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INFLUÊNCIA DE DIFERENTES MACRO- E MICROESTRUTURAS DE IMPLANTES OSSEOINTEGRÁVEIS SOBRE OS TECIDOS PERI-IMPLANTARES HUMANOS

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Orientador: Prof. Dr. Jamil Awad Shibli
Co-Orientador: Prof. Dr. Marcelo Faveri

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RESUMO

A alta taxa de sucesso obtida com as restaurações implanto-suportadas tem melhorado a sua aplicabilidade na rotina da prática clínica diária. Esta percentagem elevada de sucesso é embasada nos dados pré-clínicos anteriores obtidos a partir de estudos em animais e estudos *in vitro* que avaliaram o impacto das topografias de superfícies dos implantes no tecido ósseo. No entanto, estudos que avaliaram a histológia do tecido ósseo humano ainda são bastante escassos. Portanto, o objetivo desta revisão de literatura é descrever o panorama dos trabalhos que apresentaram dados de contato osso-implante (BIC) de implantes recuperados de maxilares humanos. Alguns aspectos sobre a topografia das superfícies dos implantes, bem como as condições sistêmicas, incluindo a osteoporose e o hábito de fumar, foram relatados, sugerindo que, a obtenção de dados do tecido ósseo humano é bastante valiosa para a melhor compreensão do processo de integração óssea. Este artigo também destaca a dificuldade de interpretação dos dados obtidos de estudos em seres humanos, devido à falta de informação detalhada sobre as superfícies dos implantes recuperados. Sem a definição das características da superfície, é difícil relacionar exatamente os padrões de superfície com as observações clínicas específicas, e portanto, as observações permanecem incompletas. Como conclusão, os dados de implantes recuperados dos maxilares humanos são muito importantes para a nossa compreensão, mas os estudos ainda são escassos e os dados fragmentados. Novos estudos ainda são necessários para que esta importante abordagem possa ser melhorada, completa e melhor desenvolvida.

Palavras-chave: Implantes dentários. Osseointegração. Propriedades de superfícies. Titânio.

ABSTRACT

The high success range obtained with implant-supported restorations has improved its applicability on routine of the daily clinical practice. This elevated percentage of success in based on the previous pre-clinical data obtained from animal and in vitro studies that evaluated the impact of implant surface topographies on bone tissue. However, the histologic evaluation on human bone tissue is scarce. Therefore, the aim of this review is depicts an actual panorama of the data presenting on bone-to-implant contact (BIC) of retrieved implants from human jaws. Some aspects of implant surface topography as well as systemic conditions as osteoporosis and smoking habit were demonstrated, suggesting that, the data obtained from human bone tissue is still valuable for the better understanding of the osseointegration process. This article also highlighted that most data in humans are difficult to interpret, due to the lack of detailed information about the surfaces found in retrieved implants. Without the definition of the surface characteristics, it is difficult to link exactly surface patterns to specific clinical observations, and all observations remain incomplete. As a conclusion, data from implants retrieved from human jaws are very important for our understanding, but studies remain scarce and data is fragmentary. This important approach should be improved, completed and developed in the future.

Keywords. Dental implant. Osseointegration. Surface properties. Titanium.

SUMÁRIO

1 INTRODUÇÃO JUSTIFICADA

Baseado nos conceitos clássicos de Bränemark et al. (1977) e Bränemark (1983) sobre a osseointegração, a utilização de implantes osseointegrados na reabilitação de indivíduos parcialmente ou totalmente desdentados tem aumentado consideravelmente nos últimos anos devido aos altos índices de sucesso (Roos-Jänsaker et al., 2006; Jacobs et al., 2010). A definição de osseointegração ou anquilose funcional considerava o percentual de contato entre o tecido ósseo e a superfície do implante sob função, quando visto em microscopia óptica normal (BIC). Entretanto, quando em microscopia eletrônica de varredura, observa-se a interposição de uma fina camada de glicoproteínas entre o tecido ósseo e o implante. Logo, a unidade funcional e estrutural de toda a Implantodontia foi alicerçada sob o conceito do contato osso-implante e consequentemente de todos os eventos e fatores que influenciam este processo.

Nos últimos 20 anos, fatores relacionados ao implante dental osseointegrado (forma do implante, tipo e composição química da superfície) e ao hospedeiro (fatores locais e sistêmicos) foram avaliados visando aprimorar e desenvolver tecnologias para as macro- e microestrturas (Mendonça et al. 2008; Coelho et al. 2009). Dentre estes fatores, a superfície dos implantes ou microestrutura é o fator que mais recebeu atenção (Wenneberg; Albrektsson, 2009). A microestrutura do implante influencia a quantidade e qualidade do contato osso-implante e consequentemente, aumenta o sucesso a longo prazo dos implantes osseointegrados, uma vez que, teoricamente, quanto melhor o contato osso-implante, maior será a longevidade destes.

A característica da superfície do implante influenciam o complexo processo de osseointegração em diferentes modos. Estudos prévios (Kasemo 1983; Johansson, Albrektsson, 1991) mostraram que a pragmática biocompatibilidade do titânio (Mendonça et al. 2008) apresentava vantagens quando comparadas a outros materiais, como por exemplo a baixa reação inflamatória entre as células adjacentes ao titânio comercialmente puro (Ticp) durante o processo de osseointegração (Suska et al. 2005). Entretanto, estudos clínicos apresentavam falhas no processo de osseointegração (Esposito et al. 1998; Esposito et al. 2007). Estas falhas foram classificadas como precoces e tardias. As precoces foram relacionadas a problemas

como falta de biocompatibilidade da superfície do implante, infecção do leito receptor, trauma cirúrgico ou qualquer outro evento que impossibilitasse a osseointegração do implante dental antes que o mesmo recebesse carga ou esforço mastigatório. As falhas tardias foram relacionadas as infecções peri-implantares associadas ou não a sobrecarga oclusal (Shibli et al. 2003; Shibli et al. 2008).

