



**UNESP**

**UNIVERSIDADE ESTADUAL PAULISTA**

**Faculdade de Odontologia de Araraquara**



**CELSO EDUARDO SAKAKURA**

**O efeito da ciclosporina –A na osseointegração**

**ARARAQUARA**

**2005**



**UNIVERSIDADE ESTADUAL PAULISTA**

**Faculdade de Odontologia de Araraquara**



**CELSO EDUARDO SAKAKURA**

O efeito da ciclosporina –A na osseointegração

*Tese apresentada à Faculdade de Odontologia de Araraquara da Universidade Estadual Paulista, como parte dos requisitos para a obtenção do título de Doutor em Odontologia – Área de PERIODONTIA.*

**Orientadora:** *Prof.<sup>a</sup> Dr.<sup>a</sup> Gulnara Scaf*

**Co-orientador:** *Prof. Dr. Elcio Marcantonio Junior*

**ARARAQUARA**

**2005**

## **DADOS CURRICULARES**

### **CELSO EDUARDO SAKAKURA**

**Nascimento:** 24. 11. 1975 – São Paulo - SP

**Filiação:** Akira Sakakura  
Tereza Maeda Sakakura

**1994-1997** Curso de Graduação em Odontologia  
Faculdade de Odontologia de Bauru – USP

**1998 – 2000** Curso de Especialização em Implantodontia  
Hospital de Reabilitação de Anomalias Crâniofaciais – USP

**2000 – 2002** Curso de Pós-graduação em Periodontia, nível Mestrado  
Faculdade de Odontologia de Araraquara – UNESP

**2002-2005** Curso de Pós-graduação em Periodontia, nível Doutorado  
Faculdade de Odontologia de Araraquara – UNESP

## **Dedico este trabalho...**

**...À minha esposa Cristiana,**

pelo amor, apoio, carinho, paciência e compreensão nos muitos momentos de minha ausência.

**...Aos meus pais, Akira e Tereza,**

meus amigos verdadeiros, pelo carinho, amor, paciência pelas “palmadas” e por todas as vezes, em que abriram mão de seus sonhos, para a realização dos meus.

## **Agradecimento especial...**

**...À Deus,**

Agradeço-te por tudo que tens feito na minha vida:  
pelos momentos alegres, tristes, vitórias e derrotas, pelas  
portas abertas e fechadas, mas, principalmente pela  
salvação em Cristo Jesus.

## **Agradecimentos especiais...**

**...À minha orientadora Prof<sup>a</sup> Dr<sup>a</sup> Gulnara Scaf,**

pela sua dedicação, competência e sabedoria.

**...Ao meu orientador Prof. Dr. Elcio**

**Marcantonio Júnior,**

pela confiança, respeito e conhecimentos transmitidos.

**...À Prof<sup>a</sup> Dr<sup>a</sup> Maria Lúcia Rubo de Rezende**

pela amizade, incentivo, apoio e exemplo na prática docente.

**...À Prof<sup>a</sup> Dr<sup>a</sup> Ann Wenzel**

pela confiança, hospitalidade e conhecimentos transmitidos durante a minha estada na Universidade de Aarhus, Dinamarca

## **Agradecimentos**

À Faculdade de Odontologia de Araraquara, da Universidade Estadual Paulista “Júlio de Mesquita Filho” – UNESP, nas pessoas de sua Diretora **Prof.<sup>a</sup> Dr.<sup>a</sup> Rosemary Adriana Chiérici Marcantonio** e seu Vice-Diretor, **Prof. Dr. José Cláudio Martins Segalla**;

Ao Coordenador do curso de Pós-Graduação em Periodontia, **Prof Dr. Elcio Marcantonio Marcantonio Júnior**, e ao Ex-Coordenador **Prof Dr. Joni Augusto Cirelli**, pela dedicação e esforço empreendidos na administração deste curso;

Aos **Professores do Curso de Pós-Graduação da Faculdade de Odontologia de Araraquara – UNESP**, pela atenção dedicada;

Aos Professores do Departamento de Periodontia da Faculdade de Odontologia de Araraquara: **Adriana C. Marcantonio, Elcio Marcantonio Júnior, Carlos Rossa Júnior, Joni Augusto Cirelli, José Eduardo Sampaio, Benedito Egbert Corrêa, Silvana e Ricardo Samih G. Abi Rached**, pela amizade e conhecimentos transmitidos.

À **Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES**, pela concessão das bolsa de estudos;

À **Fundação de Amparo à Pesquisa do Estado de São Paulo, FAPESP**, pelo apoio financeiro a este estudo;

Aos **Funcionários da Biblioteca da Faculdade de Odontologia de Araraquara – UNESP**, pela gentileza e eficiência com que sempre me atenderam;

Aos **funcionários da seção de Pós-Graduação e da Biblioteca**, pela dedicação e eficiência com que sempre me atenderam;

Aos Funcionários do Departamento de Periodontia, **Dona Maria do Rosário, Dona Teresa, Zezé, Claudia**, e especialmente, **Regina**, pela dedicação, respeito, competência com que sempre me ajudaram;



Aos meus colegas **Rogério Margonar, Juliana A. N. D. Moraes, Rafael Sartori, Rafael Faeda, Gibson Pilatti, Beatriz V. Lopes, Rhené Christiansen, Erik Gotfredsen** por participarem nas diversas etapas deste trabalho;

Aos meus colegas de turma: **Rogério, Luiz, Rodrigo, Cliciane, Esmeralda, Karina, Marinela, Cris, Ricardo e Zé Marcos**, pela convivência e amizade;

Aos meus parceiros de clínica: **Rogério, Fernando, Josiane** pela paciência e companheirismo.

À **todos os colegas e amigos do curso de Pós-Graduação de Mestrado e Doutorado nas diversas áreas**, pela amizade conquistada, especialmente àqueles com que tive oportunidade de conviver;

Aos meus irmãos **César e Patrícia**, pelo amor, apoio constante, amizade e incentivo;

Aos meus parentes dentistas **Sérgio, Cecília, Harumi e Pricila** pelo estímulo e exemplo na profissão.

Aos meus irmãos de fé **Gerson, Adriano, Lincoln, Paulo Renato, Sonia, Andréia, Adriana, Karen, Mauro, Carminda, Nilton, Maurício... e tantos outros**, pelo apoio espiritual e incentivo que sempre me deram;

**...À todos que direta ou indiretamente contribuíram para o desenvolvimento deste trabalho;**

**Muito Obrigado.**

## PREFÁCIO

Esta tese é constituída pelos seguintes artigos:

- I. Sakakura, C.E., Margonar, R., Holzhausen, M., Nociti Jr, F.H., Alba Jr, R.C., Marcantonio Jr, E. **Influence of cyclosporin a therapy on bone healing around titanium implants. A histometric and biomechanic study in rabbits.** Publicado no *Journal of Periodontology*, volume 74, p. 976-81, 2003.
- II. Sakakura, C.E., Lopes B.M.V., Margonar, R., Nociti Jr, F.H., Pilatti G.L. Marcantonio Jr, E. **Ciclosporin-A and bone density around titanium implants: a histometric study in rabbits.** Trabalho finalizado e pronto para ser submetido para publicação no *Journal of Oral Maxillofacial Implants*.
- III. Sakakura, C.E., Margonar, R., Sartori, R., Morais, J.A.D.M., Marcantonio Jr, E. **The influence of cyclosporin-a on mechanical retention of dental implants integrated in bone. A study in rabbits.** Submetido para publicação no *Journal of Periodontology*.
- IV. Sakakura, C.E., Marcantonio Jr, E., Wenzel A., Scaf G. **The influence of cyclosporin a on quality of bone around integrated dental implants. A radiographic study in rabbits.** Trabalho aceito pela revista *Clinical Oral Implant Research*.

**SUMÁRIO**

<b>RESUMO</b> -----	3
<b>INTRODUÇÃO</b> -----	4
<b>REVISÃO DA LITERATURA</b> -----	6
CICLOSPORINA-A-----	6
OSSEOINTEGRAÇÃO-----	9
Conceito-----	9
Avaliação da Osseointegração-----	9
SUBTRAÇÃO RADIOGRÁFICA DIGITAL-----	14
Conceito-----	14
Interferência (Noise)-----	15
Precisão da SRD-----	17
X-poseIT-----	18
<b>PROPOSIÇÃO</b> -----	20
<b>ESTUDO I</b> -----	21
<b>ESTUDO II</b> -----	42
<b>ESTUDO III</b> -----	56
<b>ESTUDO IV</b> -----	70
<b>DISCUSSÃO</b> -----	91
<b>CONCLUSÃO</b> -----	93
<b>REFERÊNCIAS</b> -----	94
<b>ABSTRACT</b> -----	104

SAKAKURA C.E. O efeito da ciclosporina-A na osseointegração. 2005. 104 f. Tese (Doutorado em Periodontia) – Faculdade De Odontologia de Araraquara, Universidade Estadual Paulista, Araraquara, 2005

## **RESUMO**

Agentes imunossupressores provocam alterações severas no metabolismo ósseo mineral podendo resultar em osteopenia. Tais alterações podem ser prejudiciais no processo e manutenção da osseointegração. Este estudo teve o objetivo de avaliar a influência da administração de ciclosporina –A (CSA) na osseointegração de implantes de titânio, através da avaliação: durante a cicatrização óssea ao redor de implantes dentais (Estudo I); da densidade óssea ao redor implantes dentais (Estudo II); da retenção do implante após a cicatrização óssea de implantes dentais (Estudo III) e radiográfica da qualidade óssea ao redor de implantes dentais já osseointegrados (Estudo IV). Os resultados permitiram concluir que a administração de CSA durante a cicatrização óssea resulta em diminuição da osseointegração e da densidade óssea ao redor do implante dental. Ainda, a administração de CSA após o período de cicatrização óssea ao redor do implante reduz a sua retenção mecânica ao tecido ósseo e promove a diminuição da qualidade e da densidade óssea radiográfica ao redor do implante dental.

**PALAVRAS CHAVE: osseointegração, ciclosporina-a, radiografia digital, subtração radiográfica**

## INTRODUÇÃO

Os implantes osseointegrados (IO) revolucionaram a reabilitação de desdentados parciais e totais. Quando o conceito de osseointegração foi introduzido por *Per-Ingvar Branemark* (Branemark et al. 1977) foi possível alcançar um alto índice de sucesso nessa modalidade de tratamento e diversos estudos demonstraram excelente prognóstico de longo prazo (Jemt et al. 1989; Adell et al. 1990; Block & Kent, 1994; Lazzara et al.,1996). Inicialmente, os IO foram indicados para reabilitações totais (Adell et al. 1981) e posteriormente foram utilizados com sucesso na reabilitação de desdentados parciais (Zarb and Schmitt 1993a). Gradativamente, as suas indicações foram ampliadas e hoje a restauração unitária com IO é uma modalidade estabelecida de tratamento (Zarb & Schmitt 1993b).

A integração do implante dental (ID) ao tecido ósseo é considerada um fator fundamental no tratamento com IO. A obtenção previsível dessa integração é diretamente dependente das propriedades da camada de óxido de titânio do ID e da qualidade e quantidade do tecido ósseo receptor. Portanto, fatores que alterem a qualidade e a quantidade do tecido ósseo podem influenciar na obtenção previsível da osseointegração. Dentre estes fatores, os mais importantes são tabagismo, discrasias sangüíneas, osteoporose, radioterapia, alterações psicológicas, alcoolismo, diabetes mellitus não controlada e uso de medicamentos imunossupressores (Balshi & Wolfinger 1999; Margonar et al. 2004; van Steenberghe et al. 2003). A presença destas alterações ou hábitos pode contra-indicar o tratamento com IO (van Steenberghe et al. 2003).

A ciclosporina-A (CSA) é uma droga imunossupressora utilizada amplamente na prevenção de rejeição de aloenxertos, bem como no tratamento de doenças de natureza

autoimune (Guslandi & Tittobello 1992; Julian et al. 1991; Cayco et al. 2000). Dentre os efeitos adversos da CSA sobre o periodonto, a ocorrência de crescimento gengival é o fenômeno mais relatado na literatura (Seymour & Jacobs,1992; Pilatti & Sampaio 1997). Por outro lado, a administração da CSA também provoca profundas alterações no metabolismo ósseo, resultando em osteopenia (Buchinsky et al. 1996; Movsowitz et al. 1989; Julian et al. 1991; Cayco et al. 2000). Pacientes submetidos ao tratamento com CSA, após transplantes renais ou cardíacos, freqüentemente apresentam osteopenia e conseqüentemente aumento na freqüência de fraturas femorais e vertebrais (Julian et al. 1991; Cayco et al. 2000). A relação entre CSA e osseointegração é ainda pouco conhecida. Considerando a sua influência nas alterações no metabolismo ósseo, e prováveis modificações na quantidade e qualidade óssea do leito receptor de ID, torna-se relevante investigar esse tema.

## REVISÃO DA LITERATURA

### CICLOSPORINA-A

A ciclosporina (CSA) é um decapeptídeo extraído do fungo *Tolypocladium inflatum* (Zocher et al. 1986). Essa droga se liga a proteínas intracelulares chamadas ciclofilinas, formando um complexo de calcineurin A e B, cálcio e calmodulina. Esses complexos interferem na produção de receptores para a interleucina-1 nas células T "helper" e bloqueiam a síntese de interleucina-2; evitam a produção de receptores para a interleucina-2 na superfície das células T indiferenciadas, bloqueando a produção de mais células T "helper", células T citotóxicas e células T "killer" ( Daley & Wysocki, 1984).

A CSA tem sido empregada no tratamento de uma vasta gama de doenças do sistema imunológico como diabetes mellitus tipo I, doença de Behcet, artrite reumatóide, lupus eritematoso sistêmico, doença de Crohn, colite ulcerativa, psoríase e oftalmopatia de Graves (Guslandi & Tittobello 1992; Seymour & Jacobs 1992; Franchi et al. 2004; Pozzilli et al. 1995). Mas sem dúvida, sua maior aplicação se dá nos transplantes de órgãos, onde o seu emprego diminui os índices de rejeição e aumentou drasticamente a sobrevivência dos aloenxertos (Julian et al. 1991; Cayco et al. 2000)

Como toda droga, a CSA apresenta efeitos colaterais, atualmente bem conhecidos. Dentre os efeitos adversos da CSA sobre o periodonto, a ocorrência de crescimento gengival é o fenômeno mais reportado na literatura, sendo que fatores como idade, dosagem, concentração plasmática e tempo de utilização da droga, grau de inflamação gengival e de controle da placa bacteriana parecem influenciar significativamente na sua severidade (Seymour & Jacobs 1992; Boltchi et al. 1999). Outro importante efeito colateral



da CSA é a osteopenia (Movsowitz et al. 1988; Movsowitz et al. 1989; Julian et al. 1991; Cayco et al. 2000).

Os efeitos da CSA no tecido ósseo são controversos. Nos estudos *in vitro* a CSA inibe a reabsorção óssea estimulada pelo paratormônio, prostaglandina E<sub>2</sub>, 1-25-dihidroxi vitamina D<sub>3</sub> e interleucina-I (Stewart et al. 1986; Klein et al. 1994; Sasagawa et al. 1989; Klaushofer et al. 1987). Esses efeitos diretos da CSA são dependentes de sua ação imunossupressora, uma vez que o emprego dos análogos sem efeito imunossupressor não demonstrou a mesma ação (Horowitz et al. 1984).

Por outro lado, estudos *in vivo* realizados em ratos têm demonstrado que o uso dessa droga induz a um alto metabolismo ósseo, resultando em severa osteopenia (Schlosberg et al. 1989; Movsowitz et al. 1988; Fu et al. 1999). Esse aumento no metabolismo ósseo é caracterizado pelo desequilíbrio entre a reabsorção e a formação óssea (Schlosberg et al. 1989; Movsowitz et al. 1988; Fu et al. 1999; Nassar et al. 2004), onde a taxa de reabsorção suplanta a taxa de formação resultando em perda óssea. Estudos em pacientes transplantados submetidos à terapia imunossupressora com CSA reportam que ocorrem perdas ósseas severas com aumento do risco de fraturas femorais e vertebrais típicas de osteoporose (Julian et al. 1991). Especificamente sobre o osso alveolar, FU et al. (1999) observaram inibição da formação e aumento da reabsorção óssea alveolar em sítios acometidos por periodontite. Por outro lado, Nassar et al. (2004) relataram que a CSA diminui a perda óssea inicial em sítios acometidos por doença periodontal induzida em ratos em razão das suas propriedades inibidoras do sistema imune. Especificamente sobre a osseointegração, Duarte et al (2003) relataram diminuição da formação óssea dentro das espiras dos implantes quando os animais foram submetidos a 14 dias de administração de CSA, entretanto não constataram nenhuma alteração no contato osso-implante.

A ação da CSA no metabolismo ósseo parece ser dependente da dose administrada. Doses não imunossupressivas (5mg/kg via oral) parecem não provocar efeitos deletérios no esqueleto ósseo dos ratos, entretanto doses de 15 a 30 mg/kg via oral produzem efeitos severos. Quanto maior a dose e o tempo de administração, mais evidente é a osteopenia nos animais (Movsowitz et al. 1988; Pozo et al. 1995). Outro fator que pode influenciar na severidade da osteopenia causada pela CSA é a idade. Animais mais jovens podem exibir uma tendência a osteopenia mais severa em função de um maior metabolismo ósseo presente na fase de crescimento (Katz et al. 1994). Entretanto, estudo recente conduzido por Spolidorio et al. (2004) demonstrou que a osteopenia observada em osso alveolar provocada por CSA não é dependente da idade.

## **OSSEOINTEGRAÇÃO**

### **CONCEITO**

O conceito de osseointegração foi originariamente definido por Brånemark et al. (1969) como sendo o contato direto do tecido ósseo vital com a superfície de um implante em plena função ao nível da microscopia óptica. Esses autores descreveram um tipo de fixação do implante ao osso, sem a interposição de tecido fibroso e que podia ser usado para a reabilitação de pacientes desdentados com muita previsibilidade. Posteriormente, Brånemark et al. (1985) acrescentaram que essa união deveria ser uma conexão direta, estrutural e funcional entre o osso vivo organizado e a superfície de um implante em função. Entretanto, quando a interface osso-implante é analisada por meio de microscopia eletrônica, dificilmente observa-se um real contato entre o osso e o metal, pois freqüentemente existe uma camada de proteoglicanas de 20 a 40 nm e de substância fundamental amorfa separando o osso do implante.

A obtenção da osseointegração é um fator fundamental, mas não pode ser considerada como o único critério para o sucesso do tratamento com IO. O conceito de sucesso na implantodontia atual envolve resultados estéticos, fonéticos, funcionais, sistêmicos e uso clínico mínimo de 10 anos (Albrektsson & Lekholm 1989; Esposito et al. 2005).

### **AValiação da Osseointegração**

Brånemark et al. (1969) foram os primeiros autores a relatar o contato direto entre o osso e o implante sem interposição de tecido mole ou fibroso. Esse fenômeno, o qual eles chamaram de osseointegração, foi obtido em cães que tiveram seus dentes extraídos e

receberam posteriormente parafuso de titânio com 4mm de diâmetro e 10 mm de comprimento. Após um período de cicatrização de 3 a 4 meses, esses parafusos receberam estruturas protéticas semelhantes aos dentes antigos. As análises radiográficas e histológicas indicaram estabilidade das próteses e ainda foi observado que cada parafuso era capaz de suportar 100 kg de carga na mandíbula e 30 kg a 50 kg na maxila. Além disso, os autores relataram que ao tentar separar os implantes do osso ocorria fratura óssea ao redor do parafuso e a interface osso-implante permanecia intacta.

A avaliação da interface osso-implante ganhou um novo impulso após a introdução de um método apresentado por Donath & Breuner (1982). Este método constitui no corte e desgaste para avaliação histológica de peças contendo osso, dentes, implantes cerâmicos e metálicos sem a necessidade de descalcificação prévia ao corte. Os autores modificaram uma máquina de serra para madeira acoplando um sistema de refrigeração a água, permitindo o corte de blocos de resina contendo o espécime. O desgaste desse bloco de resina era feito por meio de uma lixadeira que trabalhava automaticamente até atingir a espessura programada. Com esse equipamento os autores conseguiram cortes que variavam de 5 a 10  $\mu\text{m}$  de espessura com qualidade para avaliação por meio de microscópio óptico.

