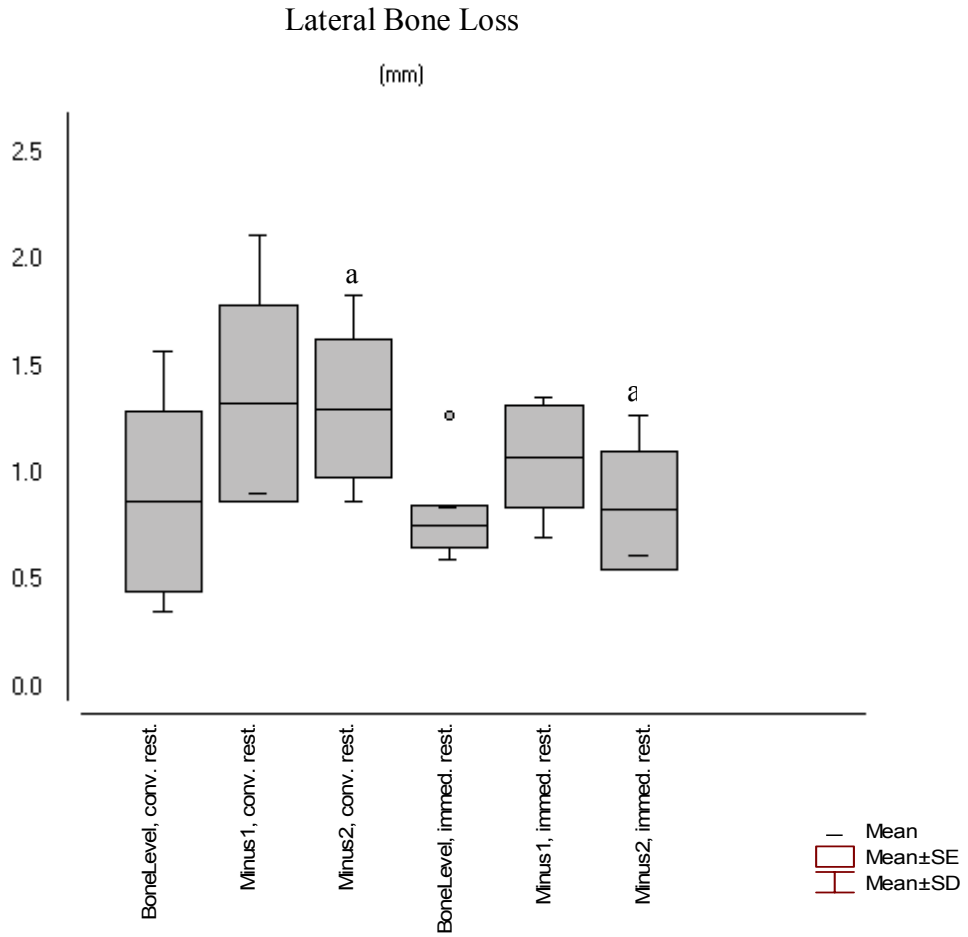

Figure 11. Box-plot from the lateral bone loss. Conv. rest. = conventional restoration; immed. rest. = immediate restoration. Identical letters indicate statistically significant intergroup differences ($p < 0.05$, Student *t* test).

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8 DISCUSSÃO

O presente estudo avaliou alterações clínicas, radiográficas e histológicas ao redor de implantes inseridos em diferentes posições verticais, submetidos a diferentes protocolos de restauração. Desta forma, após o período de acompanhamento de 90 dias, os achados mais significantes foram que a PTM foi mantida independentemente do posicionamento da JIC abaixo da crista óssea; a PTM foi melhor mantida nos sítios submetidos a restauração imediata; e a POL foi menor nos sítios submetidos à restauração imediata em comparação a restauração convencional (comparação entre os protocolos de restauração é apresentada nas tabelas A1, A2 e A3 do Anexo 3).

As implicações clínicas são que, pelo menos nas condições estudadas, a submersão de implantes de duas peças não põe em risco a altura da margem de tecido mole; e que o protocolo de restauração imediata pode ser considerado no plano de tratamento como uma alternativa para manter a altura dos tecidos moles e para controlar a largura do defeito ósseo.