Embora estas falhas na osseointegração fossem associadas a vários fatores, a principal falha era associada a deficiência na formação do tecido ósseo peri-implantar (Mendonça et al. 2008) principalmente, em áreas de osso tipo IV (Jaffin, Berman, 1991; Friberg et al.,1991; Bay; Moy, 1993; Shibli et al. 2007; Cosyn et al. 2012) e em pacientes fumantes (Aglietta et al. 2011; Yamano et al. 2010).

Não obstante, outros estudos (Tarnow et al. 1997; Piattelli et al. 1997; Balshi; Wolfinger, 1997) quase que simultâneos, avaliavam a ativação imediata e precoce de implantes osseointegrados de superfícies lisas e tratadas resultando na diminuição entre o tempo de cicatrização / osseointegração e a ativação dos implantes osseointegráveis. Juntamente com avaliações clínicas (Degidi et al., 2010) e histológicas (Degidi et al., 2009) estes estudos apresentavam dados muito promissores sobre a ativação e carregamento imediato dos implantes, com taxas de sucesso comparáveis as dos implantes carregados após período de 4 a 6 meses propostos por Adell et al. (1981) e Albrektsson et al. (1981). Estes prazos (4 meses na mandíbula e 6 meses de cicatrização na maxila) foram propostos para assegurar que os implantes osseointegrados de Ticp estivessem aptos a receber esforços mastigatórios. Estes implantes osseointegrados apenas suportariam tais esforços mastigatórios caso possuíssem ao menos 50% de osseointegração (Trisi et al., 2003; Shibli et al., 2010). Contudo, várias revisões sistemáticas (Junker et al., 2009; Wenneberg, Albrektsson, 2009; Harvey et al., 2010) têm proposto que implantes osseointegrados que apresentam superfícies tratadas ou texturizadas, podem receber carga mastigatória em um período menor com taxas de sucesso maior quando comparadas as superfícies Ticp.

Neste contexto, a Implantodontia foi sendo implementada na prática clínica, encorajando novas considerações relacionadas a topografias de implantes tanto em estudos *in vitro* quanto *in vivo* (Cooper, 2000; Shalabi et al., 2006).

1.1. Influência de diferentes superfícies de implantes

Estudos clínicos (Pinholt, 2003) e histológicos (Trisi et al. 2003, Ivanoff et al. 2003) têm proposto que implantes osseointegrados que apresentam superfícies tratadas ou texturizadas, podem receber carga mastigatória em um período menor ao preconizado anteriormente por Adell et al. (1981) e Albrektsson et al. (1981). Esses estudos clínicos e laboratoriais avaliaram o percentual de osseointegração, buscando aperfeiçoar o tipo de superfície ou microestrutura dos implantes.

O percentual de osseointegração depende além do tipo de microestrutura, da técnica cirúrgica, condições sistêmicas do indivíduo, disponibilidade e qualidade do tecido ósseo (Shalabi et al. 2006). A disponibilidade óssea é reduzida após a perda do elemento dental e reabsorção do tecido ósseo alveolar, principalmente em regiões posteriores de maxila (Friberg et al.,1991). A taxa de contato osso-implante é reportada entre 25 e 65% para superfície de Ticp e de 45 a 70% para as superfícies de titânio tratadas, tanto para estudos em animais (Coelho et al., 2009; Buser et al., 1999; Buser et al., 1991), quanto em humanos (Shibli et al., 2007; Ivanoff et al., 2003). Tais investigações mostraram ainda que implantes dentais osseointegráveis de superfícies lisas, colocados em osso tipo IV (região posterior da maxila e áreas enxertadas) apresentam altos índices de perda comparados a outras áreas de melhor densidade óssea (Linquist et al., 1997; Quirynen et al., 1991). Consequentemente, a modificação ou texturização da superfície de implante pode facilitar a cicatrização com o aumento da porcentagem do contato osso-implante em áreas de tecido ósseo pobre (Ivanoff et al., 2001; Shibli et al., 2007; Grassi et al., 2006).

As qualidades biológicas do implante dental dependem das propriedades químicas, físicas, mecânicas e topográficas da superfície. Essas diferentes propriedades interagem entre si, influenciando a atividade celular ao redor da superfície de implante (Junker et al., 2009). A partir desses dados, vários estudos têm investigado diferentes superfícies de implante, obtidas por meio de técnicas de adição (recobertas com plasma de titânio, hidroxiapatita) ou subtração (jateamento com diferentes tipos de materiais como óxido de titânio ou alumínio, tratadas com ácidos, e preparadas com laser (Weenerberg; Albrektsson, 2009).

As propriedades destas novas superfícies influenciam as células ósseas que migram e proliferam da loja cirúrgica realizada para a inserção do implante apresentando melhores taxas de contato osso-implante, devido ao aumento da área

de contato da superfície do implante (Coelho et al. 2009; Shalabi et al. 2006). Complementarmente, essa rugosidade de superfície fornece uma configuração que melhora a retenção do coágulo sangüíneo, estimula e facilita o processo de osseointegração e consequentemente permite que estes implantes possam ser submetidos à carga protética após um tempo de reparo menor (Mangano et al., 2009; Trisi et al., 2003).

1.2. Estudos histológicos em humanos

A utilização de modelos animais para testar a influência desses tipos de superfícies apresentam algumas limitações inerentes a cada modelo experimental, tais como tipo de oclusão, tempo de cicatrização e reparo celular do tecido ósseo, diferenças nas adsorções tanto dos componentes celulares e das proteínas, além de, muitas vezes, evidenciar apenas o resultado do evento biológico sem, no entanto, ser reprodutível em seres humanos (Lundgren et al., 1999; Ivanoff et al., 2001; Ivanoff et al., 2003; Shibli et al., 2007). Por esse motivo, alguns autores têm proposto a avaliação do porcentual de osseointegração em maxilares humanos utilizando implantes osseointegráveis com as mesmas superfícies disponibilizadas comercialmente, mas com dimensões reduzidas (Lundgren et al., 1999; Ivanoff et al., 2001; Ivanoff et al., 2003; Trisi et al., 2003; Rocci et al., 2003; Kohal et al., 2003).