A análise da interface osso-implante por meio de cortes não descalcificados tornou-se o padrão ouro para avaliação da osseointegração de ID. Inúmeros autores desde então utilizaram esse método para investigações sobre a osseointegração.

Além da análise histológica das lâminas realizada de maneira tradicional, a análise do contato osso-implante e da formação óssea dentro das roscas do implante é realizada por meio de programas de computador que permitem quantificar a extensão do osso em contato com o metal. Nesse caso, a osseointegração é definida como a porção do tecido ósseo em contato com o metal do implante. Entretanto, não são raras as vezes em que se observam

segmentos do implante ou metal que não estão em contato com o osso, mas também não apresentam nenhum tipo de tecido mole interposto. Isso é considerado como uma superfície osseointegrada, em virtude desse espaço ter sido provocado pela contração da peça resultado do processo de desidratação ou de polimerização da resina<sup>1</sup>.

Dependendo do tipo do osso (cortical ou trabecular) em análise e do animal (ratos, coelhos ou cães) usado para obtenção dos espécimes, têm se adotado as três ou as quatro melhores roscas consecutivas para inclusão na análise histomorfométrica (Johansson & Albrektsson 1987; Carlsson et al. 1989; Stenport et al. 2001; Ellingsen et al. 2004). Particularmente, os pesquisadores de Gotemburgo (Suécia) adotam essa metodologia na análise de espécimes obtidos de coelhos, fundamentados na característica do osso tibial desses animais. As três ou quatro primeiras roscas do implante correspondem à passagem do implante na cortical tibial do animal. Quando se adota a análise das roscas de todo o implante, freqüentemente obtém-se um valor muito baixo, por vezes não verossímil, pois se inclui na análise todo o espaço medular da tíbia do coelho, que originalmente é preenchida não por osso, mas sim por tecido medular. Por outro lado, outros autores (Cordioli et al. 2000; Sul et al. 2002) têm utilizado a análise de todas as roscas do implante com o argumento que são mais representativos. Com a inclusão de todas as roscas pode se correr o risco de diluir o efeito biológico pesquisado, portanto alguns autores têm sugerido a análise segmentada, ou seja, tanto das roscas referentes à cortical quanto à trabecular (Ellingsen et al. 2004; Duarte et al. 2005). Além disso, quando se estuda possível efeito de alterações sistêmicas no processo de osseointegração, torna-se válido analisar de forma segmentada a passagem cortical e a região trabecular do implante, uma vez que esses dois tipos de osso

---

<sup>1</sup> Comunicação pessoal – Bernd Franke, Presidente da Three Roll Mills Precision Cutting and Grinding Units (EXAKT). Alemanha.

apresentam diferenças na velocidade de seu metabolismo (Krejci 1996; Kanis 1996; Duarte et al. 2005)

Nos cortes não descalcificados também há a possibilidade de se realizar a avaliação da densidade óssea ao redor do implantes. Nociti Jr et al. (2002) propuseram a avaliação do tecido ósseo imediatamente adjacente ao implante em uma faixa restrita de 500  $\mu\text{m}$  paralela ao longo eixo do implante, como uma área de possível influência do implante. Rezende (1991) e Stenport et al. (2001) descreveram uma análise similar ao de Nociti Jr et al. (2002), mas utilizando a avaliação da densidade óssea imediatamente ao redor do implante por meio da imagem espelho das roscas do implante produzida pelo programa de computador.

Um outro parâmetro muito utilizado na literatura para avaliar a osseointegração é a mensuração do torque necessário para desrosquear/remover o implante que está supostamente osseointegrado. Esse teste foi introduzido por Carlsson et al. (1989) e consiste na adaptação de um torquímetro ao implante para mensurar a força máxima necessária para remover o implante do leito ósseo, ou seja, a resistência da osseointegração. Assim, esse método tem sido utilizado para avaliar o comportamento de diferentes superfícies (Cordioli et al. 2000; Li et al. 2002; Bernard et al. 2003; Elingsen et al. 2004), bem como os efeitos de alterações sistêmicas na osseointegração (Fujimoto et al. 1998; Johnsson et al. 2000; Stenport et al. 2001; Narai & Nagata 2003).

Assim como a análise histométrica, comparações entre os diversos estudos ficam comprometidas em função da grande variabilidade dos animais e dos examinadores. Além disso, a tíbia do coelho apresenta padrões ósseos distintos. Na região próxima da articulação do joelho, o tecido ósseo é cortical e trabecular; na região mais distal, é somente cortical o que pode gerar diferenças entre os resultados de torque de remoção. Entretanto,

podemos notar padrões de comportamento semelhantes como o aumento do torque de remoção com o passar do tempo, o que demonstra a consolidação óssea ao redor do ID (Rezende (1991); Johansson & Albrektsson 1987; Li et al. 2002; Ellingsen et al. 2004).

## **SUBTRAÇÃO RADIOGRÁFICA DIGITAL (SRD)**

### **CONCEITO DE SRD**

A subtração radiográfica digital foi introduzida na Odontologia na década de oitenta por Webber et al. (1982), Grondahl et al. (1983) e Hausmann et al. (1985). Consiste em uma operação de subtração, na qual as estruturas que não apresentaram mudanças entre um exame e outro são eliminadas, evidenciando as estruturas que apresentaram mudanças.

A imagem radiográfica digital é representada por uma matriz matemática, na qual cada casa representa um pixel, cujo valor pode variar de 0 a 255 (tons de cinza em um sistema de 8 bits). Quando duas imagens radiográficas são subtraídas, o computador realiza uma operação de subtração entre uma matriz (imagem inicial) e outra (imagem final) gerando uma terceira matriz (imagem subtraída), sendo que o valor zero representa ausência de mudança e um valor diferente de zero representa mudança, podendo ser um valor positivo ou negativo. Com o objetivo de gerar uma imagem subtraída de fácil leitura, os programas de SRD acrescentam automaticamente aos valores subtraídos o valor de 128, o que representa um tom médio de cinza (Figura 1-Estudo IV) . Assim, uma perfeita subtração de um sítio sem alteração óssea deverá mostrar ausência das estruturas anatômicas, resultando em uma imagem cujo nível de cinza deverá ser 128. Os pixels de uma região onde houve ganho de densidade deverão apresentar nível de cinza superior a 128. Pixels que apresentaram perda da densidade devem apresentar valores inferiores a 128 (Christgau et al. 1998a; Christgau et al. 1998b). Pode se resumir a operação de SRD em uma fórmula muito simples:



$n1-n2 = n3+128$ , sendo:  
n1 - imagem inicial  
n2 – imagem final  
n3 – imagem subtraída  
128 – tom médio de cinza

### **INTERFERÊNCIA (“NOISE”)**

O processo de padronização das imagens é relevante na SRD. Para a obtenção de uma imagem subtraída com validade é imperativo que as duas imagens a serem subtraídas apresentem a mesma projeção geométrica e densidade radiográfica (Weber et al. 1982; Grondahl et al. 1983; Hausmann et al. 1985; Wenzel & Sewerin 1991).

Diversos dispositivos para a padronização de séries radiográficas foram descritos previamente, geralmente baseados em métodos de moldagem dos dentes e fixação filme-paciente-tubo de raios-X (Nery et al. 1985; Rudolph & White 1988). Além disso, todo cuidado deve ser empregado no ajuste do tempo de exposição, quilovoltagem e miliamperagem do aparelho de raios-X, no processamento e na digitalização do filme radiográfico (Weber et al. 1982; Grondahl et al. 1983; Hausmann et al. 1985; Wenzel and Sewerin 1991; Christgau et al. 1998a; Schou et al. 2003).

Pequenos descuidos ocorridos nos diversos passos de padronização da imagem podem gerar resultados na imagem subtraída que não são originários do processo doença/tratamento ocorrido no período compreendido entre as duas imagens radiográficas. Esse resultado falso-positivo ou negativo conceitua-se como interferência da imagem (Wenzel & Sewerin, 1991).

Os programas de SRD devem ser capazes de compensar pequenas interferências que inevitavelmente ocorrem na obtenção de duas imagens padronizadas. Programas de SRD

prévios proporcionavam uma sobreposição manual, e pequenos desajustes geométricos eram corrigidos com movimentos rotacionais e translacionais (Wenzel 1989). O ajuste geométrico disponível nos programas atuais é baseado em pontos de referência que permitem um ajuste matemático e automático entre a imagem inicial e a final, facilitando a obtenção de uma melhor sobreposição das imagens<sup>1</sup>

Apesar da correção de pequenas interferências oriundas das inevitáveis diferenças entre a imagem inicial e final, ainda é preciso caracterizar a origem da diferença presente na imagem subtraída, ou seja, se a mudança ocorreu devido a uma alteração fisiológica do tecido ósseo ou ao tratamento instituído.

Segundo Wenzel & Sewerin (1991) uma imagem subtraída homogênea é aquela que apresenta em seus pixels uma pequena variação nos tons de cinza. O uso do histograma da região de controle (RC) da imagem subtraída fornece a amplitude de variação dos tons de cinza na imagem subtraída. Histogramas de RC com grande amplitude de tons de cinza podem significar que há grande quantidade de interferência. De qualquer forma, toda imagem subtraída apresenta interferência que não pode ser evitada e isso deve ser considerado na avaliação quantitativa das alterações na densidade óssea. Nesses casos recomenda-se considerar como interferência um ou mais desvios-padrão observados no histograma da RC da imagem subtraída. Schou et al. (2003), avaliando regeneração óssea ao redor de defeitos peri-implantares em macacos encontraram um desvio - padrão de 10 níveis de cinza nas regiões de controle e consideraram como alteração óssea de ganho/perda 128 mais ou menos dois desvios-padrão (20 níveis de cinza). Schropp et al. (2003) avaliando a remodelação óssea pós-extração dentária também utilizaram dois

---

<sup>1</sup> Comunicação Pessoal - Haiter-Neto F & Wenzel A. Noise in subtraction images made from pairs of bitewing radiographs. A comparison between two subtraction programs. Artigo aceito pela revista Dentomaxillofac Radiol.

desvios-padrão como interferência na análise por SRD. Entretanto, segundo Wenzel<sup>2</sup> não existe um número de desvio-padrão correto a ser adotado e deverá ser definido a critério do pesquisador dependendo da natureza de cada experimento, podendo apresentar variação nas magnitudes das interferências.

### **PRECISÃO DA SRD**

Mensurações realizadas em radiografias convencionais e digitais demonstram alterações ósseas somente em um plano (ex. altura óssea), por outro lado, a SRD pode proporcionar informações a respeito das alterações ósseas em até três parâmetros (altura, densidade óssea e área). Christgau et al. (1998b) demonstraram em estudo *in vitro* alta correlação entre aumento de espessura e mudança na densidade óssea detectada pela SRD. Neste estudo, foram encontradas altas correlações lineares que variaram de  $r^2 = 0,89$  a  $0,99$  para osso cortical e de  $r^2 = 0,61$  a  $0,86$ . Nesse estudo o limite de detecção da SRD foi um aumento de  $200\ \mu\text{m}$  para cortical e  $500\ \mu\text{m}$  para medular na espessura óssea enquanto que para radiografia convencional foi respectivamente  $600\ \mu\text{m}$  e  $2850\ \mu\text{m}$  comprovando a alta sensibilidade da SRD em detectar alterações na espessura óssea.

As alterações na massa de cálcio também são detectadas pela SRD. Christgau et al. (1998a), em estudo *in vitro*, demonstraram uma alta correlação linear entre alterações de cálcio na massa óssea por pixel e mudanças na densidade óssea detectada por SRD. Uma média de  $0,1-0,15\ \text{mg}$  de cálcio foi o mínimo de perda necessária para a SRD detectar.

Assim, a SRD pode ser aplicada com o objetivo de detectar discretas alterações em tecido duro como osso, esmalte e dentina (Nummikoski et al. 1992; Jeffcoat et al. 1992;

---

<sup>2</sup> Comunicação pessoal – Dr. Ann Wenzel, PhD, Dr. Odont, Head and Chair, Professor of Radiology Department, Aarhus University, Denmark

Schropp et al. 2003). Na Odontologia a SRD é utilizada quando o objetivo é detectar alterações sutis de forma mais precoce possível. Na Periodontia e Implantodontia a SRD é utilizada para o diagnóstico de perdas ósseas iniciais provocadas pelas lesões periodontais proporcionando uma intervenção mais precoce (Moreland et al. 1992; Jeffcoat et al. 1992). Essa técnica também é utilizada na avaliação dos resultados de terapias regenerativas como enxertos ósseos e regeneração óssea e tecidual guiada proporcionando não somente resultados qualitativos, mas também quantitativos (Christgau et al. 1996; Christgau et al. 1998a; Christgau et al. 1998b), bem como no estudo da remodelação óssea em humanos (Schropp et al. 2003)

#### **X-POSEIT**

Este programa de subtração radiográfica está baseado no posicionamento de ilimitados pontos de referência com o objetivo de permitir o alinhamento das duas imagens a serem subtraídas (Figura 1 – Estudo IV). Neste programa, existe uma ferramenta que permite verificar a precisão do alinhamento dos pontos de referência, possibilitando correções para um melhor alinhamento (rotação, translação, tamanho, distorção perspectiva). O programa permite, ainda, a correção gama automática com o objetivo de corrigir a densidade e o contraste das radiografias. A colocação dos pontos de referência em ambas radiografias certamente é a operação que consome mais tempo no processo de subtração das imagens. O programa também oferece a possibilidade de se definir várias regiões de interesse e/ou controle que podem ser desenhadas com auxílio do “mouse”. Tanto os pontos de referências, como as regiões de interesse/controle podem ser apagadas e refeitas.

Neste programa há a possibilidade de se definir, em nível de cinza, a interferência provocada pelo processo de exposição e processamento da radiografia. Isso é feito por meio das opções do programa, na qual se pode definir quantos desvios - padrão serão considerados na subtração.

Nas imagens subtraídas, as regiões que sofreram perda ou ganho ósseo aparecem, respectivamente, mais escuras e mais claras que a imagem subtraída. O programa permite que essas alterações sejam coloridas de acordo com a necessidade do pesquisador (Figura 1C – Estudo IV).

Todos os dados quantitativos referentes ao nível de cinza e à área, tanto da região de ganho, perda ou com ausência de alterações, são automaticamente exportados para um banco de dados para futura análise estatística. As imagens também podem ser copiadas e enviadas para outras pessoas ou programas.

## **PROPOSIÇÃO**

O objetivo desta tese foi avaliar a influência da ciclosporina-A na osseointegração.

Os objetivos específicos desta tese foram:

1. Avaliar histometricamente e biomecanicamente a influência da CSA durante a cicatrização óssea de implantes dentais (Estudo I)
2. Avaliar histometricamente a influência da CSA na densidade óssea ao redor de implantes dentais (Estudo II)
3. Avaliar a influência da CSA na retenção do implante após a cicatrização óssea de implantes dentais (Estudo III)
4. Avaliar radiograficamente a influência da CSA na qualidade óssea ao redor de implantes dentais com osseointegração estabelecida (Estudo IV)

**ESTUDO I**

Artigo intitulado “Influence Of Cyclosporin A Therapy On Bone Healing Around Titanium Implants. A Histometric And Biomechanic Study In Rabbits” Publicado no *Journal of Periodontology*, volume 74, p. 976-81, 2003.

INFLUENCE OF CYCLOSPORIN-A THERAPY ON BONE HEALING AROUND  
TITANIUM IMPLANTS. A HISTOMETRIC AND BIOMECHANIC STUDY IN RABBITS.

Celso E. Sakakura, D.D.S., M.S. \*

Rogério Margonar, D.D.S., M.S. \*

Marinella Holzhausen, D.D.S., M.S. \*

Francisco H. Nociti Jr., D.D.S., M.S., Ph.D. †

Rodolfo Candia Alba Jr., D.D.S. ‡

Elcio Marcantonio Jr., D.D.S., M.S., Ph.D. \*

\* Department of Periodontology – Dental School of Araraquara, State University of São Paulo (UNESP) Araraquara, São Paulo, Brazil.

† Dept. of Prosthodontics and Periodontics, Division of Periodontics, School of Dentistry at Piracicaba, UNICAMP, São Paulo, Brazil.

‡ Private Practice

***Author to whom correspondence should be sent:***

Elcio Marcantonio Jr, DDS, PhD, Departamento de Cirurgia e Diagnóstico, Disciplina de Periodontia; Faculdade de Odontologia de Araraquara, UNESP

Rua Humaitá, 1680, Centro, 14801-903, Araraquara, SP, Brazil,. Phone/Fax: 55 (16) 201-6314, e-mail: [elciojr@foar.unesp.br](mailto:elciojr@foar.unesp.br)

**Financial support: FAPESP - Fundação de Amparo à Pesquisa do Estado de São Paulo, grant no. 1999/09672-2 and CAPES.**

Running title: Immunosuppression and Titanium implants – Study in Rabbits



## **ABSTRACT**

**Background:** Immunosuppressive agents may induce severe changes on bone metabolism. The purpose of the present study was to evaluate the influence of the administration of cyclosporin A (CsA) on the bone tissue around titanium implants.

**Methods:** Eighteen New Zealand rabbits were randomly divided into 2 groups with nine animals each. The test group (CsA) received daily subcutaneous injection of CsA (10mg/kg bodyweight) and the control group (CTL) received saline solution by the same route of administration. Three days after the beginning of therapy, 2 implants (7.0 mm in length and 3.75 mm in diameter) were inserted bilaterally at the region of the tibial metaphysis. After 4, 8 and 12 weeks the animals were sacrificed and the biomechanical tests and the histometrical procedures, consisting of the determination of the percentages of bone-implant contact and bone area within the limits of the implant threads, were performed.

**Results:** Intergroup analysis showed that the removal torque and the percentage of bone contact with the implant surface for CsA group were significantly lower than those of the CTL group at the experimental period of 12 weeks (28.5 and 39.2 N.cm,  $p=0.01$ ; 7.76 and 18.52%,  $p=0.02$ ; respectively).

**Conclusion:** The data of the present study suggest that long term administration of cyclosporin-A may negatively influence bone healing around dental implants.

**Key words:** Dental implants / osseointegration; cyclosporin A / Systemic conditions; histometric.

## INTRODUCTION

Cyclosporin-A (CsA) is a potent immunosuppressive agent produced by the fungi *Cylindrocarpon lucidum*<sup>1</sup> that has been widely used to prevent organ rejection after allograft transplantation and in the treatment of several autoimmune diseases like pemphigus vulgaris, Behcet's disease, lichen planus, psoriasis and diabetes mellitus.<sup>2-4</sup>

Cyclosporin-A acts on the immune system by selectively suppressing the helper T cells, inhibiting the macrophage production of IL-2 and IL-1 and decreasing the cytotoxic T lymphocytes proliferation and differentiation.<sup>2, 5-10</sup>

The use of this drug can lead to a number of side effects, being the gingival overgrowth the most reported effect in the dental literature.<sup>11, 12</sup> Other adverse effects have also been reported following CsA use: nephrotoxicity, hepatotoxicity, hypertension and osteoporosis.<sup>3, 13, 14</sup>

The effects of cyclosporin A on the bone tissue appear contradictory. Some studies in rats<sup>6, 7, 15, 16, 17, 18</sup> have showed that this drug leads to a high bone turnover, resulting in severe osteopenia. Furthermore, bone loss has been reported in patients receiving CsA immunotherapy after transplantation.<sup>2, 3, 4, 13, 14</sup> The effects of CsA particularly on the alveolar bone have been reported by Fu et al. (1999)<sup>15</sup> who showed decreased bone formation and increased osteoclasia in rats. On the other hand, some in vitro studies have showed that CsA could have a protective action on the bone by inhibiting the bone resorption stimulated by parathyroid hormone, interleukin-1 (IL-1), prostaglandin E<sub>2</sub>, 1,25-dihydroxyvitamin D<sub>3</sub>, osteoclast-activating factor, thrombin and lipopolysaccharides.<sup>5, 8, 19, 20</sup>

There is little information in the literature regarding the effects of immunosuppressive agents on the osseointegration process. Duarte et al. (2001)<sup>22</sup> showed that immunosuppressive therapy with CsA and nifedipine may influence bone healing around titanium implants by

decreasing the bone area within the limits of the threads of the implant. The aim of the present study was to investigate the influence of immunosuppressive therapy with CsA alone on the biomechanical retention and osseointegration of commercially pure (CP) titanium implants in the rabbit tibia.