A metodologia deste estudo foi desenhada para esclarecer algumas contradições observadas na literatura corrente. Primeiro, o uso de implantes de duas peças é desestimulado em áreas estéticas, porque a presença da JIC ao nível da crista óssea é associada à perda óssea significante [3,15,16,18]. Segundo, a inserção da JIC apicalmente à crista óssea tem sido relacionada a reabsorção óssea adicional [15,16,18]. Por outro lado, tem-se sugerido o uso de implantes de duas peças, o qual permite que a JIC seja inserida apicalmente à crista óssea. Desta forma, pode-se otimizar o perfil de emergência, contribuir para a manutenção da altura, textura e tonalidade dos tecidos periimplantares, e permitir a substituição do conector protético em casos de recessão da margem tecidual [13,20,24].

É importante mencionar que os estudos que avaliaram implantes inseridos em diferentes posições verticais, serviram para testar diferentes tipos de implantes, com variadas distâncias da JIC à linha que separa as superfícies lisa e rugosa, e/ou foram desenvolvidos sem o emprego de próteses. Todavia, a proximidade ou distanciamento entre a JIC e a referida linha entre as superfícies lisa e rugosa interfere na quantidade de perda óssea [3]. Além disto, carregamento mecânico tem mostrado ter um papel importante no remodelamento e formação óssea [9,10,12]. Por esta razão, no presente estudo apenas um

tipo de implante de duas peças foi usado, e seus tecidos circunvizinhos foram avaliados com ênfase na manutenção de suas alturas. Duas posições verticais (1 mm e 2 mm apical à crista óssea) foram testadas em comparação com implantes inseridos ao nível da crista óssea, e o efeito da restauração imediata foi comparado com o protocolo de restauração convencional.

Os modelos de restauração imediata e convencional foram escolhidos e as próteses foram preparadas para evitar contato oclusal com a dentição oposta [7]. Mesmo assim, é inevitável que forças tenham sido transmitidas durante a alimentação e devido a ação muscular. Contatos cêntricos e excêntricos foram também evitados nos trabalhos de Ericsson et al. [11], Andersen et al. [4], e Lorenzoni et al. [19]. Adicionalmnte, no presente estudo, optou-se por esplintar as próteses para reduzir o risco de sobrecarrega, e melhorar a distribuição biomecânica.

Noventa dias após a instalação das próteses, de acordo com a avaliação clínica, a altura do tecido mole foi melhor mantida ao redor dos implantes submetidos à restauração imediata. Este achado decorre dos menores valores da distância da PTM à JPC nestes grupos ($p = 0,02$). Entretanto, na avaliação histológica, as comparações entre os grupos foram basedas na distância entre a PTM e a JIC, seguida de um ajuste com o acréscimo de 1 mm aos valores dos sítios *Menos 1*, e 2 mm aos sítios *Menos 2*. Neste caso, os métodos estatísticos não detectaram diferenças entre os grupos, provavelmente devido aos amplos valores de desvio-padrão.

O resultado da avaliação clínica corrobora a observação de estudos nos quais se observou a manutenção das papilas adjacentes a implantes carregados ou restaurados imediatamente após 36 meses [29], um ano [19], e cinco anos [4]. Por sua vez, o resultado histométrico está também de acordo com o estudo de Siar et al. [25], no qual diferença estatisticamente significante não foi observada entre os grupos ($p = 0,516$). Em tal estudo, 3 meses após a intalação das próteses, a margem da mucosa permaneceu mais coronal nos sítios carregados convencionalmente ($2,38 \pm 0,81$ mm coronal à plataforma do implante) que nos carregados imediatamente ($2,27 \pm 1,18$ mm coronal à plataforma do implante).

A PTM pode ter sido influenciada pela Reabsorção do Rebordo, a qual foi menor no grupo de implantes imediatamente restaurados ($p > 0,05$). Adicionalmente, deve-se considerar que embora o período com restauração tenha sido o mesmo para os dois grupos (90 dias), os tecidos ao redor de implantes convencionalmente restaurados

haviam sido submetidos a períodos mais longos de cicatrização previamente à instalação das próteses (120 dias) em comparação com os restaurados imediatamente (24 horas) .