Embora escassos, alguns estudos histológicos investigaram de maneira sistemática o processo de osseointegração nos maxilares humanos. Em dois estudos diferentes, Ivanoff et al. (2001) e Ivanoff et al. (2003) investigaram o efeito de diferentes superfícies sobre os maxilares humanos. Ivanoff et al. (2001) avaliaram histologicamente a osseointegração, utilizando micro-implantes com superfície de titânio jateada com óxido de titânio (TiO_2) e superfície Ticp. Vinte e sete pacientes receberam dois micro-implantes cada, um teste (TiO_2) e um controle (Ticp). Os micro-implantes foram retirados após um período de cicatrização média de 6,3 meses para a maxila e 3,9 para a mandíbula. A avaliação histomorfométrica apresentou um contato osso-implante significativamente maior para os implantes jateados, tanto na mandíbula quanto na maxila.

Valendo-se da mesma metodologia, Ivanoff et al. (2003) avaliaram histologicamente a resposta óssea frente ao micro-implante com superfície anodizada e lisa em humanos. Após 6,6 meses na maxila e 3,5 na mandíbula, os micro-implantes foram retirados e avaliados histomorfometricamente. A superfície

anodizada apresentou um maior percentual de osseointegração e preenchimento ósseo entre espiras do implante.

Trisi et al. (2003), avaliaram a influência de duas diferentes superfícies preparadas no mesmo micro-implante, inseridos em osso medular. Cada micro-implante foi inserido na região posterior da maxila de 11 pacientes parcialmente desdentados e removidos após um período de 60 dias. Utilizando luz polarizada e microscopia ótica convencional, os autores observaram um percentual de contato osso-implante de 19,00%±14,68% e 47,81%±14,01% para a superfície lisa e jateada/tratada por ácidos, respectivamente. Complementarmente, utilizando a técnica de superposição de imagens, os autores ainda observaram que a superfície lisa apresentou uma diminuição de contato osso-implante de 44,7%.

Orsini et al. (2007), avaliaram a influencia da superfície de implantes recobertas com cálcio-fostato (CaP) em uma escala nanométrica na qual estas partículas eram visualizadas ao microscópio eletrônico de varredura no aumento de 50 mil vezes. Os micro-implantes foram divididos em grupo controle (composto por micro-implantes de superfície jateada e tratada por ácidos) e grupo teste (consistiu de implantes com superfície de nanotopografia). Quinze pacientes receberam os micro-implantes na região posterior de maxila, sendo que nove pacientes receberam ambos os grupos, cinco pacientes receberam apenas um dos grupos e um paciente recebeu 4 micro-implantes (2 de cada grupo), totalizando 32 micro-implantes (16 do grupo teste e 16 do grupo controle). Os implantes foram removidos após 8 semanas de cicatrização e avaliados histomorfometricamente. O percentual de contato osso-implante variou entre 0 a 65,0% para os micro-implantes, sendo que a média foi de 19,0% e 32,2% para os grupos controle e teste respectivamente, sugerindo que a superfície incorporada com CaP poderia reduzir o tempo de cicatrização e melhorar os índices de sucesso em carregamentos oclusais precoces.

Lang et al. (2011), avaliaram os estágios iniciais de cicatrização (aos 7, 14, 21, 28 e 42 dias) de micro-implantes com superfícies hidrofílicas e hidrofóbicas. Diferentemente dos estudos anteriormente citados nos quais os implantes foram inseridos em espaços edentulos, os autores inseriram os micro-implantes em regiões retromolares de 28 voluntários (estudantes e funcionários da instituição). Em 21 pacientes, a inserção dos micro-implantes foi bilateral (um micro-implante de cada grupo). Após remoção dos implantes nos diferentes períodos, a avaliação histométrica foi realizada em apenas 30 dos 49 micro-implantes inseridos. Os micro-

implantes removidos nos períodos iniciais apresentaram dificuldades para análise, uma vez que muitas vezes ainda não havia a formação óssea que possibilitasse uma análise histométrica sendo realizada apenas análise histológica. O percentual do contato osso-implante aumentou gradativamente para ambas as superfícies, embora apenas aos 28 dias, a superfície hidrofílica apresentou diferenças significativamente maiores que o grupo hidrofóbico (p<0,05), sendo que aos 42 dias, ambas apresentavam os mesmos 62% de contato osso-implante (p>0,05). Embora o objetivo principal fosse avaliar o contato osso implante, os autores concluíram também que a taxa de osseointegração em humanos era mais lenta quando comparada ao mesmo estudo realizado em modelo animal.

1.3. Tabagismo

Além dos fatores relacionados aos implantes osseointegrados, principalmente a microestrutura, os inerentes ao hospedeiro também podem influenciar a taxa de osseointegração e consequentemente o sucesso a longo prazo das restaurações implanto-suportadas (DeLuca et al., 2006). Fatores como diabetes, tabagismo e osteoporose têm sido extensivamente avaliados por vários pesquisadores utilizando modelos histológicos em animais e alguns estudos clínicos em humanos. Estes fatores têm em comum, a influência sobre o processo de reparo do tecido ósseo.