## **MATERIALS AND METHODS**

### ANIMALS

Eighteen New Zealand white rabbits, with 9 to 12 months old (3500-4500 g) were used in the study. The animals were housed in individual cages, fed by a standard laboratory diet and given tap water *ad libitum*. The experiment was approved by the Institutional Experimentation Committee of the School of Dentistry of Araraquara, São Paulo, Brazil.

### EXPERIMENTAL PROTOCOL

After a 2-week acclimatization period, the animals were randomly divided into two groups, a test (CsA) and a control (CTL) group, with nine animals each. The CsA group received a daily subcutaneous immunosuppressive dose of 10-mg/kg bodyweight Cyclosporin-A\*, whereas the CTL group received saline solution (NaCl 0.9%) by the same route of administration. The administration of the drugs began three days before the implants placement and lasted until 3 animals were killed per group, at 4, 8 and 12 weeks postoperative.

---

\* Sandimum®, Novartis Pharma AG, Switzerland.

## IMPLANT SURGERY

The animals were anesthetized by intramuscular injections of a combination of ketamine<sup>†</sup> (0.35 mg/kg bodyweight) and xylazine<sup>‡</sup> (0.5mg/kg bodyweight). The region of the tibial metaphysis was cleansed with iodine surgical soap. Incisions of approximately 3 cm in length were performed bilaterally at the internal side of the hind-leg, just below the knee. After gentle dissection, the bone surface of the tibial metaphysis was exposed. Unicortical implant beds were prepared by using a progressive sequence of spiral drills under generous saline cooling. Two implants<sup>§</sup>, (7mm in length and 3,75 mm in diameter) one with a modified head to enable fixation of the torque meter and the other with a conventional head, were placed in each leg. The soft tissues were sutured in separate layers and the animals received a single intramuscular injection of antibiotic<sup>||</sup> (0.1 ml/kg bodyweight of an association of Penicillin with Streptomycin.) postoperatively.

## REMOVAL TORQUE

After four, eight and twelve weeks, the implants were surgically exposed under general anesthesia. The specially designed key connected the modified head implant with the torque manometer.<sup>¶</sup> An anticlockwise movement was performed in order to remove the implant. The maximal torque necessary for manual removal of each implant was measured in Newton centimeters.

---

<sup>†</sup> Francotar<sup>®</sup>; Virbac do Brasil Ltda, Brazil.

<sup>‡</sup> Rumpum<sup>®</sup> Bayer S.A. São Paulo, Brazil

<sup>§</sup> Master Screw<sup>®</sup>, Conexão, São Paulo, Brazil.

<sup>||</sup> Pentabiótico<sup>®</sup>, Wyeth-Whitehall Ltda, São Paulo, Brazil

<sup>¶</sup> 15-BTG, Tonich, Japan.

### HISTOMETRIC PROCEDURE

After the animals were killed, the conventional implants with surrounding tissue in each tibia were removed and fixed in 4% neutral formalin for 48 hours. Undecalcified sections were prepared by a technique previously described by Donath & Breuner (1992).<sup>23</sup> Subsequently, the sections were stained as follows <sup>24</sup>: i) the slide-containing specimen was placed in a vessel containing Stevenel's blue preheated to, and maintained at, 60 °C for 15 minutes; ii) the specimen was rinsed in distilled water at 60 °C and air dried; iii) a small amount of alizarin red S was placed onto the specimen surface at room temperature for 5 minutes. Then, it was washed thoroughly in running distilled water to remove excess stain and air dried. The percentage of bone contact with the implant surface and the bone area formed within the threads were measured in both sides of the implant, at the three first threads. The mean of both sides of the implant were considered.

### STATISTICAL ANALYSIS

The differences in the removal torque, in the percentage of bone contact with the implant surface or in the percentage of bone area within the threads of the implant among groups (CsA and CTL) and, among the different experimental periods (4, 8 or 12 weeks) in the same group were tested by the non-parametric Mann Whitney and Wilcoxon tests ( $p < 0.05$ ). Whether the immunosuppressive therapy would influence body weight was evaluated by intergroup analysis of the difference between final and initial weight using paired t-test.

## **RESULTS**

### CLINICAL OBSERVATIONS

The rabbits in the CsA group developed some side effects like hair and mustache growth. A significant ( $p < 0,01$ ) weight loss was also observed in the animals of the CsA group (- 994 g and - 67g, for CsA and CTL, respectively; see Fig. 1).

### REMOVAL TORQUE VALUES

The intragroup analysis (Table 1) revealed significant differences only in the CTL group between the periods of 4 and 8 weeks ( $15.8 \pm 5.0$  N.cm and  $32.3 \pm 8.5$  N.cm, respectively;  $p = 0.02$ ) and between the periods of 4 and 12 weeks ( $15.8 \pm 5.0$  N.cm and  $39.2 \pm 5.8$  N.cm , respectively;  $p = 0.02$ ).

When data were compared between CTL and CsA groups (Table 1), a significant difference was found at the experimental period of 12 weeks ( $39.2 \pm 5.8$  N.cm and  $28.5 \pm 5.7$  N.cm, respectively;  $p=0.01$ ).

### HISTOMETRIC RESULTS

Intragroup analysis (Table2 and 3) showed significant difference regarding the percentage of bone contact with the implant surface only in the CsA group between the experimental periods of 4 and 12 weeks ( $17.08 \pm 7.95\%$  and  $7.76 \pm 6.36\%$ , respectively;  $p= 0.02$ ). With regard to the percentage of bone area within the threads of the implant, there were significant differences in the CTL group between the experimental periods of 4 and 8 weeks ( $48.35 \pm 6.2\%$  and  $66.57 \pm 8.1\%$ , respectively;  $p = 0.04$ ) and, between 4 and 12 weeks ( $48.35 \pm 6.2\%$  and  $71.18 \pm 8.76\%$ , respectively;  $p = 0.02$ ).

Intergroup analysis (Tables 2 and 3) showed significant difference regarding the percentage of bone contact with the implant surface only in the experimental period of 12 weeks ( $7.76 \pm 6.30\%$  and  $18.52 \pm 5.3\%$ , for CsA and CTL groups, respectively;  $p=0.02$ ). With regard to the percentage of bone area within the threads of the implant, there were no significant differences between groups in any of the experimental periods.

## DISCUSSION

The resistance of the titanium implant to the removal force can be correlated to the degree of contact between mineralized bone and irregularities on the implant surface.<sup>25, 26</sup> Thus, the removal torque of an implant is influenced by the mechanical properties of the adjacent bone (quality and quantity) and by the degree of bone-implant contact.<sup>25, 27</sup> Bone remodeling occurs progressively, increasing the degree of bone-implant contact, consequently increasing the torque needed to remove the implant.<sup>25-28</sup>

The results of the present study for the CTL group were similar to the findings of previous studies that observed an increase in the removal torque values with time (Table 1),<sup>25-28</sup> which suggests that an increase in the degree of bone-implant contact (Table 2 and Fig. 2). On the other hand, the CsA group did not show a similar behavior. There were no significant increases in the removal force (Table1), degree of bone-implant contact (Table2) and percentage of bone area within the threads of the implant (Table 3) between observation periods. In fact, it was observed a decrease in the bone-implant contact evaluation (Table 2), which suggests a poor osseointegration process (Fig. 3), with low implant retention to the bone (see torque values – Table1).

Low values for the evaluated parameters obtained for the CsA group, mainly at the longest period (12 weeks), are probably due to the low percentage of bone-implant contact<sup>28</sup> (Table 2 and Fig. 6b). These alterations observed in the bone tissue of the CsA group animals can be explained by the action of cyclosporin-A. It is known that the immune system actively participates in bone mineral metabolism and that the T lymphocytes play a critical role in the development of CsA-induced osteopenia.<sup>29</sup> This is not surprising as the T cell is the traditional target of CsA and naturally occurring T lymphocyte perturbations are implicated in the development of primary osteoporosis in humans.<sup>29</sup> Besides, an in vitro study<sup>30</sup> corroborates with this result, by describing the necessity of thymus-derived lymphocytes presence for the production of the osteoclast-activating factor.

T lymphocytes suppression results in a high bone metabolism state, where the bone formation is supplanted by the bone resorption, leading to a decrease in the trabecular bone volume.<sup>6, 7, 29</sup> Under these conditions, a decreased percentage of bone contact with the implant surface was observed. The precise mechanism of action of CsA on bone tissue is still not well understood. It is known that these bone alterations correlate with immunosuppressive mechanisms and are mediated by cytokines.<sup>9, 10, 21, 29</sup> Moreover, possible CsA effects on osteoblasts and osteoclasts are not rejected and may result in a secondary phenomenon, leading to a high bone remodeling state with exceeding bone resorption.<sup>9</sup>

Evaluating the results obtained for control and test groups during the three experimental periods, it was observed that test group showed minimum increases for torque parameter (Table 1) and percentage of bone within the threads of the implant (Table 3) and, a decrease in the parameter percentage of bone contact with the implant (Table 2). Between the periods of 4 and 12 weeks, the values for the control group supplanted those of the test group (Tables 1, 2 and 3), which suggests that the effects of CsA administration on the osseointegration process turned evident only after an administration period longer than 4 weeks. This result is



supported by Movsowitz et al (1988)<sup>7</sup> who showed that the effect of CsA on bone is dependent on the duration and dose of CsA. Similarly, Duarte et al. (2001)<sup>22</sup> have showed that, after CsA administration for two weeks, no changes in the percentage of bone contact with the implant were detected in the experimental periods of 2 or 6 weeks. The changes were limited to a lower bone formation within the limits of the threads of the implant which, according to Johansson & Albrektsson (1987),<sup>28</sup> is not fundamental for implant anchorage, being the bone-implant contact the most important factor for higher removal torque. Considering that the CsA effects are more severe after longer administrations,<sup>7</sup> we can understand the reason why the differences between control and test group turned significant only in the last experimental period of our study (Tables 1 and 2).

Another important aspect to be observed when comparing the values for removal torque and the values for bone implant contact in the test group is that the results seem to be contradictory. Contrary to our expectations, the decrease in the percentage of bone implant contact with time, although not being significant, was not accompanied by a decrease in the removal torque values. A possible explanation for this fact may be the difference in the anatomic location of implants in the tibia. The implants designed for the biomechanical test were placed close to the knee joint, whereas those designed for the histological analysis were placed in a more distal position. Whether the difference in the porosity or in the architecture of the bone would promote different results remains to be investigated. Therefore, future studies should be considered in order to evaluate this topic.

The rabbit was the animal model used in the present investigation not only because of its small size but also because several studies are available relating its use in the study of osseointegration.<sup>25-28</sup> However, according to Gratwohl et al.,<sup>31</sup> rabbits treated with immunosuppressive doses of CsA for prolonged periods of time may develop a clinically distinct toxic syndrome characterized by loss of weight, reduced food and water intake and

reduced movements. One can speculate that this side effect could have interfered with the results of the test group. However, because there is little information in the literature, we can not determine how much of the observed effects were due to the possible toxicity of CsA.

The choice for the experimental periods of 4, 8 and 12 weeks aimed to evaluate the influence of CsA administration on the whole osseointegration process, specially on the period related to the maturation of woven bone to lamellar bone, which lasts 6 weeks in rabbits.<sup>28</sup> In order to reach a successful osseointegration, this critical period should occur without healing disturbances. Therefore, the influence of CsA could be evaluated in the most critical period of bone healing in the rabbit.

In the present study, the subcutaneous route of administration of CsA was chosen because it can promote more consistent immunologic levels than oral route once CsA is poorly absorbed from the gastrointestinal tract in the rabbit.<sup>31</sup> The dosage of CsA (10mg/kg/day body weight) used in the present study was equal to the one used by Duarte et al.<sup>22</sup> and it can promote serum levels of CsA ranging from 100 to 400ng/ml which are able to sustain an allograft transplantation in rabbits.<sup>31</sup> Similarly, serum levels of CsA ranging from 100 to 400 ng/ml are described in patients receiving immunotherapy after renal transplantation.<sup>32</sup> Besides, higher dosages could lead to systemic complications and could cause nephrotoxicity, impairing the results. We have concluded that CsA administration, for periods of time greater than 4 weeks, can decrease the osseointegration process of commercially pure (CP) titanium implants inserted in rabbit tibia. However, further investigations are necessary in order to evaluate the effect of CsA on implants which have already suffered osseointegration.

## ACKNOWLEDGMENTS

This study was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) grant no. 1999/09672-2 and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

The authors greatly appreciated the assistance of Dr. Beatriz Maria Valério Lopes in surgical phase. We also thank Solange Aranha for English review.

## REFERENCES

1. Ruegger A, Kuhn M, Lichti H, et al. Cyclosporin A, a Peptide Metabolite from *Trichoderma polysporum* (Link ex Pers.) Rifai, with a remarkable immunosuppressive activity *Helv Chim Acta*. 1976; 59:1075-92.
2. Cayco A, Wysolmerski J, Simpson C, et al. Posttransplant bone disease: evidence for a high bone resorption state. *Transplantation* 2000; 70: 1722-1728.
3. Julian BA, Laskow DA, Dubovsky J, Dubovsky EV, Curtis JJ, Quarles LD. Rapid loss of vertebral mineral density after renal transplantation. *N Engl J Med* 1991; 325: 544-50.
4. Vedi S, Greer S, Skingle SJ, et al. Mechanism of bone loss after liver transplantation: a histomorphometric analysis. *J Bone Miner Res* 1999; 14: 281-287.
5. Mccauley LK, Rosol TJ, Capen CC. Effects of cyclosporin A on rat osteoblasts (ROS 17/2.8 Cells) in vitro. *Calcif Tissue Int* 1992; 51: 291-297.
6. Movsowitz C, Epstein S, Ismail F, Fallon M, Thomas S. Cyclosporin A in the oophorectomized rat: unexpected severe bone resorption. *J Bone Miner Res* 1989; 4: 393-398.

7. Movsowitz C, Epstein S, Fallon M, Ismail F, Thomas S. Cyclosporin-A *in vivo* procedures severe osteopenia in the rat: effect of dose and duration of administration. *Endocrinol* 1988; 123: 2571-2577.
8. Orcel P, Denne MA, De Vernejoul MC. Cyclosporin-A *in vitro* decreases bone resorption, osteoclast formation, and the fusion of cells of the monocyte-macrophage lineage. *Endocrinol* 1991;128:1638-1646.
9. Rucinski B, Liu CC, Epstein S. Utilization of cyclosporine H to elucidate the possible mechanisms of cyclosporine A – Induced osteopenia in the rat. *Metabolism* 1994; 43:1114 – 1118.
10. Sasagawa K, Fushibayashi S, Okano K, et al. Different inhibitory actions of immunomodulating agents and immunosuppressive agents on bone resorption of mouse calvaria. *Int J Immunopharmac* 1989; 11: 953-959.
11. Rateitschak-Pluss EM, Hefti A, Lortscher R, Thiel G. Initial Observation that cyclosporin A induces gingival enlargement in man. *J Clin Periodontol* 1983;10: 237-46.
12. Seymour RA, Jacobs DJ. Cyclosporin and the gingival tissues. *J Clin Periodontol* 1992;19: 1-11.
13. Shane E, Rodino MA, McMahon DJ. et al. Prevention of bone loss after heart transplantation with antiresorptive therapy: a pilot study. *J Heart Lung Transplant* 1998; 17: 1089-1096.
14. Thiébaud D, Krieg MA, Gillard-Berguer D, Jacquet AF, Goy JJ, Burckhardt P. Cyclosporine induces high bone turnover and may contribute to bone loss after heart transplantation. *Eur J Clin Invest* 1996; 26: 549-555.
15. Fu E, Hsieh Y-D, Nieh S, Wikesjö, Liu D. Effects of cyclosporin A on Alveolar bone: an experimental study in the rat. *J Periodontol* 1999; 70:189-194.

16. Katz I, Li M, Joffe I, et al. Influence of age on cyclosporine A-induced alterations in bone mineral metabolism in the rat in vivo. *J Bone Miner Res* 1994; 9: 59-67.
17. Klein L, Lemel MS, Wolfe MS, Shaffer J. Cyclosporin A does not affect the absolute rate of cortical bone resorption at the organ level in the growing rat. *Calcif Tissue Int* 1994; 55: 295-301.
18. Schlosberg M, Movsowitz C, Epstein S, Ismail F, Fallon M, Thomas S. The effect of cyclosporin A administration and its withdrawal on bone mineral metabolism in the rat. *Endocrinol* 1989; 124: 2179-2184.
19. Stewart PJ, Green OC, Stern PH. Cyclosporine A inhibits calcemia hormone-induced bone resorption in vitro. *J Bone Miner Res* 1986; 1: 285-291.
20. Chowdhury MH, Shen V, Dempster, DW. Effects of cyclosporine A on chick osteoclast *in vitro*. *Calcif Tissue Int* 1991; 49: 275-279.
21. Klaushofer K, Hoffmann O, Stewart PJ, et al. Cyclosporine A inhibits bone resorption in cultured neonatal mouse calvaria. *J Pharmacol Exp Ther* 1987; 243: 584-590.
22. Duarte PM, Nogueira Filho GR, Sallum EA, Sallum AW, Nociti Junior FH. The effect of an immunosuppressive therapy and its withdrawal on bone healing around titanium implants. A histometric study in rabbits. *J Periodontol* 2001; 72:1391-7.
23. Donath K, Breuner G. A method for study of undecalcified bones and teeth with attached soft tissue. The sage-Scliff (sawing and grinding) technique. *J Oral Pathol* 1982; 11: 318-326.
24. Maniatopoulos C, Rodrigues A, Deporter DA, Melcher AH. An improved method for preparing histological sections of metallic implant *Int J Oral Maxillofac Implants* 1986; 1: 31-37.

25. Sennerby L, Thomsen P, Ericson LE. A morphometric and biomechanic comparison of titanium implants inserted in rabbit cortical and cancellous bone. *Int J Oral Maxillofac Implants* 1992; 7: 62-71.
26. Carlsson L, Röstlund T, Albrektsson B, Albrektsson T. Removal torques for polished and rough titanium implants. *Int J Oral Maxillofac Implants* 1988; 3: 21-24.
27. Ivanoff C-J, Sennerby L, Lekholm U. Influence of mono- and bicortical anchorage on the integration of titanium implants. A study in the rabbit tibia *Int Oral Maxillofac Surg* 1996; 25: 229-235.
28. Johansson C, Albrektsson T. Integration of screw implants in the rabbit: A 1-yr follow-up of removal torque of titanium implants. *Int J Oral Maxillofac Implants* 1987; 2: 69-75.
29. Buchinsky FJ, Ma Y, Mann GN, et al. T lymphocytes play a critical role in the development of cyclosporin A-induced osteopenia. *Endocrinology* 1996; 137: 2278-2285.
30. Horowitz M, Vignery A, Gershon RK, Baron R. Thymus-derived lymphocytes and their interaction with macrophages are required for production of osteoclast-acting factor in mouse. *Proc Natl Acad Sci USA* 1984; 81: 2181-2185.
31. Gratwohl A, Riederer I, Graf E, Speck B. Cyclosporine toxicity in rabbits. *Lab Anim* 1986; 20: 213-220.
32. Boltchi FE, Rees TD, Iacopino AM. Cyclosporine A - induced gingival overgrowth: A comprehensive review. *Quintessence int* 1999; 30: 775-783.

## TABLES

**Table 1** – Mean and standard deviation of removal torque (N.cm) for each experimental group in each experimental period.

	Experimental groups		<i>p</i> value
	CTL	CsA	
4 weeks	15.8 ± 5.0	21.8 ± 5.1	0.09
8 weeks	32.3 ± 8.5 <sup>†</sup>	24.7 ± 2.3	0.12
12 weeks	39.2 ± 5.8 <sup>‡</sup>	28.5 ± 5.7	0.01*

\*Significant difference at  $p < 0.05$ , Mann-Whitney test.