Nos grupos convencionalmente restaurados, valores de PS e NIR, avaliados pela análise clínica, foram maiores à medida que a JIC foi sendo inserida mais apicalmente. Este achado corrobora o estudo histométrico de Todescan et al. [28], no qual maiores extensões de epitélio e tecido conjuntivo foram observados ao redor de implantes inseridos 1 mm abaixo da crista óssea, em comparação com aqueles inseridos ao nível da crista óssea. Todavia, esta situação não foi observada dentre os grupos restaurados imediatamente, uma vez que os sítios *Menos 1* tiveram valores de PS ($p = 0,01$) e NIR maiores ($p < 0,05$) que os sítios *Ao Nível* e *Menos 2*. Contudo, o protocolo de restauração não influenciou este parâmetro. Esta observação corrobora o estudo clínico de Romeo et al. [23], o qual comparou PS de implantes imediatamente e tardiamente carregados suportando prótese total do tipo "overdenture" por dois anos.

Histometricamente, a extensão dos tecidos moles (PTM-pCOI) incluiu a soma dos valores da ES, EJ, e TC. Dentre estes parâmetros, os valores de TC foram os que mais variaram (de 1,49 mm a 2,76 mm), e sua extensão parece ter sido responsável pelo preenchimento do espaço criado pela perda óssea vertical. O valor do EJ variou menos (0,85 mm a 1,04 mm), o que não está de acordo com o estudo de Hermann et al. [17], no qual a porção mais apical do epitélio é localizada apicalmente à JIC em todos os casos. De acordo com Abrahamsson et al. [1], o epitélio juncional migra apicalmente à JIC devido à ruptura da mucosa local, causando proliferação epitelial e reabsorção óssea, que ocorre para permitir a formação de um contato de tecido conjuntivo com o implante em uma dimensão apropriada.

No caso de restauração imediata, esta ruptura não ocorre, pois o conector protético é parafusado logo após a instalação do implante. Contudo, no presente estudo, uma tendência de posicionamento do epitélio juncional coronalmente à JIC também foi observada em sítios submetidos à restauração convencional (Figuras A1 e A2, no Anexo 2). Pode-se sugerir que uma troca de componente protético realizada em condições de anti-sepsia e utilizando conector protético estéril, não necessariamente resulta na migração apical do epitélio juncional. Este achado não está de acordo com o estudo de Abrahamsson et al. [1], no qual remoções periódicas, seguidas de desinfecções com álcool, e reinstalações de conectores protéticos influenciaram a altura dos tecidos moles e duros periimplantares.

A extensão de tecido mole coronal à JIC foi calculada histometricamente pela medida da PTM-JIC. Sabe-se que a confecção de restaurações estéticas em área com tecido mole fino, com espessura inferior a 2 mm, é um desafio [24]. No presente estudo, o uso de implantes inseridos ao nível da crista óssea (grupo *Ao Nível*) resultou em alturas médias de tecido mole de 1,47 ± 0,97 mm e 1,56 ± 0,81 mm, respectivamente para restauração convencional e imediata. Por outro lado, áreas onde a altura de tecido mole exceda 4 mm podem estar associadas à formação de defeitos infra-ósseos, bolsas periimplantares, complicações na segunda fase cirúrgica, dificuldade na instalação dos conectores protéticos, e excesso de cimento na instalação das restaurações [24]. No presente estudo, os valores médios do grupo *Menos 1* variaram entre 2,41 ± 0,79 mm e 3,04 ± 0,56 mm, enquanto que os dos grupos *Menos 2* atingiram 3,51 ± 0,89 mm e 3,43 ± 1,40 mm, respectivamente para restauração convencional e imediata. A estabilidade destes resultados, e sua significância clínica devem ser avaliadas em períodos de acompanhamento mais longos.

A medida da distância entre o rebordo e o pCOI foi usada para avaliar a dimensão vertical do defeito ósseo formado, e tanto na avaliação radiográfica quanto na histométrica, houve uma tendência a maiores valores à medida que os implantes foram inseridos em posições mais apicais. Estatisticamente, os menores valores observados foram os dos grupos *Ao Nível* ($p < 0,05$).