O tabagismo tem sido relacionado como fator de risco para a doença periodontal e para a doença peri-implantar (Bain, 2003). Outros estudos tem ainda correlacionado o tabagismo com a perda precoce dos implantes, aumento da perda óssea marginal e problemas de cicatrização do tecido mole (Schwartz-Arad et al., 2002). Alguns estudos avaliando a cicatrização de alvéolos utilizando modelos animais (César-Neto et al., 2005; Correa et al., 2009) e em humanos (Saldanha et al., 2006) mostraram o efeito deletério da inalação da fumaça de cigarro e da injeção de nicotina e cotinina sobre a aposição de tecido ósseo a superfície do implante. Estes estudos mostram que o cigarro influencia a cicatrização do tecido ósseo peri-implantar tanto pelo efeito local (calor e fumaça) quanto pelo efeito sistêmico da inalação de todas as substâncias tóxicas presente no cigarro, reduzindo o contato osso-implante e aumentando a taxa de perda de implantes antes da conexão da prótese (falhas precoces). A fumaça do cigarro é composta por mais de 4.000 substâncias tóxicas

que atuam diretamente no organismo. Substâncias como nicotina, monóxido de carbono, benzeno, aldeídos e cianetos que possuem efeitos deletérios sobre a cicatrização e eventos celulares relacionados a aposição e *turnover* celular ósseo (W-Dahl et al. 2004). A nicotina é um potente vasoconstritor que reduz o fluxo sanguíneo e o de nutrientes ao sitio cirúrgico, além de inibir a proliferação de fibroblastos, macrófagos e células sanguíneas. O monóxido de carbono diminui a capacidade das células sanguíneas de transportar oxigênio, aumentando consequentemente a quantidade de cianeto e levando a hipóxia tecidual.

Complementarmente, a formação e regeneração óssea é estreitamente relacionada a angiogênese, pela invasão de vasos e artérias ao sitio cirúrgico em cicatrização. A influência negativa do cigarro sobre o processo de angiogênese, desenvolvimento dos leucócitos e sobre os níveis e funções de algumas citocinas, como por exemplo o osteoprotegerina, que reduzida, diminui a taxa de aposição óssea e que em parte, poderiam justificar os resultados obtidos em estudos clínicos que demonstram os baixos índices de sucesso assim como maior perda óssea marginal peri-implantar em pacientes fumantes (Schwartz-Arad et al., 2002; DeLuca et al., 2006).

2 PROPOSIÇÃO

O objetivo geral deste estudo foi avaliar o impacto de diferentes superficies de implantes e macroestruturas sobre o tecido humano após 60 dias de cicatrização. Especificamente, os objetivos foram:

1) Avaliar o efeito da superfície tratada com duplo ataque de combinação de diferentes ácidos (H2SO4,H3PO4, HCl, and HF) – Mangano *et al*. Early Bone Response to Dual Acid-Etched and Machined Dental Implants Placed in the Posterior Maxilla: A Histologic and Histomorphometric Human Study. Implant Dent. 2017 Feb;26(1):24-29. doi: 10.1097/ID.0000000000000511.

2) Revisar a literature que avaliou o comportamento de dos efeitos de diferentes superficies e diferentes condições sistemicas e locais - Shibli et al. Impact of Different Implant Surfaces Topographies on Peri-Implant Tissues: An Update of Current Available Data on Dental Implants Retrieved from Human Jaws. Curr Pharm Biotechnol. 2017;18(1):76-84. doi: 10.2174/1389201017666161221120618.

3) Efeito da nanosuperfície com calcio sobre os tecidos humanos Mangano *et al*. Early bone formation around immediately loaded implants with nanostructured calcium-incorporated and machined surface: a randomized, controlled histologic and histomorphometric study in the human posterior maxilla. Clin Oral Investig. 2017 Nov;21(8):2603-2611. doi: 10.1007/s00784-017-2061-y.

4) Impacto de diferentes macroestruturas sobre o tecido ósseo humano - Mangano *et al*. Early Bone Formation around Immediately Loaded Transitional Implants Inserted in the Human Posterior Maxilla: The Effects of Fixture Design and Surface. Biomed Res Int. 2017;2017:4152506. doi: 10.1155/2017/4152506. Epub 2017 Feb 9. PMID: 28280731; PMCID: PMC5322419.

Early Bone Response to Dual Acid-Etched and Machined Dental Implants Placed in the Posterior Maxilla: A Histologic and Histomorphometric Human Study

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Purpose: *To compare the early bone response to implants with dual acid-etched (DAE) and machined (MA) surface, when placed in the posterior human maxilla.*

Materials and Methods: *Fourteen patients received 2 implants in the posterior maxilla: 1 DAE and 1 MA. After 2 months, the implants were retrieved for histologic/histomorphometric evaluation. The bone-to-implant contact (BIC%), bone density in the threaded area (BDTA %), and the bone density (BD%) were calculated. The Wilcoxon matched-pairs signed rank test was used to evaluate differences (BIC%, BDTA%, and BD%) between the surfaces.*

Results: *In the MA implants, a mean (±SD) BIC%, BDTA%, and BD% of 21.76 (±12.79), 28.58 (±16.91), and 21.54 (±11.67), respectively, was reported. In the DAE implants, a mean (±SD) BIC %, BDTA%, and BD% of 37.49 (±29.51), 30.59 (±21.78), and 31.60 (±18.06), respectively, was reported. Although the mean BIC% of DAE implants value was almost double than that of MA implants, no significant differences were found between the 2 groups with regard to BIC% (P = 0.198) and with regard to BDTA% (P = 0.778) and BD% (P = 0.124).*

Conclusions: *The DAE surface increased the periimplant endosseous healing properties in the native bone of the posterior maxilla. (Implant Dent 2017;26:24–29)*

Key Words: bone healing, histology, histomorphometry, humans

Nowadays, dental implants represent a predictable and effective solution for the rehabilitation of partially and totally edentulous patients, with satisfactory high survival and success rates, as confirmed by several clinical studies in the medium and long term.[1–3] However, the survival and success rates of implants placed in areas of poor bone quality, such as the posterior maxilla, are still lower than those of implants placed in the anterior areas of the maxilla, or in the mandible, where the bone density is higher.[4,5]

The demand for improved dental implant survival at sites of lower bone density, such as the posterior maxilla, has stimulated researchers to introduce implant design alterations and therefore surface modifications, to increase the early bone response and accelerate osseointegration.[5,6]

In fact, the implant surface is the first part of the biomedical device to interact with the host: body fluids and cells interact with the implant surface, and micrometer-scale features (such as cavities, grooves, ridges, and wells) play an important role in determining molecular and cellular responses.[6,7] Accordingly, in the last years, a variety of rough-surfaced implants have been introduced in the market.[8–10]