<sup>†</sup>  $p = 0.02$ , significant difference between the periods of 4 and 8 weeks in the CTL group, Wilcoxon test.

<sup>‡</sup>  $p = 0.02$ , significant difference between the periods of 4 and 12 weeks in the CTL group, Wilcoxon test.

**Table 2** – Mean and standard deviation of bone contact (%) with the implant surface for each experimental group according to experimental period.

	Experimental groups		<i>p</i> value
	CTL	CsA	
4 weeks	9.76 ± 4.7%	17.08 ± 7.95%	0.26
8 weeks	13.08 ± 3.77%	12.5 ± 3.66%	0.74
12 weeks	18.52 ± 5.3%	7.76 ± 6.30% <sup>†</sup>	0.02*

\*Significant difference at  $p < 0.05$ , Mann-Whitney test.

<sup>†</sup>  $p = 0.02$ , significant difference between the periods of 4 and 12 weeks in the CsA group, Wilcoxon test.

**Table 3** – Mean and standard deviation of bone area (%) within the limits of the implant threads for each experimental group according to experimental period.

	Experimental groups		<i>p</i> value
	CTL	CsA	
4 weeks	48.35 ± 6.2%	46.27 ± 6.17 %	0.52
8 weeks	66.57 ± 8.1% <sup>†</sup>	51.07 ± 10.34 %	0.26
12 weeks	71.18 ± 8.7% <sup>‡</sup>	59.50 ± 5.47 %	0.1

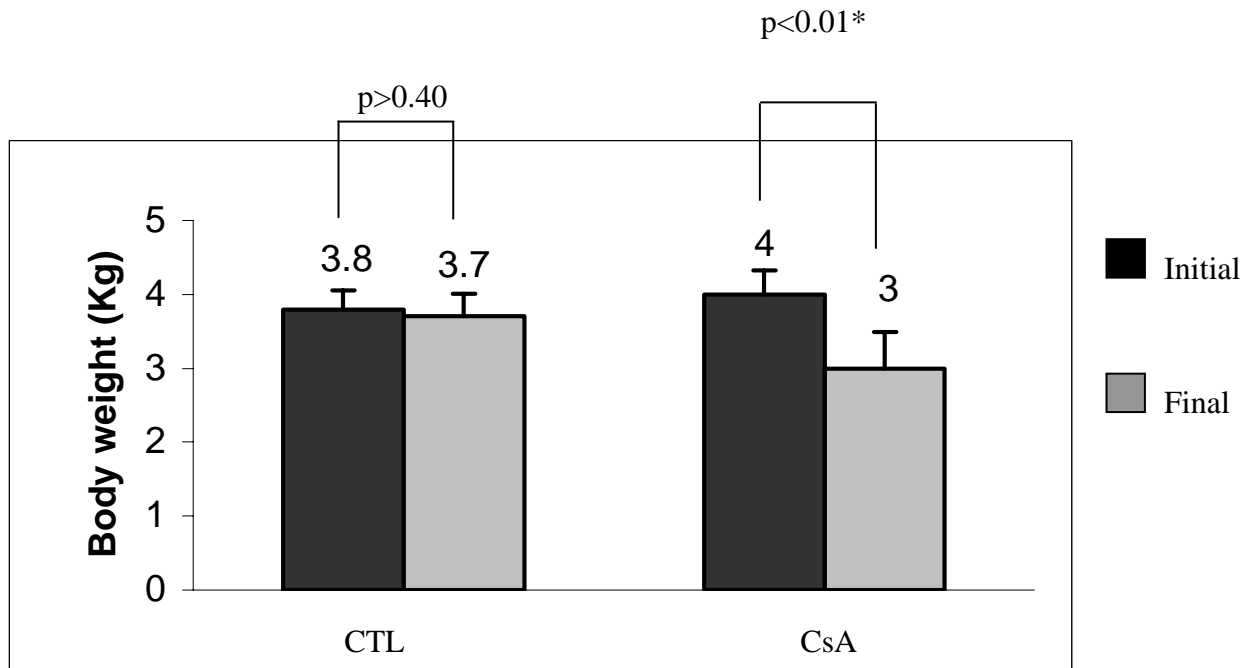
<sup>†</sup> *p*= 0.02, significant difference between the periods of 4 and 8 weeks in the CTL group, Wilcoxon test.

<sup>‡</sup> *p*= 0.02, significant difference between the periods of 4 and 12 weeks in the CTL group, Wilcoxon test.

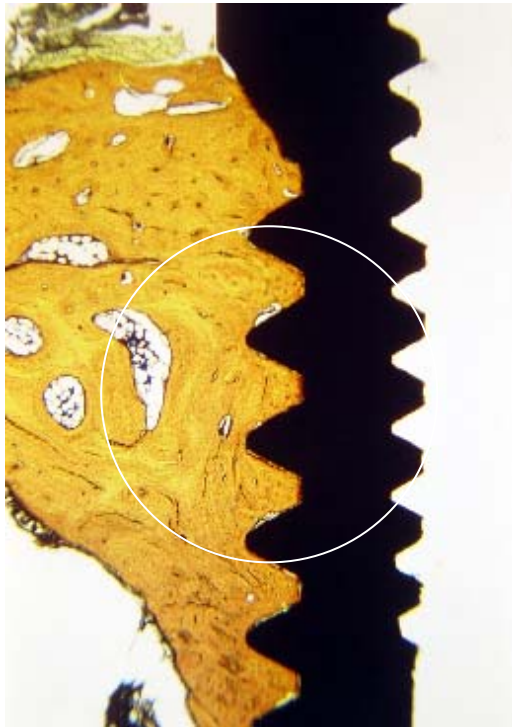


**FIGURES**

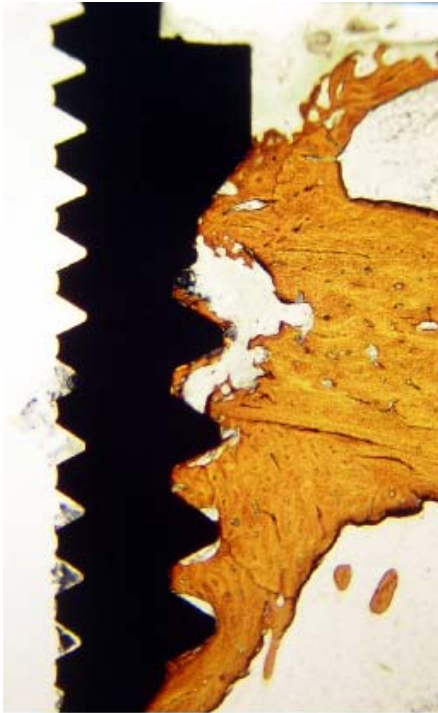
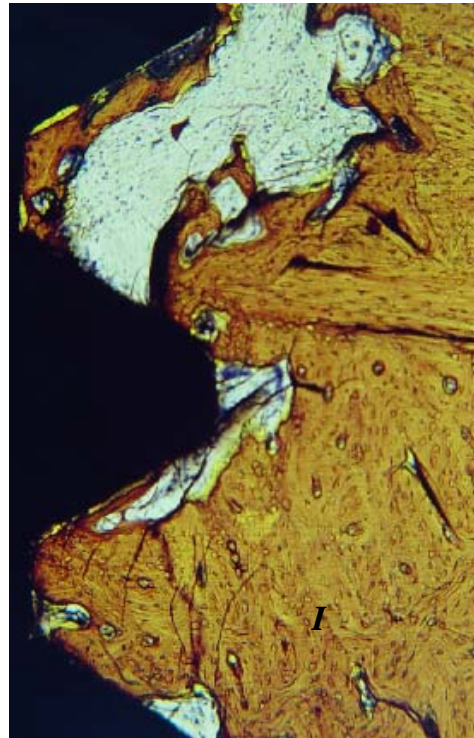
**Figure 1** – Mean and standard deviation of the initial and final body weight (Kg) for CTL and CsA groups.



\* Significance difference at  $p < 0.05$ , T-test.

**Figure 2a****Figure 2b**

**Figure 2** – Photomicrograph illustrating the histological aspect of the bone formed within the limits of the threads of an implant for CTL group, 12 weeks / Original magnification = 25x (Figure 2a). Note the direct bone contact with the implant surface / Original magnification =100x. Stevenel's blue and alizarin red S (Figure 2b).

**Figure 3a****Figure 3b**

**Figure 3** Photomicrograph illustrating the histological aspect of the bone formed within the limits of the threads of an implant for CsA group, 12 weeks / Original magnification = 25x (Figure 3a). Note the small extension of bone contact with the implant surface / Original magnification = 100x (Figure 3b). Stevenel's blue and alizarin red S.

**ESTUDO II**

Artigo intitulado “Ciclosporin-A and bone density around titanium implants: a histometric study in rabbits”. Submetido para avaliação no Journal of Periodontology

**CYCLOSPORIN-A AND BONE DENSITY AROUND TITANIUM IMPLANTS: A HISTOMETRIC STUDY IN RABBITTS**

Celso Eduardo Sakakura, DDS, MS\*

Beatriz M.V. Lopes, DDS, MS\*

Rogério Margonar DDS, MS\*

Francisco Humberto Nociti Júnior, DDS, MS, PhD<sup>†</sup>

Gibson Luiz Pilatti, DDS, MS, PhD<sup>&</sup>

Elcio Marcantonio Júnior, DDS, MS, PhD\*

\* Department of Periodontology – Araraquara Dental School, São Paulo State University (UNESP) Araraquara, São Paulo, Brazil.

<sup>†</sup> Department of Prosthodontics and Periodontics, Division of Periodontics, Piracicaba Dental School, UNICAMP, São Paulo, Brazil.

<sup>&</sup> Department of Periodontology – Ponta Grossa Dental School, Paraná State University (UEPG) Ponta Grossa, Paraná, Brazil.

***Author to whom correspondence should be sent:***

Elcio Marcantonio Jr, DDS, PhD, Departamento de Cirurgia e Diagnóstico, Disciplina de Periodontia; Faculdade de Odontologia de Araraquara, UNESP

Rua Humaitá, 1680, Centro, 14801-903, Araraquara, SP, Brazil,. Phone/Fax: 55 (16) 3301-6378, e-mail: [elciojr@foar.unesp.br](mailto:elciojr@foar.unesp.br)

Financial support: FAPESP - Fundação de Amparo à Pesquisa do Estado de São Paulo, grant no. 1999/09672-2 and CAPES.

## **ABSTRACT**

**Background:** Cyclosporine A (CsA) is an immunosuppressive agent commonly used to prevent organ transplantation rejection. It has been demonstrated that CsA may negatively affect osseointegration around dental implants. Therefore, the aim of this study was to evaluate the influence of CsA administration on bone density around titanium dental implants.

**Materiais e Métodos:** Twelve New Zealand rabbits were randomly divided into 2 groups with six animals each. The test group (CsA) received daily subcutaneous injection of CsA (10mg/kg bodyweight) and the control group (CTL) received saline solution by the same route of administration. Three days after the beginning of immunosuppressive therapy, one machined dental implant (7.0 mm length and 3.75 mm in diameter) was inserted bilaterally at the region of the tibial metaphysis. After 4 and 8 weeks the animals were sacrificed and the histometrical procedures were performed and the bone density was measured at first four threads.

**Results:** Bone density showed a statistically significant increase from the 4-week to the 8 week-period in the control group ( $37.41\% \pm 14.85$  versus  $58.23\% \pm 16.38$  –  $p < 0.01$ ). However, bone density consistently decreased in the test group over the time ( $46.31\% \pm 17.38$  versus  $16.28 \pm 5.08$  –  $p < 0.05$ ). In the 8-week period, there was a statistically significant difference in bone density between the control and the test group ( $58.23 \pm 16.38$  e  $16.28 \pm 5.08$  –  $p = 0.001$ ).

**Conclusions:** Within the limits of this study, long-term CsA administration may reduce bone density around titanium dental implants during the osseointegration process.

**Key-words:** dental implant; bone density; cyclosporin-a; immunosuppression

## INTRODUCTION

The principle of osseointegration is based on intimate bone-implant contact. The bone volume and quality are fundamental factors to achieve osseointegration during the healing and maintained over the years under load conditions<sup>1</sup>.

Several systemic diseases such as *diabetes mellitus*<sup>2,3</sup>, osteoporosis<sup>4,5</sup>, radiotherapy<sup>6</sup>, smoking habits<sup>7</sup> and some drugs therapy<sup>8,9</sup> can induce alterations on bone metabolism leading in a poor quality and impairing the healing of bone tissue..

Cyclosporine A (CsA) is an immunosuppressive agent commonly used to prevent organ transplantation rejection and treat other immunologic diseases<sup>10</sup>. CsA acts on immune system inducing T-helper lymphocytes suppression. This mechanism may affect bone tissue, since immune system, particularly T-lymphocytes, play a critical role on bone remodeling<sup>11-14</sup>. Some animal studies have demonstrated that this drug leads to a high bone turnover, resulting in unbalance of resorption and formation, leading to osteopenia<sup>12-14</sup>. Studies<sup>15-17</sup> in transplanted patients receiving CsA therapy showed high incidence of osteoporosis confirming this deleterious effect on bone metabolism in human.

The influence of CsA in osseointegration has been studied by Duarte et al 2001<sup>8</sup> and Sakakura et al (2003)<sup>9</sup> and it has been shown that CsA may negatively affect osseointegration, reducing the bone-to-implant contact and bone formation within implants threads. In other hand, there are no studies reporting the influence of CsA on bone density, which may be considered an important indicator of the quality of bone tissue formed around dental implants. Therefore, the aim of this study was to evaluate the influence of CsA administration on bone density around titanium dental implants.



## MATERIALS AND METHODS

### ANIMALS

Twelve New Zealand white rabbits, with 9 to 12 months of age (3500-4500 g) were used in the study. The animals were housed in individual cages, fed by a standard laboratory diet and given tap water *ad libitum*. The experiment was approved by the Institutional Experimentation Committee of the Araraquara Dental School, São Paulo, Brazil.

### EXPERIMENTAL PROTOCOL

After a 2-week acclimatization period, the animals were randomly divided into two groups, a test (T) and a control (CTL) group, with six animals each. The T group received a daily subcutaneous immunosuppressive dose<sup>18</sup> of 10-mg/kg bodyweight Cyclosporin-A\*, whereas the CTL group received saline solution (NaCl 0.9%) by the same route of administration. The administration of the drugs began three days before the implants placement and lasted until the day of sacrifice ( 4 and 8 weeks).

### IMPLANT SURGERY

The animals were anesthetized by intramuscular injections of a combination of ketamine<sup>†</sup> (0.35 mg/kg bodyweight) and xylazine<sup>‡</sup> (0.5mg/kg bodyweight). The region of the tibial metaphysis was cleansed with iodine surgical soap. Incisions of approximately 3

---

\* Sandimmun<sup>®</sup>, Novartis Pharma AG, Switzerland.

† Francotar<sup>®</sup>; Virbac do Brasil Ltda, Brazil.

‡ Rumpum<sup>®</sup> Bayer S.A. São Paulo, Brazil

cm in length were performed bilaterally at the internal side of the hind-leg, just below the knee. After gentle dissection, the bone surface of the tibial metaphysis was exposed. Unicortical implant beds were prepared by using a progressive sequence of spiral drills under generous saline cooling. One implant<sup>§</sup>, (7mm length and 3,75 mm in diameter), was placed in each leg. The soft tissues were sutured in separate layers and the animals received a single intramuscular injection of antibiotic<sup>||</sup> (0.1 ml/kg bodyweight of an association of Penicillin with Streptomycin.) postoperatively.

#### HISTOMETRIC PROCEDURE

After the animals were killed, the conventional implants with surrounding tissue in each tibia were removed and fixed in 4% neutral formalin for 48 hours. Undecalcified sections were prepared by a technique previously described by Donath & Breuner (1992)<sup>19</sup> Subsequently, the sections were stained as follows: i) the slide-containing specimen was placed in a vessel containing Stevenel's blue preheated to, and maintained at, 60 °C for 15 minutes; ii) the specimen was rinsed in distilled water at 60 °C and air dried; iii) a small amount of alizarin red S was placed onto the specimen surface at room temperature for 5 minutes. Then, it was washed thoroughly in running distilled water to remove excess stain and air dried. The bone density (i.e, proportion of mineralized bone in a 500 µm-wide zone lateral to the implant.) tissue was measured in both sides of the implant, at the first four threads. The mean of both sides of the implant was considered for statistical analysis (Figure 2).

---

<sup>§</sup> Master Screw<sup>®</sup>, Conexão, São Paulo, Brazil.

<sup>||</sup> Pentabiótico<sup>®</sup>, Wyeth-Whitehall Ltda, São Paulo, Brazil

## STATISTICAL ANALYSIS

Since data were normally distributed, as demonstrated by Kolmogorov and Smirnov test, unpaired t test was used to access difference in bone density between the groups in each experimental period (4-week and 8-week period). Paired t test was used to compare differences in bone density between the 4-week and the 8-week period in each experimental group separately. The level of statistical significance was set to  $\alpha = 0.05$ .

## **RESULTS**

A statistically significant increase in bone density between the 4-week and the 8-week period could be seen in the control group ( $37.41\% \pm 14.85$  versus  $58.23\% \pm 16.38$  –  $p < 0.01$ ). However, bone density consistently decreased in the test group along the time ( $46.31\% \pm 17.38$  versus  $16.28 \pm 5.08$  –  $p < 0.05$ ). In the 8-week period, there was a statistically significant difference in bone density between the control and the test group ( $58.23 \pm 16.38$  e  $16.28 \pm 5.08$  –  $p = 0.001$ ). No statistically significant difference could be found between test and control groups in the 4-week period ( $46.31\% \pm 17.38$  versus  $37.41\% \pm 14.85$ ) (Figure 1).

## DISCUSSION

The increase of bone density around dental implants over time could be seen in control group showing the bone quality improvement. However, in the T group, a statistically significant decrease in bone density was observed around the implants from the 4-week to the 8-week period ( $46.31\% \pm 17.38$  e  $16.28 \pm 5.08$  –  $p < 0.05$ ). This suggests that CsA administration during 8-week period may have negatively affected the bone quality around dental implant. The possible reasons for explain these results are related with the mechanism of immunosuppression caused by CSA. It is known that the immune system actively participates in bone mineral metabolism and that the T lymphocytes play a critical role in the development of CsA-induced osteopenia.<sup>20</sup> This is not surprising as the T cell is the traditional target of CsA, and naturally occurring T lymphocyte perturbations are implicated in the development of primary osteoporosis in humans.<sup>20</sup> Besides, an *in vitro* study<sup>21</sup> corroborates with this result, by describing the necessity of thymus-derived lymphocytes presence for the production of the osteoclast-activating factor.

The lymphocytes suppression results in a high bone metabolism state, where the bone formation is supplanted by the bone resorption, leading to a decrease in the trabecular bone volume.<sup>12, 13, 20</sup> Under these conditions, a decrease in bone density around dental implant was observed despite of the osteoinduction properties of titanium oxide on the implant surface. The precise mechanism of action of CsA on bone tissue is still not well understood. It is known that these bone alterations correlate with immunosuppressive mechanisms and are mediated by cytokines.<sup>14, 20, 22, 23</sup> Moreover, possible CsA effects on osteoblasts and osteoclasts should not be disregarded, which may result in a secondary phenomenon, leading to a high bone remodeling state with exceeding bone resorption.<sup>14</sup>

The side effects of CsA on bone tissue seem to be time and dose-dependent. Higher doses and long time administration may lead to severe alterations in bone metabolism.<sup>13</sup>

The percentage of bone tissue formed at the first four threads was used to assess bone density due to the close proximity to the cortical bone of the tibial metaphysis. Despite the fact that CsA exerts its effect mainly on trabecular bone, bone density was dramatically reduced in the cortical bone, as demonstrated in this study. This suggests that in areas where trabecular bone predominates, such as in the maxilla, bone density around dental implants may be severely damaged. Fu et al<sup>24</sup> (1999) found an increased alveolar bone resorption in rats receiving CsA, with greater bone loss in sites affected by periodontitis.