Com relação à distância entre a JIC e o pCOI, a qual avaliou a dimensão da perda óssea vertical abaixo da JIC, houve uma tendência a menores valores à medida que os implantes foram inseridos em posições mais apicais, porém diferenças estatisticamente significantes não foram observadas entre os grupos. Esta tendência foi também documentada por Todescan et al. [28] que avaliaram por um período de acompanhamento de 3 meses, implantes inseridos 1 mm acima, 1 mm abaixo, e ao nível da crista óssea sob condições de não-carregamento. Contudo, não está de acordo com estudos prévios, nos quais implantes não carregados foram acompanhados por 6 meses, e observou-se uma perda óssea de aproximadamente 2 mm abaixo da JIC, a qual ocorreria para restabelecer o espaço biológico [3,8]. Pode-se sugerir que o período de acompanhamento do presente estudo não foi suficiente para rearranjar a anatomia ao redor de implantes inseridos nas posições mais apicais, uma vez que maiores quantidades de reabsorção óssea seriam esperadas ao redor destes grupos. Entretanto, de acordo com Hermann et al. [18], a alteração da localização da crista ao redor de implantes de duas peças

ocorre dentro das 4 primeiras semanas após a instalação do conector protético, mesmo para implantes inseridos 1 mm abaixo da crista óssea.

Com relação aos resultados da POL, os grupos *Ao Nível* tiveram os menores valores. Este achado pode ser explicado pela ocorrência de reabsorção óssea horizontal em alguns sítios destes grupos. Consequentemente, a ausência de defeitos em forma de cálice parece ter puxado para baixo os valores desta medida. Na avaliação radiográfica, considerando os implantes submetidos à restauração convencional, os valores da POL do grupo *Ao Nível* foram menores em comparação com os do grupo *Menos 2*; enquanto que esta diferença não foi observada entre os submetidos à restauração imediata. Além disto, na análise histométrica constatou-se que o protocolo de restauração exerce um efeito sobre este parâmetro, de tal forma que implantes submetidos à restauração imediata tiveram defeitos ósseos mais estreitos que os submetidos à restauração convencional, o que foi estatisticamente significante ($p = 0,006$). Este fato pode ser explicado pela estimulação causada pelo carregamento mecânico [9,10,12].

A largura do defeito ósseo é um importante parâmetro a ser considerado durante a escolha do posicionamento tridimensional ideal de um implante. De acordo com Tarnow et al. [27], em humanos, a POL é estimada em 1,34 a 1,40 mm. Então, entre implantes adjacentes, uma distância de 3 mm deve ser mantida, para prevenir a sobreposição de perdas ósseas laterais, que levam a reabsorção da crista óssea na região da papila interproximal, e a um aumento da distância entre a crista óssea e o ponto de contato das próteses, o que resultaria na migração apical da margem de tecido mole.

Uma vez que existe uma clara relação entre o posicionamento apico-coronal, mesio-distal e vestíbulo-lingual, é importante considerar que posições mais apicais poderiam ser restritas aos casos em que um espaço adequado no sentido mesio-distal e vestíbulo-lingual estão disponíveis. Neste caso, a arquitetura da margem do tecido mole será suportada pela crista óssea do dente ou implante adjacente [14,26]. Em áreas não estéticas, o uso de implantes posicionados apicalmente à crista óssea não é justificado, e a JIC (no caso de implantes de duas peças) ou o limite entre as superfícies lisa e rugosa (no caso de implantes de uma peça) deveriam ser posicionados ao nível da crista óssea ou mais coronalmente.

Finalmente, o desenvolvimento deste estudo em animais, permitiu a criação de condições controladas, e a comparação entre diferentes grupos. Todavia, dados de estudos com período de acompanhamento mais longo, e estudos em humanos devem ser conduzidos para suportar os achados apresentados, e avaliar sua significância clínica.

9 CONCLUSÃO

Dentro dos limites do presente estudo, pode-se concluir que a instalação de implantes apicalmente à crista óssea não interfere na manutenção da altura dos tecidos periimplantares moles e duros. Além disto, a restauração imediata pode ser benéfica para manter a altura dos tecidos moles periimplantares, e para minimizar a largura do defeito ósseo.

Estes resultados sugerem que o posicionamento apical de implantes pode ser utilizado com sucesso, principalmente em combinação com protocolo de restauração imediata. Estudos adicionais são sugeridos para avaliar o significado clínico destes resultados em longo prazo.