Acid-etching and sandblasting are 2 of the most commonly used methods for the preparation of rough implant surfaces.[11–13]

In the acid-etching procedure, dental implants are immersed in acidic solutions; the result obtained, namely the erosion of the surface with formation of peaks and cavities of various dimensions, depends on the concentration of the acidic solutions, the immersion time, and the temperature.[14] In general, acid-etched surfaces are obtained by combined

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treatment with strong acids, such as hydrochloric acid (HCl) and sulfuric acid (H_2SO_4), or with hydrofluoric acid (HF) and nitric acid (HNO_3).[15]

Dual acid-etched (DAE) surface implants are a good example of the application of these treatments, and the clinical application of these implants has been extensively documented, with high survival and success rates.[16–18] DAE surfaces promote the organization of fibrin clot and the adhesion of platelets in the early healing phases.[19] In several animal studies, DAE implants have shown improved histologic and histomorphometric bone response, when compared with machined (MA) dental implants,[20–22] and higher removal torque values.[23,24]

Until now, however, only a few histologic and histomorphometric studies have investigated the early bone response to DAE implants placed in humans.[25–28] Most of these studies were based on few samples,[26,28] retrieved from different subjects,[26] and only in a few of them the implants were inserted and retrieved from the posterior maxilla.[25,27]

Hence, the aim of the present controlled histologic and histomorphometric study was to compare the early periimplant endosseous healing properties of DAE and MA implants, when placed in native mature bone of the posterior maxilla.

MATERIALS AND METHODS

Study Design

The present controlled histologic and histomorphometric study evaluated the early bone response to DAE surface implants and MA implants, inserted in the posterior human maxilla. Each patient received 2 transitional implants (n = 1 DAE implant: *test*; and n = 1 MA implant: *control*). These implants were left submerged for an undisturbed healing period of 2 months and finally retrieved for the histologic and histomorphometric evaluation. Bone-to-implant contact (BIC %, defined as the amount of mineralized bone in direct contact with the implant surface), bone density in the threaded area (BDTA%, defined as the fraction of mineralized bone tissue within the threaded area), and bone density (BD%, defined as bone density in a 500-μm-wide zone lateral to the implant surface) were the histomorphometric parameters evaluated in this study.

Patient Selection

A total of 14 subjects (6 men, 8 women; aged between 45 and 74 years, mean age 59.0 ± 8.5 years) who were referred to the Oral Implantology Clinic, Dental Research Division, Guarulhos University, SP, Brazil, for oral rehabilitation with dental implants, were included in the present study. Inclusion criteria were good systemic and oral health and sufficient native bone to place implants of 3.25 mm diameter and 10 mm length. Exclusion criteria were pregnancy, nursing, smoking, and any systemic condition that could affect bone healing. All participants received detailed explanations about the nature of the study and signed a written informed consent form. The Institutional Clinical Research Ethics Committee of Guarulhos University approved the protocol of the present study (CEP UnG #203/2013), which was conducted according to the principles outlined in the World Medical Association's Declaration of Helsinki on experimentation involving human subjects, as revised in 2008.

Implant Design and Surface Treatment

The transitional implants used in the present study (BT Konic; Biotec-BTK, Dueville, Vicenza, Italy) were made of titanium grade 4 (ASTM F67—ISO 5832-2). All these implants (test and control) were macroscopically identical, with a tapered design, 3.25 mm in diameter, and 10 mm in length. The test implants had the surface treated with a DAE procedure. A mix of strong inorganic acids (H_2SO_4, H_3PO_4, HCl, and HF) was used, in 2 different acid baths. After each acid bath, implants were rinsed and washed with distilled water, to neutralize and remove acid residuals. Finally, implants were taken to a cleaning room ISO 7 class to be decontaminated through a plasma spray decontamination process, in argon atmosphere. The DAE implant surface was studied with scanning electron microscopy (SEM) (Fig. 1). The following standard roughness parameters were measured: Ra (the arithmetic mean of the absolute height of all points), Rq (the square root of the sum of the squared mean difference of all points), and Rt (the difference between the highest and the lowest points). The SEM evaluation of DAE surface implants revealed a mean Ra of 1.12 (±0.41) μm, a mean Rq of 1.34 (±0.69) μm, and a mean Rt of 3.86 (±1.40) μm, respectively. The control implants had a MA surface.

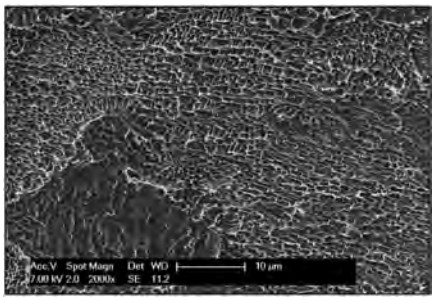

Fig. 1. SEM evaluation of the DAE implant surface. The surface presented micron-sized shallow cavities uniformly covered by submicroscopic pittings limited by razor-sharp cusps and edges.

Surgical Protocol

Twenty-eight transitional implants (n = 14 test implants and n = 14 control implants) were inserted in this study. All implants were placed under aseptic conditions. After local anesthesia, a crestal incision connected with 2 releasing vertical incisions was made. Mucoperiosteal flaps were raised and conventional implants were inserted, in accordance with the surgical and prosthetic plan prepared for each patient. Then, 2 transitional implants (n = 1 test implant and n = 1 control implant) were inserted in each patient. The transitional implants were placed in the posterior maxilla (in the areas of second premolars/first molars), distally to the most posterior conventional implant. The assignment of test and control implants (right posterior maxilla or left posterior maxilla) was random, as determined by a coin toss. The implant sites were prepared according to the manufacturer's recommendations, under profuse irrigation with sterile saline. The stability of all implants was checked using a dedicated instrument (Osstell Mentor; Osstell,

Goteborg, Sweden): if an implant showed insufficient primary stability (<35), a backup surgical site had to be prepared. The flaps were then sutured. Clindamycin 300 mg (Clindamin C; Teuto, Anapolis, Goias, Brazil) was administered 3 times a day for a week, to avoid postsurgical infection. Postoperative pain was controlled with 600 mg of ibuprofen (Actron; Bayer Scherig Pharma, Berlin, Germany) every 12 hours for 2 days. To enable subjects to control postoperative dental biofilm, 0.12% chlorhexidine rinses (Chlorhexidine; OralB, Boston, MA) were prescribed, twice a day for 14 days. The sutures were removed after 10 days. All transitional implants were left submerged for an undisturbed healing period of 2 months. After this, during the 2-stage surgery to uncover the conventional implants, the 2 transitional implants (test and control) and the surrounding tissues were retrieved from each patient, using a 4.5-mm-wide trephine bur.