Although it could be demonstrated a negative side effect of CsA on cortical bone healing around dental implants corroborating the previous findings<sup>8,9</sup>, further studies should be developed in order to investigate the effects of this drug on trabecular bone and around osseointegrated functionally loaded dental implants. Within the limits of this study, long-term CsA immunosuppression may reduce bone density around titanium dental implants during the osseointegration process.

## **CONCLUSIONS**

Within the limits of this study, long-term CsA immunosuppression may reduce bone density around titanium dental implants during the osseointegration process.

**REFERENCES**

1. Albrektsson T. Bone tissue response. In: Brånemark P-I, Zarb G, Albrektsson T, eds. Tissue integrated prosthesis osseointegration in clinical dentistry. Chicago: Quintessence, 1985: 129-144.
2. Margonar R, Sakakura CE, Holzhausen M, Pepato MT, Alba Jr RC, Marcantonio Jr E. The Influence of Diabetes Mellitus and Insulin Therapy on Biomechanical retention around Dental Implants: A Study in Rabbits. *Implant Dentistry*. 2003, 4: 333-339.
3. Takeshita F, Murai K, Iyama S, et al. Uncontrolled diabetes hinders bone formation around titanium implants in rat tibiae. A light and fluorescence microscopy, and image processing study. *J Periodontol*. 1998;69:314–320.
4. Dao TT, Anderson JD, Zarb GA. Is osteoporosis a risk factor for osseointegration of dental implants? *Int J Oral Maxillofac Implants*. 1993; 8; 137-144.
5. Duarte PM, Cesar-Neto JB, Sallum AW, Sallum EA, Nociti FH Jr. Effect of estrogen and calcitonin therapies on bone density in a lateral area adjacent to implants placed in the tibiae of ovariectomized rats. *J Periodontol*. 2003;74(11):1618-24.
6. Granström G. Radiotherapy, osseointegration and hyperbaric oxygen therapy. *Periodontol 2000* 2003; 33; 145-162.
7. Cesar-Neto JB, Duarte PM, Sallum EA, Barbieri D, Moreno H Jr, Nociti FH Jr. A comparative study on the effect of nicotine administration and cigarette smoke inhalation on bone healing around titanium implants. *J Periodontol*. 2003 Oct;74(10):145-9.

8. Duarte PM, Nogueira Filho GR, Sallum EA, Sallum AW, Nociti Junior FH. The effect of an immunosuppressive therapy and its withdrawal on bone healing around titanium implants. A histometric study in rabbits. *J Periodontol* 2001; 72:1391-7.
9. Sakakura CE, Margonar R, Holzhausen M, Nociti Jr FH, Alba Jr RC, Marcantonio Jr E. influence of cyclosporin a therapy on bone healing around titanium implants. A histometric and biomechanic study in rabbits. *J Periodontol*. 2003; 74: 974-979.
10. Ruegger A, Kuhn M, Lichti H, et al. Cyclosporin A, a Peptide Metabolite from *Trichoderma polysporum* (Link ex Pers.) Rifai, with a remarkable immunosuppressive activity *Helv Chim Acta*. 1976; 59:1075-92.
11. Mccauley LK, Rosol TJ, Capen CC. Effects of cyclosporin A on rat osteoblasts (ROS 17/2.8 Cells) in vitro. *Calcif Tissue Int* 1992; 51: 291-297.
12. Movsowitz C, Epstein S, Ismail F, Fallon M, Thomas S. Cyclosporin A in the oophorectomized rat: unexpected severe bone resorption. *J Bone Miner Res* 1989; 4: 393-398.
13. Movsowitz C, Epstein S, Fallon M, Ismail F, Thomas S. Cyclosporin-A *in vivo* procedures severe osteopenia in the rat: effect of dose and duration of administration. *Endocrinol* 1988; 123: 2571-2577.
14. Rucinski B, Liu CC, Epstein S. Utilization of cyclosporine H to elucidate the possible mechanisms of cyclosporine A – Induced osteopenia in the rat. *Metabolism* 1994; 43:1114 – 1118.
15. Cayco A, Wysolmerski J, Simpson C, et al. Posttransplant bone disease: evidence for a high bone resorption state. *Transplantation* 2000; 70: 1722-1728.

16. Julian BA, Laskow DA, Dubovsky J, Dubovsky EV, Curtis JJ, Quarles LD. Rapid loss of vertebral mineral density after renal transplantation. *N Engl J Med* 1991; 325: 544-50.
17. Vedi S, Greer S, Skingle SJ, et al. Mechanism of bone loss after liver transplantation: a histomorphometric analysis. *J Bone Miner Res* 1999; 14: 281-287.
18. Gratwohl A, Riederer I, Graf E, Speck B. Cyclosporine toxicity in rabbits. *Lab Anim* 1986; 20: 213-220.
19. Donath K, Breuner G. A method for study of undecalcified bones and teeth with attached soft tissue. The sage-Scliff (sawing and grinding) technique. *J Oral Pathol* 1982; 11: 318-326.
20. Buchinsky FJ, Ma Y, Mann GN, et al. T lymphocytes play a critical role in the development of cyclosporin A-induced osteopenia. *Endocrinology* 1996; 137: 2278-2285.
21. Horowitz M, Vignery A, Gershon RK, Baron R. Thymus-derived lymphocytes and their interaction with macrophages are required for production of osteoclast-acting factor in mouse. *Proc Natl Acad Sci USA* 1984; 81: 2181-2185.
22. Sasagawa K, Fushibayashi S, Okano K, et al. Different inhibitory actions of immunomodulating agents and immunosuppressive agents on bone resorption of mouse calvaria. *Int J Immunopharmac* 1989; 11: 953-959.
23. Klaushofer K, Hoffmann O, Stewart PJ, et al. Cyclosporine A inhibits bone resorption in cultured neonatal mouse calvaria. *J Pharmacol Exp Ther* 1987; 243: 584-590.
24. Fu E, Hsieh Y-D, Nieh S, Wikesjö, Liu D. Effects of cyclosporin A on alveolar bone: an experimental study in the rat. *J Periodontol* 1999; 70:189-194.



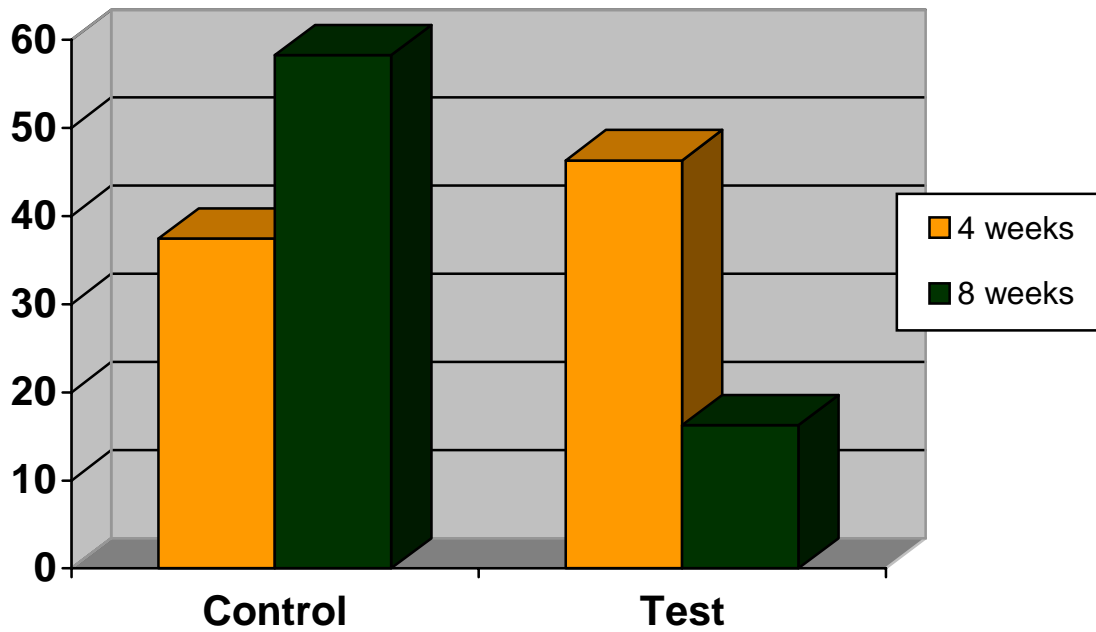
**FIGURES**

Figure 1 – Mean (%) and standard deviation for bone density around implants for control and test groups at 4 and 8 weeks post surgery.

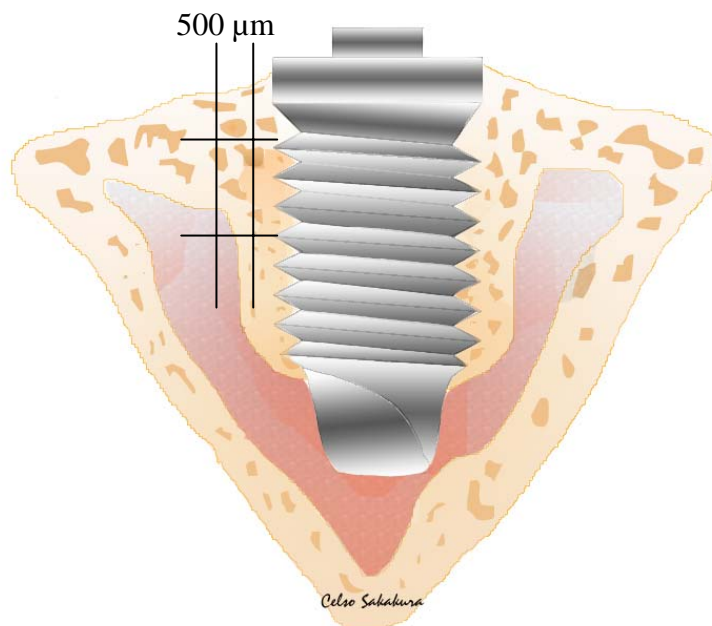


Figure 2 –Schematic illustration demonstrating the region of bone density analysis.

**ESTUDO III**

Artigo intitulado: “**The influence of cyclosporin-a on mechanical retention of dental implants integrated to the bone. A study in rabbits**”. Submetido ao *Journal of Periodontology*, onde está sob processo de revisão

**THE INFLUENCE OF CYCLOSPORIN-A ON MECHANICAL RETENTION OF DENTAL IMPLANTS INTEGRATED TO THE BONE. A STUDY IN RABBITS**

Celso E. Sakakura, DDS, MS, PhD\*

Rogério Margonar, DDS, MS, PhD\*

Rafael Sartori, DDS, MS student\*

Juliana Najarro Dearo de Moraes, DDS, MS, PhD student\*

Elcio Marcantonio Junior DDS, MS, PhD, Assistance Professor\*

**AUTHOR TO WHOM CORRESPONDE SHOULD BE SENT (*can be published*):**

Elcio Marcantonio Jr, DDS, PhD, Departamento de Cirurgia e Diagnóstico, Disciplina de Periodontia; Faculdade de Odontologia de Araraquara, UNESP

Rua Humaitá, 1680, Centro, 14801-903, Araraquara, SP, Brazil,. Phone/Fax: 55 (16) 3301-6314, e-mail: [elciojr@foar.unesp.br](mailto:elciojr@foar.unesp.br)

This study was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) Grant # 2003/04253-9 and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

Disclosure - The authors claim to have no financial interests in any company or any of the products described in this manuscript.

---

\* \* Department of Periodontology – Dental School of Araraquara, State University of São Paulo (UNESP) Araraquara, São Paulo, Brazil.

Numbers of figures – 2

**Running Title:** the influence of cyclosporin-a on dental implant mechanical retention

**ABSTRACT:**

**Background:** Immunosuppressive agents may induce severe changes on bone metabolism and may impair the osseointegration process during the implant healing. No data are available concerning the influence of cyclosporin A on healed bone around dental implants. The aim of this study was evaluate the influence of cyclosporine-A administration in the mechanical retention of bone integrated to dental implants.

**Material and Methods:** Eighteen female *New Zealand* Rabbits were submitted to an implant surgery. Each animal received one commercial dental implant of 10mm x 3.75mm. After 12 weeks of undisturbed healing period, 6 animals were randomly sacrificed and the removal torque was performed (Group A). Besides, another 6 animals were submitted to a daily injection of cyclosporine-A in a 10mg/Kg dosage (Group B) and the other 6 animals received saline solution as a control (Group C). After 12 weeks of cyclosporine-A administration both group B and C were sacrificed and submitted to a removal torque test.

**Results:** the removal torque results were 30.5 ( $\pm 9.8$ ) N.cm for group A; 50.16 ( $\pm 17.5$ ) N.cm for group B; 26 ( $\pm 7.8$ ) N.cm for group C. The statistical analysis showed significant difference between group A and B ( $p < 0.05$ ) and between B and C ( $p < 0.01$ ).

**Conclusion:** The cyclosporine-A administration may impair the mechanical retention of dental implants integrated to the bone.

**Key Words:** Cyclosporin A, Osseointegration, Dental Implant, Removal Torque

## **INTRODUCTION**

Cyclosporin A is a potent immunosuppressive drug used to treat patients who received organ transplantation.<sup>1</sup> Besides, this drug might be used for the treatment of type 2 diabetes, psoriasis, malaria, multiple sclerosis, rheumatoid arthritis, sarcoidosis and several other immunologic diseases.<sup>2</sup> The long term use of cyclosporin A is related to several side-effects which included nephrotoxicity, neurologic disturbances, hypertension and gingival overgrowth.<sup>1-3</sup> Another side-effect reported in allogeneic organ transplantation is osteoporosis, and the use of cyclosporin A associated with other immunosuppressive drugs such as steroidal anti-inflammatory may be responsible for its pathogenesis.<sup>4-6</sup> Several studies both in animals<sup>7-8</sup> and humans<sup>4-6</sup> have been reported that cyclosporin A increases the bone turnover producing higher resorption than formation, increasing the incidence of bone fractures.<sup>5,6</sup>

There are few studies reporting some relationship between cyclosporin A and dental implants. Duarte et al.<sup>9</sup> showed a negative impact on bone formation within implant threads when the animals were submitted to a short-term cyclosporin A administration. Sakakura et al.<sup>10</sup> reported also a negative influence on bone-to-implant contact and removal torque values when the cyclosporin was administered for longer periods. These previous studies were designed to assess the influence of cyclosporin-A administration *during* the bone healing immediately after implant placement. There are, however, no data available on the effects of cyclosporin on bone already healed around dental implants. Thus, the aim of this study was to evaluate the influence of cyclosporin-A administration on the mechanical retention of bone integrated to dental implants.

## **MATERIALS AND METHODS**

### ANIMALS

Eighteen *New Zealand* white rabbits, with 9 to 12 months old (3500-4500 g) were used in the study. The animals were housed in individual cages, fed by a standard laboratory diet and given tap water *ad libitum*. The experiment was approved by the Institutional Experimentation Committee of the School of Dentistry of Araraquara, São Paulo, Brazil.

### EXPERIMENTAL PROTOCOL

After a 2-week acclimatization period, the animals were submitted into an implant surgery. After a 12 weeks of implant healing 6 animals were randomly sacrificed (Group A) and other 6 animals were randomly selected and submitted into a daily subcutaneous immunosuppressive dose of 10-mg/kg<sup>11</sup> bodyweight Cyclosporin-A\* (group C), whereas the 6 other animals received saline solution (NaCl 0.9%) by the same route of administration (Group B).

### IMPLANT SURGERY

The animals were anesthetized by intramuscular injections of a combination of ketamine<sup>†</sup> (0.35 mg/kg bodyweight) and xylazine<sup>‡</sup> (0.5mg/kg bodyweight). The region of the tibial metaphysis was cleansed with iodine surgical soap (figure 1A). Incisions of approximately 3 cm in length were performed at the internal side of the hind-leg, just below the knee. After gentle dissection, the bone surface of the tibial metaphysis was exposed. Bicortical implant beds were prepared by using a progressive sequence of spiral drills under generous saline

---

\* Sandimmun<sup>®</sup>, Novartis Pharma AG, Switzerland.

† Francotar<sup>®</sup>; Virbac do Brasil Ltda, Brazil.

‡ Rumpum<sup>®</sup> Bayer S.A. São Paulo, Brazil

cooling. One implants with machine surface<sup>4</sup> (10mm in length and 3,75 mm in diameter) with a modified head to enable fixation of the torque meter were placed (figure 1B). The soft tissues were sutured in separate layers (figure 1C) and the animals received a single intramuscular injection of antibiotic<sup>1</sup> (0.1 ml/kg bodyweight of an association of Penicillin with Streptomycin.) postoperatively.

## REMOVAL TORQUE

Immediately after the animals sacrifice the tibias were surgically removed and the implants were submitted into a removal torque test. The specially designed key were connected the modified head implant with the torque-gauge wrench<sup>¶</sup>. An anticlockwise movement was performed in order to remove the implant and the maximal torque value necessary for manual removal of each implant was measured in Newton centimeters.

## STATISTICAL ANALYSIS

To test the differences in body weight and torque values between the three groups the Mann Whitney test was used. The value of  $P < 0.05$  was consider the limit for a significant difference.

---

<sup>4</sup> Master, Conexão Sistemas de Prótese Ltda, São Paulo, Brazil.

<sup>1</sup> Pentabiótico®, Wyeth-Whitehall Ltda, São Paulo, Brazil

<sup>¶</sup> 15-BTG, Tonich, Japan.



## **RESULTS**

### CLINICAL FINDINGS

The healing was uneventful in all rabbits after implant placement. However, the remaining animals subjected to cyclosporine A administration experienced a significant weight loss ( $p < 0.05$ ). The mean and standard deviation were  $4216 \pm 728$ g,  $4783 \pm 432$ g and  $3266 \pm 531$ g, respectively group A, B and C

### TORQUE VALUES

All the implants showed values compatible with osseointegration status. The removal torque and the standard deviation results were  $30.5(\pm 9.8)$  N.cm group A;  $50.16(\pm 17.5)$  N.cm for group B;  $26(\pm 7.8)$  N.cm for group C. The statistical analysis showed significant difference between group A and B ( $p < 0.05$ ) and between B and C ( $p < 0.01$ ) (Figure 2).

## **DISCUSSION**

The present study evaluated the influence of cyclosporin-A administration on mechanical retention of dental implants already osseointegrated. The compound administration began after 12 weeks after implant placement, at this time it was supposed that all implants were osseointegrated, since the 6 animals were randomly selected and submitted to a removal torque assessment (group A) showing similar torque values reported in previous studies<sup>10,12,13</sup>. The others remaining six animals (group B) receiving saline solution showed 24 weeks after implant placement an improvement in mechanical retention comparing to group A (12 weeks). However, in group C, which the animals were subjected to a compound

administration during 12 weeks showed lower mechanical retention values. Indeed, the torque values were almost half than values of group B and even lower than group A. These findings suggested that the continuing processes of bone attachment into the surface of implant were impaired, probably because the cyclosporin-A administration might result in dramatic alteration in bone metabolism.

These alterations can be explained by the action of cyclosporin-A. It is known that the immune system actively participates in bone mineral metabolism and that the T lymphocytes play a critical role in the development of CsA-induced osteopenia.<sup>14</sup> This is not surprising as the T cell is the traditional target of CsA and naturally occurring T lymphocyte perturbations are implicated in the development of primary osteoporosis in humans.<sup>14</sup> Besides, an in vitro study<sup>15</sup> corroborates with this result, by describing the necessity of thymus-derived lymphocytes presence for the production of the osteoclast-activating factor.

T lymphocytes suppression results in a high bone metabolism state, where the bone formation is supplanted by the bone resorption, leading to a decrease in the trabecular bone volume.<sup>7, 8, 14</sup> Under these conditions, a decreased percentage of bone contact with the implant surface could be expected resulting in a lower mechanical retention of implant to the bone<sup>10</sup>. The precise mechanism of action of CsA on bone tissue is still not well understood. It is known that these bone alterations correlate with immunosuppressive mechanisms and are mediated by cytokines.<sup>14</sup> Moreover, possible CsA effects on osteoblasts and osteoclasts are not rejected and may result in a secondary phenomenon, leading to a high bone remodeling state with exceeding bone resorption.<sup>15</sup>

The fixture mechanical retention is directly dependent of amount of bone attached into implant surface. Implants showing larger area of implant-to-bone contact shows greater removal torque values.<sup>10,12,13, 17,18</sup> Regarding to implants subjected to a cyclosporin-A administration and considering the bone as one of the most dynamic tissues in the body, we

can speculate that the bone attached to implant surface might be resorbed during the cyclosporin A administration, or there was mineral contents loosening leading in lower bone strength, resulting in lower mechanical retention. This confirmed the hypothesis that even bone already healed around dental implants may be affected by CsA. This fact should be considered in the clinical situation for patients who are candidates for implant placement and who had implants and began CsA treatment. However, there is no data regarding neither if such bone alterations could impair the prostheses supporting nor if this bone alterations remain for a long periods or is only a transitional phenomena.