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11 ANEXOS

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Anexo 2

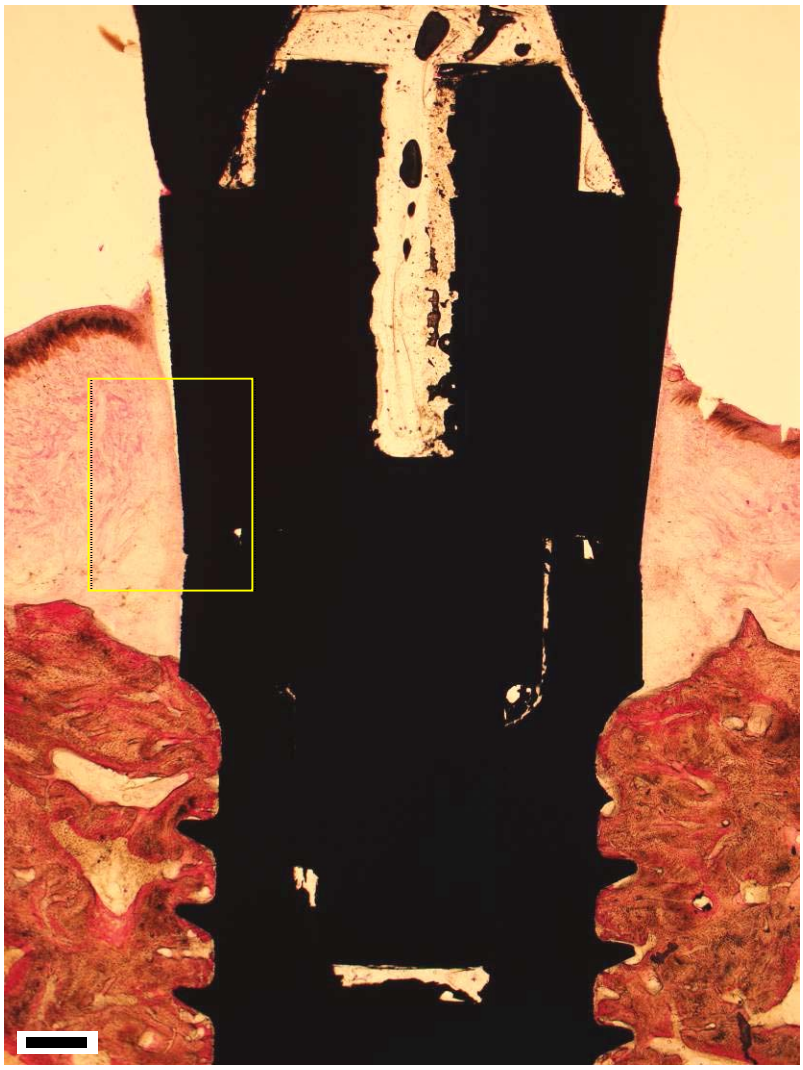

FIGURA A1. - Secção histológica mesio-distal de implante do grupo *Ao Nível* submetido à restauração convencional, cujo detalhe é apresentado na Figura A2 (Azul de toluidina e fucsina ácida, aumento original 2X, Barra preta = 0,5 mm).