Histologic and Histomorphometric Evaluation

The biopsies were fixed by immediate immersion in 10% buffered formalin and processed (Precise 1 Automated System; Assing, Rome, Italy) to obtain thin ground sections, as previously described.[26,27] The specimens were dehydrated in an ascending series of alcohol rinses and embedded in glycol-methacrylate resin (Technovit 7200 VLC; Kulzer, Wehrheim, Germany). After polymerization, the specimens were sectioned lengthwise along the larger axis of the implants with a high-precision diamond disk at about 150 μm, and ground down to about 30 μm. Two to 3 slides were obtained from each implant, stained with basic fuchsin and toluidine blue. The specimens were analyzed under a transmitted light microscope (Laborlux S; Leitz, Wetzlar, Germany) that was connected to a high-resolution video camera (3CCD-JVC KY-F55B; JVC, Yokohama, Japan) and interfaced to a monitor and a personal computer PC (Intel Pentium III 1200 MMX; Intel, Santa Clara, CA). This optical system was associated with a digitizing pad (D-Pad; Matrix Vision GmbH, Oppenweiler, Germany) and a histometry software package with image-capture functionalities (Image-Pro Plus 4.5; Media Cybernetics, Immagini & Computer Snc, Milan, Italy). For the histomorphometric evaluation, the BIC%, defined as the amount of mineralized bone in direct contact with the implant surface, was measured around all implant surfaces. The BDTA%, defined as the fraction of mineralized bone tissue within the threaded area, and the BD% in a 500-μm-wide zone lateral to the implant surface were measured bilaterally, as previously reported.[29]

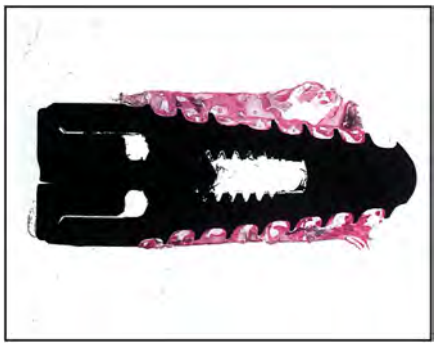

Fig. 2. Test implant (DAE surface). Newly formed trabecular bone with small marrow spaces was found along the implant perimeter, lacking only in the apical portion, maybe due to procedure of surgical removal. Low-density (D3-D4) preexisting bone, typical of the posterior maxilla, was also evident (histological staining: acid fuchsin-toluidine blue, ×18).

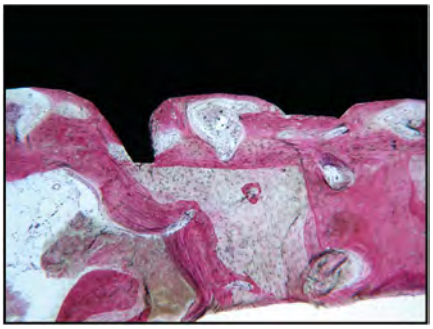

Fig. 3. Test implant (DAE surface). Newly formed bone could be observed inside the concavities of the implant threads, where active osteoblasts secerning osteoid matrix were present. In some areas, this matrix was undergoing mineralization. Preexisting bone with a low affinity for fuchsin and not in contact with the implant surface could also be seen (histological staining: acid fuchsin-toluidine blue, ×40).

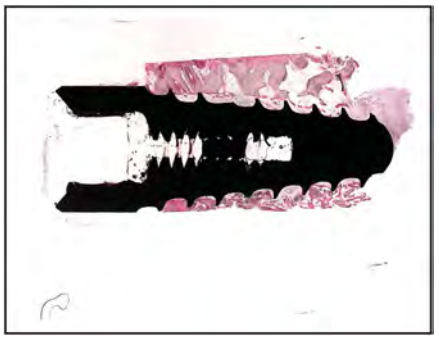

Fig. 4. Control implant (MA surface), preexisting bone was in contact with the implant surface mainly in the coronal portion. Trabeculae of newly formed bone going toward the implant surface were observed in the middle and apical portions (histological staining: acid fuchsin-toluidine blue, ×18).

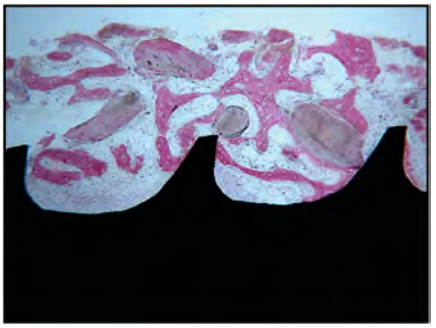

Fig. 5. Control implant (MA surface), only in few fields newly formed trabecular bone in contact with the implant surface was present (histological staining: acid fuchsin-toluidine blue, ×40).

Statistical Analysis

All collected data were inserted in a sheet for statistical analysis (Excel 2003; Microsoft, Redmond, WA). Mean, SDs, median, and 95% confidence intervals of histomorphometric values (BIC%, DBTA%, and BD%) were calculated for each implant and then for each group of implants (test vs control implants). The Wilcoxon matched-pairs signed rank test was used to evaluate differences (BIC%, BDTA%, and BD%) between the implant surfaces. The level of significance was set at 0.05. All computations were carried out with a statistical analysis software (SPSS 17.0; SPSS Inc., Chicago, IL).