Therefore, within limits of this study we conclude that the cyclosporin A administration might impair the mechanical retention of dental implants integrated to the bone.

## **ACKNOWLEDGMENTS**

The authors greatly appreciated the assistance of Celso Luiz Borsato for technical support in the Animal Laboratory.

## **REFERENCES**

1. Faulds D, Goa KL, Benfield P. Cyclosporin. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in immunoregulatory disorders. *Drugs*. 1993 Jun;45(6):953-1040. Review. Erratum in: *Drugs* 1993 Sep;46(3):377.
2. Adams D, Davies G. Gingival hyperplasia associated with cyclosporin A. A report of two cases. *Br Dent J*. 1984 Aug 11;157(3):89-90.

3. Seymour RA, Ellis JS, Thomason JM. Risk factors for drug-induced gingival overgrowth. *J Clin Periodontol*. 2000 Apr;27(4):217-23. Review.
4. Cayco A, Wysolmerski J, Simpson C, et al. Posttransplant bone disease: evidence for a high bone resorption state. *Transplantation* 2000; 70: 1722-1728.
5. Julian BA, Laskow DA, Dubovsky J, Dubovsky EV, Curtis JJ, Quarles LD. Rapid loss of vertebral mineral density after renal transplantation. *N Engl J Med* 1991; 325: 544-50.
6. Vedi S, Greer S, Skingle SJ, et al. Mechanism of bone loss after liver transplantation: a histomorphometric analysis. *J Bone Miner Res* 1999; 14: 281-287.
7. Movsowitz C, Epstein S, Ismail F, Fallon M, Thomas S. Cyclosporin A in the oophorectomized rat: unexpected severe bone resorption. *J Bone Miner Res* 1989; 4: 393-398.
8. Movsowitz C, Epstein S, Fallon M, Ismail F, Thomas S. Cyclosporin-A in vivo procedures severe osteopenia in the rat: effect of dose and duration of administration. *Endocrinol* 1988; 123: 2571-2577.
9. Duarte PM, Nogueira Filho GR, Sallum EA, Sallum AW, Nociti Junior FH. The effect of an immunosuppressive therapy and its withdrawal on bone healing around titanium implants. A histometric study in rabbits. *J Periodontol* 2001; 72:1391-7.
10. Sakakura CE, Margonar R, Holzhausen M, Nociti FH Jr, Alba RC Jr, Marcantonio E Jr. Influence of cyclosporin A therapy on bone healing around titanium implants: a histometric and biomechanic study in rabbits. *J Periodontol*. 2003 Jul;74(7):976-81.
11. Gratwohl A, Riederer I, Graf E, Speck B. Cyclosporine toxicity in rabbits. *Lab Anim* 1986; 20: 213-220.

12. Ivanoff C-J, Sennerby L, Lekholm U. Influence of mono- and bicortical anchorage on the integration of titanium implants. A study in the rabbit tibia *Int Oral Maxillofac Surg* 1996; 25: 229-235.
13. Johansson C, Albrektsson T. Integration of screw implants in the rabbit: A 1-yr follow-up of removal torque of titanium implants. *Int J Oral Maxillofac Implants* 1987; 2: 69-75.
14. Buchinsky FJ, Ma Y, Mann GN, et al. T lymphocytes play a critical role in the development of cyclosporin A-induced osteopenia. *Endocrinology* 1996; 137: 2278-2285.
15. Horowitz M, Vignery A, Gershon RK, Baron R. Thymus-derived lymphocytes and their interaction with macrophages are required for production of osteoclast-acting factor in mouse. *Proc Natl Acad Sci USA* 1984; 81: 2181-2185.
16. Rucinski B, Liu CC, Epstein S. Utilization of cyclosporine H to elucidate the possible mechanisms of cyclosporine A – Induced osteopenia in the rat. *Metabolism* 1994; 43:1114 – 1118.
17. Cordioli G, Majzoub Z, Piattelli A, Scarano A. Removal torque and histomorphometric investigation of 4 different titanium surfaces: an experimental study in the rabbit tibia. *Int J Oral Maxillofac Implants*. 2000 Sep-Oct;15(5):668-74.
18. Klokkevold PR, Nishimura RD, Adachi M, Caputo A. Osseointegration enhanced by chemical etching of the titanium surface. A torque removal study in the rabbit. *Clin Oral Implants Res*. 1997 Dec;8(6):442-7.

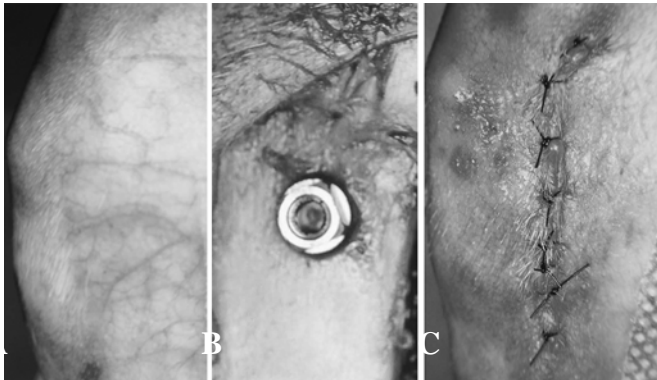
**FIGURES**

Figure 1 –A) Rabbit Tibia Metaphysis; B) Dental implant installed with modified head to fit in torque meter gouge; C) Tissues sutured.

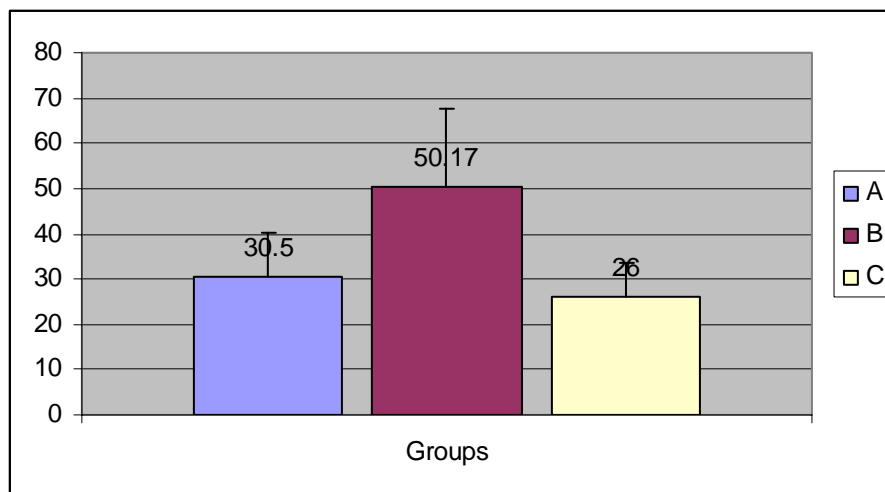


Figure 2 – Removal torque values (N.cm) for group A, B and C

**ESTUDO IV**

Artigo intitulado **“The Influence Of Cyclosporin A On Quality Of Bone Around Integrated Dental Implants. A Radiographic Study In Rabbits”** Trabalho aceito pelo *Clinical Oral Implants Research*.

**THE INFLUENCE OF CYCLOSPORIN A ON QUALITY OF BONE AROUND  
INTEGRATED DENTAL IMPLANTS. A RADIOGRAPHIC STUDY IN RABBITS**

Celso Eduardo Sakakura, DDS, MSc, PhD<sup>5</sup>

Elcio Marcantonio Junior, DDS, MSc, PhD\*

Ann Wenzel, DDS, PhD, Dr. Odont.<sup>6</sup>

Gulnara Scaf, DDS, MSc, PhD\*

**RUNNING-TITLE: Influence of cyclosporin A on bone around titanium implants.**

**KEY WORDS: cyclosporin; subtraction radiograph; titanium implants; bone density.**

**AUTHOR RESPONSIBLE FOR CORRESPONDENCE:**

Gulnara Scaf DDS, MSc, PhD, Departamento de Diagnóstico e Cirurgia, Faculdade de Odontologia de Araraquara, UNESP, Rua Humaitá, 1680, 14801-903, Araraquara, SP, Brazil, Phone: 55 (16) 3322-3285, Fax: 55 (16) 3301-6369 e-mail: [scaf@foar.unesp.br](mailto:scaf@foar.unesp.br)

---

<sup>5</sup> Department of Oral Diagnosis and Surgery, School of Dentistry, State University of São Paulo, UNESP, Araraquara, SP, Brazil.

<sup>6</sup> Head and Chair, Professor of Radiology Department, Aarhus University, Denmark



**ABSTRACT**

Sakakura CE, Marcantonio Jr E, Wenzel A, Scaf G. The influence of cyclosporin A on quality of bone around integrated dental implants. A radiographic study in rabbits. *Clin Oral Impl Res*

**Objectives:** To evaluate the influence of cyclosporin-A administration on bone around integrated dental implants assessed by a bone quality index and by quantitative subtraction radiography.

**Material and Methods:** A total of 36 machine surface commercial implants were placed in eighteen adult rabbits. After a 3-month healing period without any disturbance, the animals were randomly divided into three groups of 6 animals each. Group A was sacrificed at this time. Cyclosporine A was injected subcutaneously in an immunosuppressive dose of 10mg/kg/day in a test group (Group T), and a Group B served as a control receiving only vehicle. After 3 months of cyclosporine administration, the animals of both Group B and T were sacrificed. Radiographs were obtained at implant surgery and at the day of sacrifice with a CMOS sensor. Bone quality around the implants was compared between the groups using a bone quality index and quantitative subtraction radiography.

**Results:** The bone analysis showed that in Group T, the bone quality changed dramatically from a dense cortical to a loose trabecular bone structure ( $p < 0.0001$ , qui-square test) while in Groups A and B there were no significant differences. Quantitative digital subtraction radiography showed significant ( $p < 0.05$ ) lower gray shade values (radiographic density) in a region of bone formation around the implants in Group T ( $118 \pm 12$ ) than in Group A ( $161 \pm 6$ ) and B ( $186 \pm 10$ ).

**Conclusion:** Within the limits of this study, cyclosporine A administration has a negative effect on the quality of bone around integrated dental implant.

## **INTRODUCTION**

Cyclosporin A is a potent immunosuppressive drug used to treat patients who have received organ transplants (Faulds et al. 1993). Besides, this drug might be used for the treatment of type 2 diabetes, psoriasis, malaria, multiple sclerosis, rheumatoid arthritis, sarcoidosis and several other immunologic diseases (Adams e Davies 1984). The long term use of cyclosporin A is connected with several side-effects, which includes nephrotoxicity, neurological disturbances, hypertension and gingival overgrowth (Faulds et al. 1993; Adams e Davies 1984; Seymour et al. 2000). Another side-effect reported in allogenic organ transplantation is osteoporosis, and the use of cyclosporin A in association with other immunosuppressive drugs such as steroidal anti-inflammatory medicaments may be responsible for its pathogenesis (Cayco et al. 2000; Julian et al. 1991; Vedi et al. 1999). Several studies, both *in vitro* (Buchinsky et al. 1996; Horowitz et al. 1984) and clinical have reported that cyclosporin A increases the bone turnover resulting in a higher resorption than formation rate, increasing the incidence of bone fractures (Cayco et al. 2000; Julian et al. 1991; Vedi et al. 1999). Further, the use of this compound has been associated with a bone mineral loss in animal studies (Movsowitz et al. 1988; Movsowitz et al. 1989, Schlosberg et al. 1989).

There are few studies reporting the relationship between cyclosporin A and dental implants. Duarte et al. 2001 showed a negative impact on bone area amount within implant threads when the animals were submitted to a short-time cyclosporin A administration, and similarly, Sakakura et al. 2003 reported a negative influence on bone-to-implant contact when the compound was administered for longer periods. These previous studies were designed to assess the influence of cyclosporin A administration *during* bone healing immediately after placement of the implants. There are, however, no data available on the effect of cyclosporin A on bone around already integrated dental implants.

In order to detect early destruction of periodontal and peri-implant bone, several diagnostic tools have been introduced, however for clinical applications, non-invasive diagnostic tools are required. Therefore, the periodontal probing and the intraoral radiograph are the most widely used diagnostic methods to assess the changes in clinical attachment levels and bone height (Suomi et al. 1968; Papelassi & Diamanti-Kipiotti 1997)

However, the sensitivity of conventional periapical radiography for detection of small changes in bone mineral content has been shown to be low, and over- and under-estimation of periodontal defects often occurred (Suomi et al. 1968; Papelassi & Diamanti-Kipiotti 1997). The introduction of digital subtraction radiography has facilitated detection of small bone changes, since structures that are unchanged over the time period between two recordings may be eliminated (Jeffcoat et al. 1992). Using this method, disease progress or treatment effects may be assessed in a quantitative manner (Schou et al. 2003a, Schou et al. 2003b; Schou et al. 2003c). Christgau et al. 1998 reported a high accuracy of subtraction radiography to detect small changes in calcium mass in alveolar as well as cortical bone.

The aim of this study was to evaluate the influence of cyclosporin A administration on bone around integrated dental implants assessed by a bone quality index and by quantitative subtraction radiography.

## **MATERIAL AND METHODS**

### **ANIMALS**

Eighteen New Zealand white rabbits, 9 to 12 months old (3500-4500 g), were used in the study. The animals were housed in individual cages, fed by a standard laboratory diet and given tap water *ad libitum*. The experiment was approved by the Institutional Experimentation Committee of the School of Dentistry.

## EXPERIMENTAL PROTOCOL

After a 4-week acclimatization period, the animals were submitted to implant surgery. After 12 weeks of implant healing, 6 randomly selected animals were sacrificed (Group A). Another 6 randomly selected animals were submitted to a daily subcutaneous immunosuppressive dose (Gratwohl 1986) of 10-mg/kg bodyweight cyclosporin A\* (Group T) whereas the 6 remaining animals received a saline solution (NaCl 0.9%) by the same route of administration (Group B). Group B and T were sacrificed after another 12 weeks.

## IMPLANT SURGERY

The animals were anesthetized by intramuscular injections of a combination of ketamine<sup>†</sup> (0.35 mg/kg bodyweight) and xylazine<sup>‡</sup> (0.5 mg/kg bodyweight). The region of the tibial metaphysis was cleansed with iodine surgical soap. Incisions of approximately 3 cm in length were performed at the internal side of the hind leg, just below the knee. After gentle dissection, the bone surface of the tibial metaphysis was exposed. The implant beds were prepared by using a progressive sequence of spiral drills under generous saline cooling. Two machine surface implants<sup>7</sup> (10 mm in length and 3.75 mm in diameter) were fixed in the right leg. The soft tissues were sutured in separate layers, and the animals received a single intramuscular injection of antibiotics<sup>l</sup> (0.1 ml/kg bodyweight of a mixture of Penicillin and Streptomycin) postoperatively.

## RADIOGRAPHY

Radiographs were taken immediately after implant surgery (baseline images) and at sacrifice (final images) using a CMOS sensor<sup>8</sup> with the vertical long axis of the implant fixed

---

\* Sandimmun<sup>®</sup>, Novartis Pharma AG, Switzerland.

† Francotar<sup>®</sup>, Virbac do Brasil Ltda, Brazil.

‡ Rumpum<sup>®</sup> Bayer S.A. São Paulo, Brazil

<sup>7</sup> Master, Conexão Sistemas de Prótese Ltda, São Paulo, Brazil.

<sup>l</sup> Pentabiótico<sup>®</sup>, Wyeth-Whitehall Ltda, São Paulo, Brazil

<sup>8</sup> Schick Sensor, Schick Technologies Inc., Long Island, NY, USA

perpendicular to the central ray and parallel to the sensor at 40 cm focal spot-to-object distance. The x-ray unit was operated at 65 kVp, 10 mA and 0.3 seconds. In order to obtain a geometrically standardized projection, a sensor holder device was developed, which was fixed to the dental implant keeping a rigid connection between the animal's leg and the sensor.

#### QUALITATIVE BONE INDEX

All radiographs were submitted to a qualitative assessment using a 2-score bone quality index to classify the bone around the implant. The scores were defined as: 1) cortical bone 2) trabecular bone (Fig. 1A and B). One independent observer assessed all the images twice and blind in a random sequence.

#### QUANTITATIVE DIGITAL SUBTRACTION

The CMOS images were saved in TIFF format, exported from the CDR program<sup>9</sup> and imported to a digital subtraction program<sup>10</sup>. In order to prepare the baseline and the final image for the subsequent subtraction, ten reference points were positioned around the implant with the aid of the computer mouse (Fig. 3A). These reference points were placed on the edge of threads (Schou et al. 2003a; Schou et al. 2003b; Schou et al. 2003c) along the implant length in both sides of the fixture (five points in each side). The same reference points were placed on the same landmarks in corresponding images taken after sacrifice (Wenzel 1989) (Fig. 3B). The precision of the reference points placed in the two radiographs was evaluated by means of an "accuracy tool" in the software. The points were re-positioned if the distance between two points was larger than 2 pixels.

---

<sup>9</sup> CDR DICOM for Windows, Schick Technologies Inc., Long Island, NY, USA

<sup>10</sup> X-Poseit, version 3.1.17, Image Interpreter System, Lystrup, Denmark

In order to restrict the areas of analysis to those of interest for the study, the following regions of interest were outlined using the computer mouse on the baseline radiographs and automatically transferred to the subtraction images:

1. Osseointegrated region (OR). One rectangular window with 10 pixels of width and three implant threads high was drawn comprising the bone immediately close to the fixture (the implant cortical passage area). The region was drawn in the mesial and distal side of the implant (Fig. 1A).
2. Control region (CR). A region of cortical bone localized far away from the fixture was chosen as the CR. In this region one square window of approximately 1000 square pixels<sup>2</sup> was drawn (Fig. 1A) in order to take into account the “noise” in the image, i.e. recording errors not accounted for by the program and the physiologic bone changes, bone modeling and remodeling.

The standard deviation of the histogram distribution of the shades of gray in the CR of the subtraction image was used to determine the threshold between what was interpreted as a “signal” and what could be determined as a “noise” in the subtraction image (Wenzel & Sewerin 1991). When setting one standard deviation of gray shades as the noise threshold, this corresponded to an average of 3.68 shades of gray (range 2.84 to 5.68).

The subtraction was performed automatically by the program, and all data concerning the subtraction in regions of interest was automatically stored in the program data bank.

## DATA TREATMENT

The observer’s reproducibility using the bone quality index was calculated as percentage agreement between the first and second assessment. For the presentation of the results only the first assessment was used and the chi-square tests were performed between quality scores after implant placement and before sacrifice.

The subtraction program automatically generated the distribution of shades of gray in the OR and the CR. The mean value between the mesial and distal OR was calculated and used for further statistical analysis. By the definition of the threshold for signal and noise, mean levels for bone loss and bone gain and the area related to bone loss/gain were calculated by the subtraction program and the data sent to a data bank. The data were thereafter extracted and analyzed using SPSS software<sup>11</sup>. Differences in mean shades of gray and area between Groups T, B and A were assessed using the Mann-Whitney U-test. The level of statistical significance was set to  $\alpha = 0.05$ .

## **RESULTS**

### **CLINICAL ASSESSMENT**

The healing was uneventful in all rabbits after implant placement. However, some rabbits in Group T showed side effects during cyclosporin A administration, and they were omitted from the study. Further, the remaining animals subjected to cyclosporine A administration experienced a significant weight loss ( $p < 0.05$ ). The means and standard deviations were  $4216 \pm 728\text{g}$ ,  $4783 \pm 432\text{g}$  and  $3266 \pm 531\text{g}$ , in Group A, B and T, respectively.