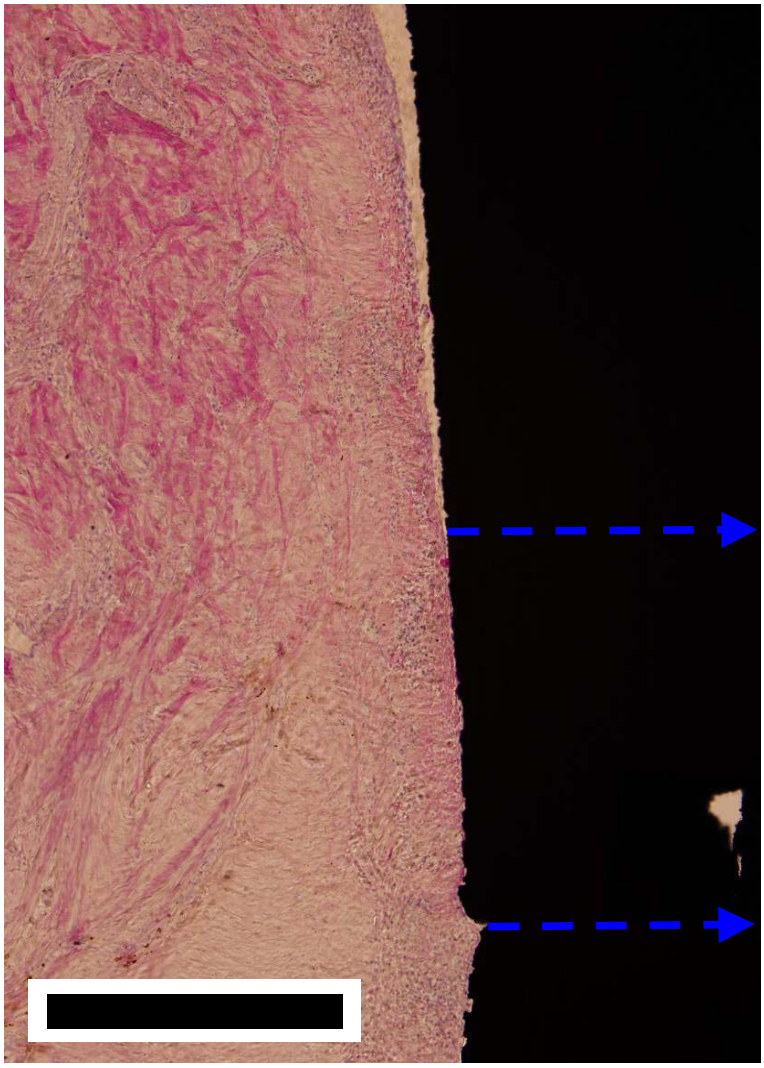

FIGURA A2 - Maior aproximação da área demarcada na Figura A1, com posição mais apical do epitélio juncional coronal à junção implante-conector protético. Secção histológica mesio-distal de implante do grupo *Ao Nível* submetido à restauração convencional (Azul de toluidina e fucsina ácida, aumento original 10X, Barra preta = 0,5 mm).

Anexo 3

Tabela A1 - Médias (± desvio-padrão) dos dados da análise clínica, agrupando os dados de cada hemi-mandíbula

	Restauração convencional	Restauração imediata	P
IG (%)	0,0 ± 0,0	5,6 ± 23,6	ns
ISS (%)	25,0 ± 34,2	30,6 ± 34,9	ns
PTM-JPC (mm)	1,6 ± 0,7	0,9 ± 0,9	0,02
PS (mm)	3,0 ± 0,6	3,2 ± 0,6	ns
NIR (mm)	4,7 ± 0,7	4,1 ± 0,8	ns

ns = não significante.

IG = Índice gengival;
ISS = índice de sangramento à sondagem;
PTM = posição do tecido marginal;
JPC = junção prótese-conector protético;
PS = profundidade de sondagem;
NIR = nível de inserção relativo.

Tabela A2 - Médias (mm ± desvio-padrão) dos dados da análise radiográfica, agrupando os dados de cada hemi-mandíbula

	Restauração convencional	Restauração imediata	P
Reabsorção do Rebordo*	0,9 ± 0,6	0,5 ± 0,7	ns
JIC-pCOI	1,2 ± 0,4	1,1 ± 0,6	ns
Rebordo-pCOI	1,3 ± 0,7	1,5 ± 0,8	ns
POL	1,1 ± 0,4	1,0 ± 0,4	ns

* Valor ajustado da Rebordo-JIC, adicionando 1 mm ao grupo *Menos 1*, e 2 mm ao grupo *Menos 2*.

ns = não significante.

JIC = junção implante-conector protético;
pCOI = primeiro contato osso-implante;
POL = perda óssea lateral.

Tabela A3 - Médias (mm ± desvio-padrão) dos dados da análise histométrica, agrupando os dados de cada hemi-mandíbula

	Restauração convencional	Restauração imediata	P
ES	0,6 ± 0,4	0,5 ± 0,2	ns
EJ	0,9 ± 0,8	1,0 ± 0,7	ns
TC	2,0 ± 0,7	2,2 ± 1,1	ns
PTM-pCOI	3,7 ± 1,0	3,6 ± 1,1	ns
PTM-JIC*	1,5 ± 0,8	1,7 ± 1,0	ns
JIC-pCOI	1,2 ± 0,4	1,1 ± 0,6	ns
Rebordo-pCOI	1,5 ± 0,8	1,7 ± 1,0	ns
POL	1,2 ± 0,4	0,9 ± 0,3	0,006

* Valor ajustado da PTM-JIC, adicionando 1 mm ao grupo *Menos 1*, e 2 mm ao grupo *Menos 2*.

ns = não significante.

ES = extensão do epitélio sulcular;
EJ = extensão do epitélio juncional;
TC = extensão do tecido conjuntivo;
PTM = posição do tecido marginal;
pCOI = primeiro contato osso-implante;
JIC = junção implante-conector protético;
POL = perda óssea lateral.

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Araraquara, 01 de março de 2007,
Ana Emília Farias Pontes