Table 1. Mean, SD, Range, and Confidence Interval of BIC%, BDTA, and BD% of DAE and MA Implants Placed in the Posterior Maxilla (n = 14 Subjects)

	DAE Surface (Test Implants)			MA Surface (Control Implants)			
%	Mean (SD)	Range	CI 95%	Mean (SD)	Range	CI 95%	*P*
BIC	37.49 (29.51)	0–78.08	22.04–52.94	21.76 (12.79)	0–44.21	15.07–28.45	0.198
BDTA	30.59 (21.78)	0–60.5	19.19–41.99	28.58 (16.91)	0–56.78	19.73–37.43	0.778
BD	31.60 (18.06)	0–55.9	22.14–41.06	21.54 (11.67)	0–38	15.43–27.65	0.124

Wilcoxon matched-pairs signed rank test (level of significance set at 0.05).
CI indicates confidence interval.

RESULTS

Clinical Observations

After 2 months of healing, a total of 28 transitional implants (n = 14 test implants and n = 14 control implants) were retrieved and evaluated. Three implants (one test implant and 2 control implants) were not clinically stable and showed no osseointegration, although they did not show any sign of infection. The remaining 25 implants were clinically stable at the time of retrieval.

Histologic and Histomorphometric Results

The bone surrounding both implant groups was healthy. Woven bone with several osteocyte lacunae and preexisting bone were present; the woven newly formed bone was separated from the preexisting bone by cement lines. Some bone remodeling was observed, at early stages, even in the coronal portions of the specimens.

In the test group (DAE surface), newly formed trabecular bone with small marrow spaces was found throughout the implant body, with the exception of the apical portion; this is because of the surgical removal. Newly formed bone was found also in the coronal part of the implant. Preexisting bone is also evident, with a quality comprised between D3 and D4 (Fig. 2). Inside of the implant threads, the concavities were colonized by new bone formation, with the presence of active osteoblasts secerning osteoid matrix; in some areas, this matrix was undergoing mineralization. Preexisting bone, not in contact with the implant surface, showed low quality and low affinity for fuchsin (Fig. 3).

In the control group (MA surface), the implant was in contact with the bone tissue mainly in the coronal portion, where preexisting bone could be detected. In the middle and apical portions, newly formed trabeculae coming from the old bone and going toward the implant surface could be observed (Fig. 4). Inside of the concavities, only in few fields newly formed trabecular bone in contact with the implant surface was found (Fig. 5).

In the MA implants, the histomorphometric evaluation revealed mean (±SD) BIC%, BDTA%, and BD% of 21.76 (±12.79), 28.58 (±16.91), and 21.54 (±11.67), respectively. In the DAE implants, the histomorphometric analysis revealed mean (±SD) BIC%, BDTA%, and BD% of 37.49 (±29.51), 30.59 (±21.78), and 31.60 (±18.06), respectively (Table 1). For the MA implants, the BIC% ranged from 0 to 44.21; for the DAE implants, the BIC% ranged from 0 to 78.08. Although the mean BIC% of DAE implants value was almost double than that of MA implants, the Wilcoxon matched-pairs signed rank test found no significant differences between the 2 groups of implants, with regard to BIC% ($P = 0.198$). The BDTA% was similar in the 2 groups, as it ranged from 0 to 54.51 for the MA implants, and from 0 to 60.5 for the DAE implants. Again, the Wilcoxon matched-pairs signed rank test failed to find a significant difference between the 2 groups of implants, with regard to BDTA% ($P = 0.778$). Finally, for the MA implants, the BD% ranged from 0 to 38; for the DAE implants, the BD% ranged from 0 to 55.9. Although BD% was higher in the test group than in the control group, this difference was not statistically significant ($P = 0.124$).

DISCUSSION

At present, the relationship between surface topography and osseointegration is well recognized.[8–10] In fact, the nature of the implant surface is known to influence the rate of osteoblast proliferation, matrix synthesis, and local autocrine factor production, which all, ultimately, influence the rate of osseointegration.[8–10] Rough surfaces have demonstrated better adsorption of biomolecules from biological fluids, which has the potential to alter the cascade of events that leads to bone healing and intimate apposition with the device.[7,9,12] *In vitro* reports indicate that rough surfaces improve the initial cellular response, including cytoskeletal organization and cellular differentiation with matrix deposition.[6,7,9,12,19] Histologically, it has been demonstrated that rough surfaces can effectively promote better and faster osseointegration when compared with MA surfaces.[20–22,25–29] From a clinical point of view, several studies have reported excellent long-term survival/success rates for rough surface implants.[2,16–18]

In challenging implant cases, such as immediate loading, immediate implant placement in postextraction sockets, and placement of implants in "poor" quality bone, the acceleration of early periimplant bone healing might be very useful; [4,5] however, the precise nature of surface characteristics needed for optimal osseointegration remains to be elucidated.[6,8,12,19] Among different surface treatments, acid etching seems to be one of the most popular, and DAE implants have been used for several years, with satisfactory high survival and success rates.[16–18]

At present, histologic and histomorphometric assessments are the most accurate methods to investigate the bone healing processes and the morphological characteristics of the bone-implant interface.[30] Unfortunately, only a few studies in the present literature have dealt with histologic

and histomorphometric evaluation of human-retrieved DAE implants: this is because of ethical issues related to implant retrieval from human subjects.[25–29]