### **RADIOGRAPHY**

#### **OVERALL RADIOGRAPHIC CHARACTERISTICS**

The baseline images showed healthy bone without any disturbance such as tumor or osteopenia. The leg rabbit bone consists of two thin cortical plates separated by a medullar tissue. At sacrifice, a dramatic change had occurred in the bone close to the implants in all groups as displayed in the final images (Fig. 1). The bone change was characterized by

---

<sup>11</sup> SPSS for Windows, ver. 10, SPSS, USA

formation of new bone along the implant threads, mainly at the top and at the bottom while at baseline, a medullar tissue was seen in this region. By the naked eye, an osteoconduction initiated by the implant titanium oxide layer could thus be demonstrated. In Group T, a thinning of the cortical bone plate was seen as well. Radiographically, all the implants were interpreted as osseointegrated since there was no radiolucent area between the implant and surrounding bone.

#### QUALITATIVE BONE INDEX

There was an agreement of 88.2% between first and second assessment. Bone quality in the baseline images in Groups A and B was scored as cortical bone in 75% and 33.3% for Group A and B, respectively. In all baseline images (100%) from Group T, bone was scored as cortical. Groups A and B showed no difference between their baseline and final images ( $p=1.00$ ), however in Group T, the bone quality had changed totally, since all final images were scored as trabecular bone ( $p<0.0001$ ). This means that cyclosporine A administration had a dramatic effect on the quality of bone after 12 weeks of administration (Table 1).

#### QUANTITATIVE DIGITAL SUBTRACTION

Subtraction images, using one standard deviation of the histogram defining the distribution of gray levels in the CR as the threshold for noise, demonstrated that a significant difference existed in mean shade of gray between all groups; Group B (mean  $186 \pm 10$ ), Group A (mean  $161 \pm 6$ ) and Group T (mean  $118 \pm 12$ )(Fig. 2). This means that there was an increase in density around the implant, interpreted as more mineral content, with increasing age of the animal when the animals were not disturbed (Groups A vs. B). Moreover, the cyclosporine A administration resulted in a significant decrease ( $p<0.05$ ) in mean gray shade level (mineral content) compared with the control group of the same age (Groups T vs. B),



and this reduction in mineral content resulted in gray shades even significantly lower than in the young control Group A ( $p < 0.05$ ). For the gray shades related to the loss there are no differences among the three groups (fig 3).

Regarding the area of the peri-implant bone, the differences observed among the three groups were not statistically significant, neither for formation of new bone (Fig. 4) nor loss of bone (fig 5).

## **DISCUSSION**

The results from this study showed a negative impact of cyclosporin A (CsA) on the quality of bone around dental implants, which had already healed for twelve weeks. The purpose of control Group A was to observe the radiographic appearance of undisturbed bone healing for twelve weeks before the CsA administration started. From the appearance of the final images in Group A, it was interpreted that the implants had been integrated in bone at this time.

There was in this study a continuing increase in bone density with age in the control groups since the mean shades of gray in the osseointegrated region around the implant in Group B were significantly larger than in Group A. However, there was no difference among them regarding area of bone gain or loss. This is in accordance with findings reported by Johansson & Albrektsson 1987, who found no significant increase in bone area and bone-to-implant contact after 12 weeks of implant healing in rabbits.

From our findings in Group T, we suggest that cyclosporin A administered in immunosuppressive doses gave rise to alterations in the quality of bone in close proximity to dental implants. After a twelve-week period of CsA administration, the bone may be characterized as loose with a trabecular appearance and with a substantial loss of mineral

content. The changes were demonstrated by the bone quality index, which provided strong evidence of deterioration in the quality of bone around the implants since all animals receiving CsA changed from a score 1 to a score 2, cortical to trabecular, bone structure. Since the bone quality index is a subjective assessment, the conductors of the study might have been biased since they could easily recognize the animals in the groups, so an observer (a dentist) not familiar with implant surgery, nor with the nature of the study scored the images using the bone index. This result from the qualitative assessment seems to be similar to that shown in previous reports (Duarte et al. 2001; Sakakura et al. 2003), which demonstrated the negative influence of CsA administration on bone-to-implant contact and the rate of bone formation between the threads.

In clinical studies, quantitative digital subtraction radiography has been increasingly used to detect small changes in peri-implant bone (Engelke et al. 1990; Jeffcoat et al. 1992; Schou et al. 2003a; Schou et al. 2003b, Schou et al. 2003c). Subtraction radiography was used in the present study to add quantitative data to the information obtained from the bone quality index. The subtraction images provided evidence that bone in close proximity to dental implants had a lower radiographic density in Group T than in both control groups, which was interpreted as lower mineral content (Christgau et al. 1998). Although not statistically significant, the area of bone gain around the implants in Group T was also smaller than in the control Groups A and B. The mineral loss involved not only the surrounding bone, but also a new area of bone formation along the implant threads. This confirmed the hypothesis that, even bone already healed around dental implants, may be affected by CsA. This may not be surprising since bone is one of the most dynamic tissues in the body. It is known that the immune system actively participates in bone mineral metabolism, and that the T-lymphocytes play a critical role in the development of CsA-induced osteopenia (Buchinsky et al. 1996). The T-cell is the target of CsA and naturally occurring T-lymphocyte perturbation is involved

in the development of primary osteoporosis in man. Besides, a previous *in vitro* study (Horowitz et al. 1984) corroborates these results since it reported the need for thymus-derived lymphocytes in the production of an osteoclast activating factor. Thus, the CsA administration results in a high bone turnover, which is characterized by higher resorption than formation and increased osteoclast activity (, Movsowitz et al. 1988 and 1989; Schlosberg et al. 1989; Buchinsky et al. 1996; Cayco et al. 2000).

In opposition to previous studies, the present effect of CsA was obtained in animals, which had received implants that were already healed in bone. Therefore, it may be expected that CsA has a negative impact on the quality of both *healing* (Duarte et al. 2001; Sakakura et al. 2003) and *healed* bone around implants. This fact should be considered in the clinical situation for patients who are candidates for implant placement, and patients who have already had implants installed when they start CsA treatment. However, our study does not provide data that such bone alterations would impair the maintenance of implant-supported prostheses, nor that the bone changes remain for a longer period after cease of treatment since it may be a transitional phenomenon.

Within the limits of this study, we conclude that cyclosporin A administration given in immunosuppressive doses to animals with integrated dental implants may result in a deterioration in radiographically assessed bone quality with thinning of the cortical bone and a decrease in bone density.

### **ACKNOWLEDGMENT**

This study was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) Grant # 2003/04253-9 and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the exchange Scholarship.

We would like to thank Celso Luiz Borsato for technical support in the Araraquara Dental School animal laboratory. We also wish to express our gratitude to Hanne Hintze, René Christiansen, Erik Gotfredsen and Dorte Vilhelmsen, all from the department of Oral Radiology, Aarhus Dental School, University of Aarhus, Denmark, for their assistance.

### **DISCLOSURE**

The authors claim to have no financial interests in any company or any of the products described in this manuscript.

**REFERENCES**

1. Adams, D., Davies, G. (1984) Gingival hyperplasia associated with cyclosporin A. A report of two cases. *Br Dent J* 157:89-90.
2. Buchinsky, F.J., Ma, Y., Mann, G.N., et al. (1996) T lymphocytes play a critical role in the development of cyclosporin A-induced osteopenia. *Endocrinology* 137: 2278-2285.
3. Cayco, A., Wyslowski, J., Simpson, C., et al. (2000) Posttransplant bone disease: evidence for a high bone resorption state. *Transplantation* 70: 1722-1728.
4. Christgau, M., Hiller, K.A., Schmalz, G., Kolbeck, C., Wenzel, A. (1998) Accuracy of quantitative digital subtraction radiography for determining changes in calcium mass in mandibular bone: an in vitro study. *J Periodontol* 69:138-49.
5. Duarte, P.M., Nogueira Filho, G.R., Sallum, E.A., Sallum, A.W., Nociti Junior, F.H. (2001) The effect of an immunosuppressive therapy and its withdrawal on bone healing around titanium implants. A histometric study in rabbits. *J Periodontol* 72: 1391-7.
6. Engelke, W., de Valk, S., Ruttimann, U. (1990) The diagnostic value of subtraction radiography in the assessment of granular hydroxylapatite implants. *Oral Surg Oral Med Oral Pathol* 69:636-41.
7. Faulds, D., Goa, K.L., Benfield, P. (1993) Cyclosporin. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in immunoregulatory disorders. *Drugs* 45:953-1040.
8. Gratwohl, A., Riederer, I., Graf, E., Speck, B. (1986) Cyclosporine toxicity in rabbits. *Lab Anim* 20: 213-220.

9. Horowitz, M., Vignery, A., Gershon, R.K., Baron, R. (1984) Thymus-derived lymphocytes and their interaction with macrophages are required for production of osteoclast-acting factor in mouse. *Proc Natl Acad Sci* 81: 2181-2185.
10. Jeffcoat, M.K., Reddy, M.S., van den Berg, H.R., Bertens, E. (1992) Quantitative digital subtraction radiography for the assessment of peri-implant bone change. *Clin Oral Implants Res* 3:22-7.
11. Johansson, C., Albrektsson, A. (1987) Integration of screw implants in the rabbit: a 1-year follow-up of removal torque of titanium implants. *Int J Oral Maxillofac Implants* 2:69-75.
12. Julian, B.A., Laskow, D.A., Dubovsky, J., Dubovsky, E.V., Curtis, J.J., Quarles, L.D. (1991) Rapid loss of vertebral mineral density after renal transplantation. *N Engl J Med* 325: 544-50.
13. Movsowitz, C., Epstein, S., Fallon, M., Ismail, F., Thomas, S. (1988) Cyclosporin-A in vivo procedures severe osteopenia in the rat: effect of dose and duration of administration. *Endocrinol* 123: 2571-2577.
14. Movsowitz, C., Epstein, S., Ismail, F., Fallon, M., Thomas, S. (1989) Cyclosporin A in the oophorectomized rat: unexpected severe bone resorption. *J Bone Miner Res* 4: 393-398.
15. Pepelassi, E.A., Diamanti-Kipioti, A. (1997) Selection of the most accurate method of conventional radiography for the assessment of periodontal osseous destruction. *J Clin Periodontol* 8:557-67.
16. Sakakura, C.E., Margonar, R., Holzhausen, M., Nociti Jr, F.H., Alba Jr, R.C., Marcantonio Jr, E. (2003) Influence of cyclosporin A therapy on bone healing around titanium implants: a histometric and biomechanic study in rabbits. *J Periodontol* 74: 976-81.

17. Schlosberg, M., Movsowitz, C., Epstein, S., Ismail, F., Fallon, M.D., Thomas, S. (1989) The effect of cyclosporin A administration and its withdrawal on bone mineral metabolism in the rat. *Endocrinology* 124: 2179-84.
18. Schou, S., Holmstrup, P., Jorgensen, T., Skovgaard, L.T., Stoltze, K., Hjorting-Hansen, E., Wenzel, A. (2003) Implant surface preparation in the surgical treatment of experimental peri-implantitis with autogenous bone graft and ePTFE membrane in cynomolgus monkeys. *Clin Oral Implants Res* 14: 412-22.
19. Schou, S., Holmstrup, P., Jorgensen, T., Skovgaard, L.T., Stoltze, K., Hjorting-Hansen, E., Wenzel, A. (2003) Anorganic porous bovine-derived bone mineral (Bio-Oss) and ePTFE membrane in the treatment of peri-implantitis in cynomolgus monkeys. *Clin Oral Implants Res* 14: 535-47.
20. Schou, S., Holmstrup, P., Jorgensen, T., Stoltze, K., Hjorting-Hansen, E., Wenzel, A. (2003) Autogenous bone graft and ePTFE membrane in the treatment of peri-implantitis. I. Clinical and radiographic observations in cynomolgus monkeys. *Clin Oral Implants Res* 14: 391-403.
21. Seymour, R.A., Ellis, J.S, Thomason, J.M. (2000) Risk factors for drug-induced gingival overgrowth. *J Clin Periodontol* 27: 217-23.
22. Suomi, J.D., Plumbo, J., Barbano, J.P. (1968) A comparative study of radiographs and pocket measurements in periodontal disease evaluation. *J Periodontol* 39: 311-5.
23. Vedi, S., Greer, S., Skingle, S.J., et al. (1999) Mechanism of bone loss after liver transplantation: a histomorphometric analysis. *J Bone Miner Res* 14: 281-287.
24. Wenzel, A., Sewerin, I. (1991) Sources of noise in digital subtraction radiography. *Oral Surg Oral Med Oral Pathol* 71:503-8.

25. Wenzel, A. (1989) Effect of manual compared with reference point superimposition on image quality in digital subtraction radiography. *Dentomaxillofac Radiol* 18: 145-50.



**TABLES**

Table 1 – Percentage of cortical and trabecular bone index at baseline and final periods.

Grupo	Baseline (%)		Final (%)	
	Cortical	Trabecular	Cortical	Trabecular
A	75	25	75 <sup>a</sup>	25 <sup>a</sup>
B	33.3	66.7	16.7 <sup>a</sup>	83.3 <sup>a</sup>
T	100	0	0 <sup>b</sup>	100 <sup>b</sup>

a – no significant difference ( $p > 0.05$ )

b – significant difference ( $p < 0.0001$ )

qui-square test

## FIGURES

Figure 1

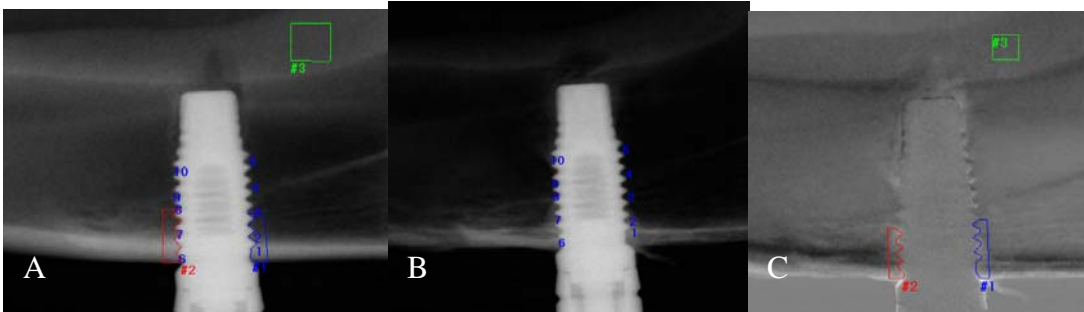


Figure 1 – A) Image of cortical bone index and the baseline digital image – references points in blue. Region of Interest (1 and 2 – blue and red) and the Region of Control (3 in green). B) Image of trabecular bone index and the final digital image - references point placed in along the implant in same position of image in A. C) The subtraction image with regions of interest (dark – means bone loss and white means gain).

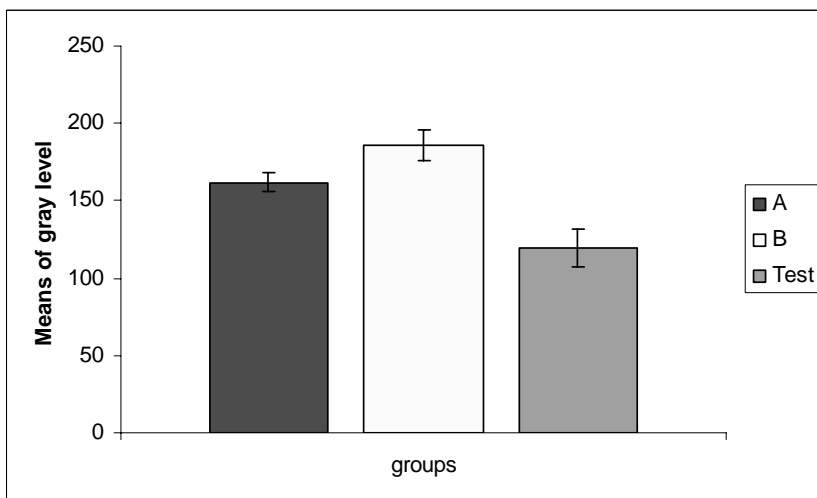


Figure 2 – Mean of gray level evaluated by quantitative digital subtraction related to bone gain.

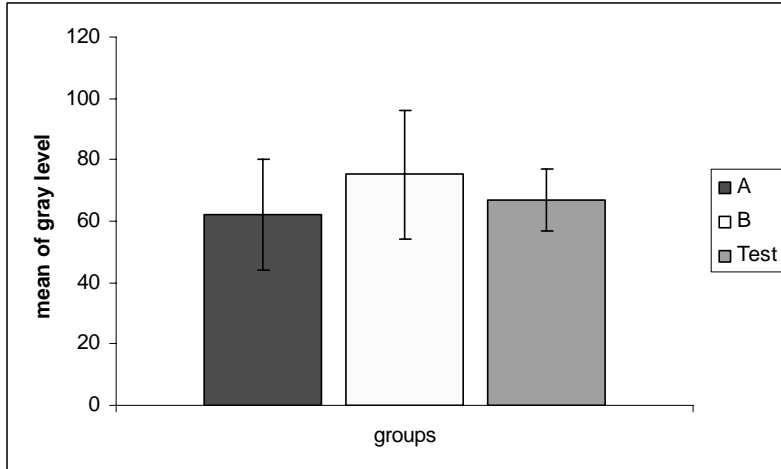


Figure 3 – Mean of gray level evaluated by quantitative digital subtraction related to bone loss.

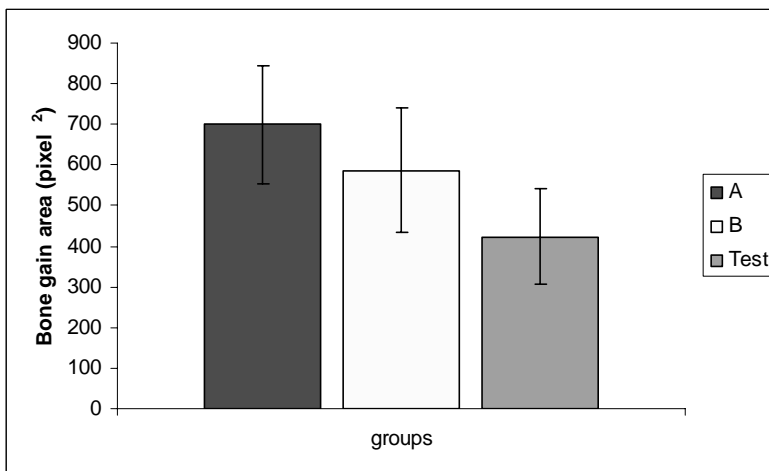


Figure 4 – Bone gain area evaluated by quantitative digital subtraction.

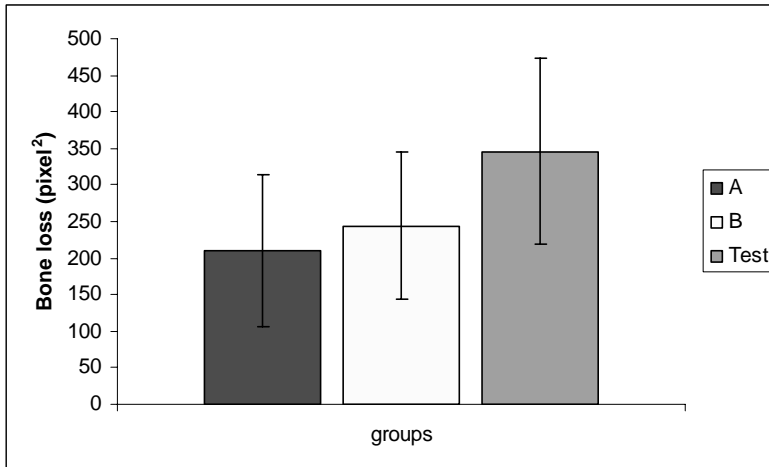


Figure 5 - Bone loss area evaluated by quantitative digital subtraction.

## DISCUSSÃO

### MODELO EXPERIMENTAL

O uso do coelho como animal experimental foi justificado pela possibilidade de utilização de implantes comerciais em um animal de fácil manejo e disponibilidade. Além disso, diversos autores e centros de pesquisas utilizam esse modelo experimental para estudar o fenômeno da osseointegração (Johansson & Albrektsson 1987; Carlsson et al. 1989; Stenport et al. 2001; Ellingsen et al. 2004).

A metáfise tibial foi o local de implantação escolhido por ser de fácil acesso e de resistência mecânica. A instalação dos implantes no meio da tíbia pode enfraquecer a estrutura óssea e resultar em fraturas quando submetidas a esforços mecânicos. A instalação dos implantes na metáfise tibial deve ser criteriosa em relação ao tipo de osso que se deseja estudar. Caso o objeto de estudo seja osso trabecular deve se instalar o implante mais próximo possível da cartilagem patelar por ser esse osso de características trabecular. No caso de osso cortical, deve se procurar uma região à cerca de três centímetros da cartilagem.

O osso cortical da metáfise tibial do coelho é caracterizado por duas finas corticais ósseas preenchidas por tecido medular. Portanto, somente a porção coronal e apical do implante ficam primariamente em contato com o leito ósseo.

Um aspecto negativo que encontramos nesse modelo animal refere-se à intolerância que alguns animais desenvolveram a CSA. Após um período de 20 a 30 dias, alguns coelhos desenvolveram uma síndrome chamada *wasting syndrome* (Gratwohl et al. 1986) caracterizada pela inanição do animal, levando a considerável perda de peso corporal. Esses animais foram removidos do estudo. Dessa maneira, o estudo envolvendo coelhos e CSA deve sempre ser realizado com um número de animais de margem de segurança

## RESULTADOS

Os achados desse estudo apontam para um efeito deletério da CSA sobre o tecido ósseo perimplantar. Os efeitos negativos foram observados tanto durante o processo de cicatrização (osseointegração) como após esse período (remodelação óssea) e puderam ser comprovados por diversos parâmetros utilizados. Os estudos III e IV demonstraram, que apesar dos implantes apresentarem osseointegração estabelecida, o tecido ósseo perimplantar apresentou alterações frente a CSA resultando em uma menor retenção mecânica e menor densidade radiográfica.

Apesar dos achados dos estudos aqui apresentados apontarem para um efeito deletério da CSA sobre a osseointegração, fica difícil extrapolar os resultados para a prática clínica. Não é possível dimensionar se a redução na osseointegração é clinicamente significativa ou se a presença de carga sobre o implante apresenta algum tipo de efeito protetor ou catalisador sobre a manutenção ou perda da osseointegração. Os resultados aqui apresentados devem ser interpretados sob a ótica de como a CSA pode interferir no processo de osseointegração.

## **CONCLUSÕES**

A administração de ciclosporina –A em doses imunossupressoras provoca efeitos negativos sobre a osseointegração.

Considerando o momento de administração de CSA:

1. Concomitante a “cicatrização” do implante provoca:
  - 1.1. Diminuição do contato osso/implante
  - 1.2. Diminuição da formação óssea dentro da espira
  - 1.3. Diminuição da retenção mecânica do implante ao osso
  
2. Após a “cicatrização” do implante provoca:
  - 2.1. Diminuição da retenção mecânica do implante ao osso
  - 2.2. Diminuição da qualidade e densidade óssea radiográfica ao redor dos implantes

**REFERÊNCIAS**

- 1.
2. Adell R, Eriksson B, Lekholm U, Branemark PI, Jemt T. Long-term follow-up study of osseointegrated implants in the treatment of totally edentulous jaws. *Int J Oral Maxillofac Implants*. 1990 Winter;5(4):347-59.
3. Adell R, Lekholm U, Rockler B, Branemark PI. A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. *Int J Oral Surg*. 1981 Dec;10(6):387-416.
4. Albrektsson T, Lekholm U. Osseointegration: current state of the art. *Dent Clin North Am*. 1989 Oct;33(4):537-54.
5. Balshi TJ, Wolfinger GJ. Dental implants in the diabetic patient: a retrospective study. *Implant Dent*. 1999;8(4):355-9.
6. Bernard JP, Szmukler-Moncler S, Pessotto S, Vazquez L, Belser UC. The anchorage of Branemark and ITI implants of different lengths. I. An experimental study in the canine mandible. *Clin Oral Implants Res*. 2003 Oct;14(5):593-600.
7. Block MS, Kent JN. Long-term follow-up on hydroxylapatite-coated cylindrical dental implants: a comparison between developmental and recent periods. *J Oral Maxillofac Surg*. 1994 Sep;52(9):937-43
8. Boltchi FE, Rees TD, Iacopino AM. Cyclosporine A-induced gingival overgrowth: A comprehensive review. *Quintessence Int* 1999;30:775-783.
9. Brånemark PI, Adell R, Breine U, Hansson BO, Lindstrom J, Ohlsson A. Intraosseous anchorage of dental prostheses. I. Experimental studies. *Scand J Plast Reconstr Surg*. 1969;3(2):81-100.



10. Brånemark PI, Hansson BO, Adell R, Breine U, Lindstrom J, Hallen O, Ohman A. Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. *Scand J Plast Reconstr Surg Suppl.* 1977;16:1-132.
11. Brånemark PI, Zarb GA, Albrektsson T. *Tissue Integrated Prosthesis: Osseointegration in clinical dentistry.* Chicago, Quintessence, 1985.
12. Buchinsky, F.J., Ma, Y., Mann, G.N., et al. (1996) T lymphocytes play a critical role in the development of cyclosporin A-induced osteopenia. *Endocrinology* 137: 2278-2285.
13. Carlsson LV, Albrektsson T, Berman C. Bone response to plasma-cleaned titanium implants. *Int J Oral Maxillofac Implants.* 1989 Fall;4(3):199-204
14. Cayco, A., Wysolmerski, J., Simpson, C., et al. Posttransplant bone disease: evidence for a high bone resorption state. *Transplantation.* 2000; 70: 1722-1728.
15. Christgau M, Hiller KA, Schmalz G, Kolbeck C, Wenzel A. Accuracy of quantitative digital subtraction radiography for determining changes in calcium mass in mandibular bone: an in vitro study. *J Periodontal Res.* 1998 Apr;33(3):138-49.
16. Christgau M, Hiller KA, Schmalz G, Kolbeck C, Wenzel A. Quantitative digital subtraction radiography for the determination of small changes in bone thickness: an in vitro study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1998 Apr;85(4):462-72.
17. Christgau M, Wenzel A, Hiller KA, Schmalz G. Quantitative digital subtraction radiography for assessment of bone density changes following periodontal guided tissue regeneration. *Dentomaxillofac Radiol.* 1996 Jan;25(1):25-33.

18. Cordioli G, Majzoub Z, Piattelli A, Scarano A. Removal torque and histomorphometric investigation of 4 different titanium surfaces: an experimental study in the rabbit tibia. *Int J Oral Maxillofac Implants*. 2000 Sep-Oct;15(5):668-74.
19. Daley TD, Wysocki GP. Cyclosporine therapy. Its significance to the periodontist. *J Periodontol*. 1984 Dec;55(12):708-12.
20. Donath K, Breuner G. A method for study of undecalcified bones and teeth with attached soft tissue. The Sage-Scliff (sawing and grinding) technique. *J Oral Pathol* 1982;11:318-326.
21. Duarte PM, Cesar Neto JB, Goncalves PF, Sallum EA, Nociti FH. Estrogen deficiency affects bone healing around titanium implants: a histometric study in rats. *Implant Dent*. 2003;12(4):340-6.
22. Duarte PM, de Vasconcelos Gurgel BC, Sallum AW, Filho GR, Sallum EA, Nociti FH Jr. Alendronate therapy may be effective in the prevention of bone loss around titanium implants inserted in estrogen-deficient rats. *J Periodontol*. 2005 Jan;76(1):107-14.
23. Ellingsen JE, Johansson CB, Wennerberg A, Holmen A. Improved retention and bone-to-implant contact with fluoride-modified titanium implants. *Int J Oral Maxillofac Implants*. 2004 Sep-Oct;19(5):659-66.
24. Esposito M, Coulthard P, Thomsen P, Worthington HV. The role of implant surface modifications, shape and material on the success of osseointegrated dental implants. A Cochrane systematic review. *Eur J Prosthodont Restor Dent*. 2005 Mar;13(1):15-31.
25. Franchi C, Cainelli G, Frigerio E, Garutti C, Altomare GF. Association of cyclosporine and 311 nM UVB in the treatment of moderate to severe forms of

- psoriasis: a new strategic approach. *Int J Immunopathol Pharmacol*. 2004 Sep-Dec;17(3):401-6.
26. Fu E, Hsieh Y-D, Nieh S, Wikesjö, Liu D. Effects of cyclosporin A on Alveolar bone: an experimental study in the rat. *J Periodontol* 1999; 70:189-194.
27. Fujimoto T, Niimi A, Sawai T, Ueda M. Effects of steroid-induced osteoporosis on osseointegration of titanium implants. *Int J Oral Maxillofac Implants*. 1998 Mar-Apr;13(2):183-9.
28. Gratwohl A, Riederer I, Graf E, Speck B. Cyclosporine toxicity in rabbits. *Lab Anim* 1986; 20: 213-220.
29. Grondahl K, Grondahl HG, Webber RL. Digital subtraction radiography for diagnosis of periodontal bone lesions with simulated high-speed systems. *Oral Surg Oral Med Oral Pathol*. 1983 Mar;55(3):313-8.
30. Guslandi M, Tittobello A. Cyclosporin for Crohn's disease? *Drugs*. 1992 Apr;43(4):440-2.
31. Hausmann E, Christersson L, Dunford R, Wikesjo U, Phyto J, Genco RJ. Usefulness of subtraction radiography in the evaluation of periodontal therapy. *J Periodontol*. 1985 Nov;56(11 Suppl):4-7.
32. Horowitz M, Vignery A, Gershon RK, Baron R. Thymus-derived lymphocytes and their interaction with macrophages are required for production of osteoclast-activating factor in mouse. *Proc Natl Acad Sci (USA)* 1984;81:2181-2185.
33. Jeffcoat MK, Reddy MS, van den Berg HR, Bertens E. Quantitative digital subtraction radiography for the assessment of peri-implant bone change. *Clin Oral Implants Res*. 1992 Mar;3(1):22-7.

34. Jemt T, Lekholm U, Adell R. Osseointegrated implants in the treatment of partially edentulous patients: a preliminary study on 876 consecutively placed fixtures. *Int J Oral Maxillofac Implants*. 1989 Fall;4(3):211-7.
35. Johansson C, Albrektsson T. Integration of screw implants in the rabbit: A 1-year follow-up of removal torque of titanium implants. *Int J Oral Maxillofac Implants* 1987;2:69-75.
36. Johnsson AA, Sawaii T, Jacobsson M, Granstrom G, Turesson I. A histomorphometric and biomechanical study of the effect of delayed titanium implant placement in irradiated rabbit bone. *Clin Implant Dent Relat Res*. 2000;2(1):42-9.
37. Julian, B.A., Laskow, D.A., Dubovsky, J., Dubovsky, E.V., Curtis, J.J., Quarles, L.D. (1991) Rapid loss of vertebral mineral density after renal transplantation. *N Engl J Med* 325: 544-50.
38. Kanis JA. The menopause and the skeleton: key issues. *Baillieres Clin Obstet Gynaecol*. 1996 Sep;10(3):469-81.
39. Katz I, Li M, Joffe I, et al. Influence of age on cyclosporine A-induced alterations in bone mineral metabolism in the rat in vivo. *J Bone Miner Res* 1994; 9: 59-67.
40. Klaushofer K, Hoffmann O, Stewart PJ, et al. Cyclosporine A inhibits bone resorption in cultured neonatal mouse calvaria. *J Pharmacol Exp Ther* 1987;243:584-590.
41. Klein L, Lemel MS, Wolfe MS, Shaffer J. Cyclosporin A does not affect the absolute rate of cortical bone resorption at the organ level in the growing rat. *Calcif TissueInt* 1994;55:295-301.

42. Krejci CB. Osteoporosis and periodontal disease: is there a relationship? *J West Soc Periodontol Periodontal Abstr.* 1996;44(2):37-42.
43. Lazzara R, Siddiqui AA, Binon P, Feldman SA, Weiner R, Phillips R, Gonshor A. Retrospective multicenter analysis of 3i endosseous dental implants placed over a five-year period. *Clin Oral Implants Res.* 1996 Mar;7(1):73-83.
44. Li D, Ferguson SJ, Beutler T, Cochran DL, Sittig C, Hirt HP, Buser D. Biomechanical comparison of the sandblasted and acid-etched and the machined and acid-etched titanium surface for dental implants. *J Biomed Mater Res.* 2002 May;60(2):325-32.
45. Margonar R, Sakakura CE, Holzhausen M, Pepato MT, Alba RC, Marcantonio E. The influence of diabetes mellitus and insulin therapy on biomechanical retention around dental implants: a study in rabbits. *Implant Dent.* 2003;12(4):333-9.
46. Moreland LW, Reddy MS, Koopman WJ, Webber RL, Alarcon GS, Jeffcoat MK. Digital subtraction radiography for the assessment of bone changes in rheumatoid arthritis. *J Rheumatol.* 1992 Nov;19(11):1697-703.
47. Movsowitz, C., Epstein, S., Fallon, M., Ismail, F., Thomas, S. (1988) Cyclosporin-A in vivo procedures severe osteopenia in the rat: effect of dose and duration of administration. *Endocrinol* 123: 2571-2577.
48. Movsowitz, C., Epstein, S., Ismail, F., Fallon, M., Thomas, S. (1989) Cyclosporin A in the oophorectomized rat: unexpected severe bone resorption. *J Bone Miner Res* 4: 393-398.
49. Narai S, Nagahata S. Effects of alendronate on the removal torque of implants in rats with induced osteoporosis. *Int J Oral Maxillofac Implants.* 2003 Mar-Apr;18(2):218-23.

50. Nassar CA, Nassar PO, Abi Rached RS, Holzhausen M, Marcantonio E Jr, Spolidorio LC. Effect of cyclosporin A on alveolar bone homeostasis in a rat periodontitis model. *J Periodontal Res.* 2004 Jun;39(3):143-8.
51. Nery EB, Olson JW, Henkin JM, Kalbfleisch JH. Film-holder device for radiographic assessment of periodontal tissues. *J Periodontal Res.* 1985 Jan;20(1):97-105.
52. Nociti FH Jr, Stefani CM, Sallum EA, Duarte PM, Sallum AW. Nicotine and bone density around titanium implants: a histometric study in rabbits. *Implant Dent.* 2002;11(2):176-82.
53. Nummikoski PV, Martinez TS, Matteson SR, McDavid WD, Dove SB. Digital subtraction radiography in artificial recurrent caries detection. *Dentomaxillofac Radiol.* 1992 May;21(2):59-64.
54. Pilatti GL, Sampaio JE. The influence of chlorhexidine on the severity of cyclosporin A-induced gingival overgrowth. *J Periodontol.* 1997 Sep;68(9):900-4. Erratum in: *J Periodontol* 1998 Jan;69(1):102.
55. Pozzilli P, Visalli N, Buzzetti R, Baroni MG, Boccuni ML, Fioriti E, Signore A, Mesturino C, Valente L, Cavallo MG, et al. Adjuvant therapy in recent onset type 1 diabetes at diagnosis and insulin requirement after 2 years. *Diabete Metab.* 1995 Feb;21(1):47-9.
56. Rezende, M L R. *Reações a curto prazo do tecido ósseo da tíbia de coelhos a implantação de parafusos de titânio comercialmente puro.* 1991. 152f (Doutorado em PeriodontiaA) Faculdade de Odontologia de Bauru - Universidade de São Paulo.
57. Rudolph DJ, White SC. Film-holding instruments for intraoral subtraction radiography. *Oral Surg Oral Med Oral Pathol.* 1988 Jun;65(6):767-72.

58. Sakakura, C.E., Margonar, R., Holzhausen, M., Nociti Jr, F.H., Alba Jr, R.C., Marcantonio Jr, E. (2003) Influence of cyclosporin A therapy on bone healing around titanium implants: a histometric and biomechanic study in rabbits. *J Periodontol* 74: 976-81.
59. Sasagawa K, Fushibayashi S, Okano K, et al. Different inhibitory actions of immunomodulating agents and immunosuppressive agents on bone resorption of mouse calvaria. *Int J Immunopharmac* 1989;11:953-959.
60. Schlosberg M, Movsowitz C, Epstein S, Ismail F, Fallon M, Thomas S. The effect of cyclosporin A administration and its withdrawal on bone mineral metabolism in the rat. *Endocrinol* 1989;124:2179-2184.
61. Schou S, Holmstrup P, Jorgensen T, Stoltze K, Hjorting-Hansen E, Wenzel A. Autogenous bone graft and ePTFE membrane in the treatment of peri-implantitis. I. Clinical and radiographic observations in cynomolgus monkeys. *Clin Oral Implants Res.* 2003 Aug;14(4):391-403.
62. Schropp L, Wenzel A, Kostopoulos L, Karring T. Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. *Int J Periodontics Restorative Dent.* 2003 Aug;23(4):313-23.
63. Seymour RA, Jacobs DJ. Cyclosporin and the gingival tissues. *J Clin Periodontol.* 1992 Jan;19(1):1-11.
64. Spolidorio LC, Spolidorio DM, Nassar PO, Nassar CA, Holzhausen M, Almeida OP. Influence of age on combined effects of cyclosporin and nifedipine on rat alveolar bone. *J Periodontol.* 2004 Feb;75(2):268-72.

65. Stenport VF, Olsson B, Morberg P, Tornell J, Johansson CB. Systemically administered human growth hormone improves initial implant stability: an experimental study in the rabbit. *Clin Implant Dent Relat Res*. 2001;3(3):135-41.
66. Stewart PJ, Green OC, Stern PH. Cyclosporine A inhibits calcemia hormone-induced bone resorption in vitro. *J Bone Miner Res* 1986;1:285-291.
67. Sul YT, Johansson CB, Albrektsson T. Oxidized titanium screws coated with calcium ions and their performance in rabbit bone. *Int J Oral Maxillofac Implants*. 2002 Sep-Oct;17(5):625-34.
68. van Steenberghe D. The use of oral implants in compromised patients. *Periodontol* 2000. 2003;33:9-11.
69. Webber RL, Ruttimann UE, Grondahl HG. X-ray image subtraction as a basis for assessment of periodontal changes. *J Periodontal Res*. 1982 Sep;17(5):509-11.
70. Wenzel, A. (1989) Effect of manual compared with reference point superimposition on image quality in digital subtraction radiography. *Dentomaxillofac Radiol* 18: 145-50.
71. Wenzel, A., Sewerin, I. Sources of noise in digital subtraction radiography. *Oral Surg Oral Med Oral Pathol* (1991) 71:503-8.
72. Zarb GA, Schmitt A. The longitudinal clinical effectiveness of osseointegrated dental implants in posterior partially edentulous patients. *Int J Prosthodont*. 1993 Mar-Apr;6(2):189-96.
73. Zarb GA, Schmitt A. The longitudinal clinical effectiveness of osseointegrated dental implants for single-tooth replacement. *Int J Prosthodont*. 1993 Mar-Apr;6(2):197-202.



74. Zocher R, Nihira T, Paul E, Madry N, Peeters H, Kleinkauf H, Keller U. Biosynthesis of cyclosporin A: partial purification and properties of a multifunctional enzyme from *Tolypocladium inflatum*. *Biochemistry*. 1986 Feb 11;25(3):550-3.

SAKAKURA, C.E. The influence of cyclosporin-A on osseointegration. 2005. 104 f. Tese (Doutorado em Periodontia) – Faculdade de Odontologia de Araraquara, Universidade Estadual Paulista, Araraquara, 2005.

#### **ABSTRACT**

Immunosuppressive agents may induce severe changes on bone mineral metabolism resulting in osteopenia. These alterations may impair the osseointegration processes. The purposes of this study were evaluate the cyclosporin-A (CSA) influence: on bone healing around dental implants (Study I); on bone density around dental implants (Study II); on mechanical retention of dental implants integrated to the bone (Study III) and on radiographic bone density and quality around dental implants integrated to the bone (Study IV). The CSA administration may impair the osseointegration and bone density during the bone healing. Besides, the administration of CSA after bone healing may impair the dental implant mechanical retention and decrease the radiographic bone density and bone quality around dental implants integrated to the bone.

**KEY WORDS:** Osseointegration, cyclosporin-A, subtraction radiography, digital radiography