Lazzara et al[25] conducted a human histologic/histomorphometric study to compare the percentage of BIC% at 6 months for DAE and MA titanium implant surfaces. Eleven patients were selected for installation of 1 DAE and 1 MA mini-implants (2 mm diameter × 5 mm length), in the posterior maxilla (type III and type IV bone), during conventional dental implant surgery.[25] After 6 months of undisturbed healing, the mini-implants and surrounding hard tissue were removed.[25] Histomorphometric analysis indicated that the mean BIC% value for the DAE surfaces (72.96 ± 25.13) was significantly higher than the mean BIC% value for the MA surfaces (33.98 ± 31.04).[25] The authors concluded that in poorer quality bone (posterior maxilla), implants with DAE surface can guarantee a faster bone healing when compared with implants with MA surface.[25] In a more recent histological study, the authors retrieved 2 DAE implants from the mandible, to repair damage to the inferior alveolar nerve.[26] After 6 months of healing, both implants seemed to be surrounded by newly formed bone.[26] No gaps or fibrous tissues were present at the bone-implant interface.[26] The mean BIC% (61.3 ± 3.8) was high.[26] In another study, the authors documented the osseointegration of 2 DAE implants after 2 months of healing, with different loading conditions.[28] A completely edentulous patient received a total of 11 DAE implants in the mandible.[28] Six implants were immediately loaded to support a provisional fixed partial denture and 5 were left submerged. After 2 months, 2 submerged and 1 immediately loaded implants were retrieved for histologic/histomorphometric analysis.[28] The BIC% was 38.9 for the submerged implants and 64.2 for the immediately loaded one. The authors concluded that osseointegration can be achieved after 2 months by DAE implants placed in the mandible, either when immediately loaded or when submerged and unloaded.[28] Finally, in a recent work, DAE surface was compared with bioceramic molecular-impregnated surface.[29] Ten subjects received 2 transitional mini-implants implants (1 of each surface) during conventional implant surgery in the posterior maxilla.[29] After an undisturbed healing period of 2 months, the implants and the surrounding tissue were removed by means of a trephine and were nondecalcified processed for ground sectioning and analysis of BIC%, BDTA%, and osteocyte index (Oi).[29] At the end, histometric evaluation showed significantly higher BIC % and Oi for bioceramic molecular-impregnated implants ($P < 0.05$), whereas BA% was not significantly different between groups. The authors concluded that bioceramic molecular-impregnated surface can positively modulate bone healing at early implantation times compared with the DAE surface.[29]

In our present study, we have decided to evaluate DAE (test) and MA implants (control) with an intra-individual comparison to overcome the possible anticipated variability between individuals. This represents a clear advantage of our present study, as an intraindividual comparison between different surface is a rarity in the literature.[25,29,30]

In our study, in the MA implants, the histomorphometric evaluation revealed mean (±SD) BIC%, BDTA%, and BD% of 21.76 (±12.79), 28.58 (±16.91), and 21.54 (±11.67), respectively. In the DAE implants, the histomorphometric analysis revealed mean (±SD) BIC%, BDTA%, and BD% of 37.49 (±29.51), 30.59 (±21.78) and 31.60 (±18.06), respectively. Although the mean BIC% of DAE implants value was almost double than that of MA implants, the statistical analysis found no significant differences between the 2 groups, with regard to BIC% ($P = 0.198$). These statistical results may be influenced by the fact that also nonosseointegrated samples (3) have been included in our analysis, and most of all, the overall number of samples (14 per type) was low; if the sample had been larger, we would probably have expected a statistically significant difference between the 2 groups in the BIC%. No significant differences were found in our study between DAE and MA implants with regard to BDTA% ($P = 0.778$) and BD% ($P = 0.124$). Anyhow, our results indicate that DAE surface can potentiate healing process and new bone apposition, compared with MA surface.

It should be noted that most of the human histologic/histomorphometric studies that are currently available in the literature have focused on hard and soft tissue reactions around experimental implants with smaller dimensions than those of regular dental implants.[25,29,31] To date, only a few studies have evaluated the histological response around standard-diameter implants,[13,30] and most of these were based on implants removed for fracture.[13,32] In our present study, we have used transitional implants of standard dimensions (3.25 mm diameter × 10 mm length). This may represent an advantage of our study because we have evaluated how the healing processes take place in a situation closer to the real one. However, the retrieval of our transitional implants was carried out in such a way that the resulting prepared canal in bone could be used for dental implants with a larger diameter.[30]

CONCLUSIONS

At present, only a few histologic and histomorphometric studies have evaluated the bone healing around dental implants in humans; however, the histological data from the retrieved human implants are absolutely necessary to obtain useful information about the bone healing processes around dental implants, as well as the bone-implant interface. Within its limits (such as the small sample size), the present study reports that the DAE surface improved the periimplant early healing processes in the native bone of the maxilla when compared with the MA surface. Further studies on a larger sample of patients are needed to confirm these results.

DISCLOSURE

The authors claim to have no financial interest, either directly or

indirectly, in the products or information listed in the article.

APPROVAL

The present study was approved by the Institutional Clinical Research Ethics Committee of Guarulhos University (number of approval: CEP UnG #203/2013).

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REVIEW ARTICLE

Impact of Different Implant Surfaces Topographies on Peri-Implant Tissues: An Update of Current Available Data on Dental Implants Retrieved from Human Jaws

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Abstract: The high success range obtained with the implant-supported restorations has improved its applicability on routine of the daily clinical practice. This elevated percentage of success is related to the previous pre-clinical data obtained from animal and *in vitro* studies that evaluated the impact of implant surface topographies on bone tissue. However, the histological evaluation of human bone tissue is scarce. Therefore, the aim of this review is to depict an actual panorama of the data available on bone-to-implant contact (BIC) of retrieved implants from human jaws. Some aspects of implant surface topography as well as systemic conditions as osteoporosis and smoking habit were demonstrated to have a strong impact, suggesting that the data obtained from human bone tissue is still valuable for the better understanding of the osseointegration process. This article also highlighted that most data in humans are difficult to interpret, due to the lack of detailed information about the surfaces found in retrieved implants. Without the definition of the surface characteristics, it is difficult to link exactly the surface patterns to specific clinical observations, and all observations remain de facto incomplete. As a conclusion, data from implants retrieved from human jaws are very important for our understanding, however the studies remain scarce and data is fragmented. This important approach should be improved, completed and developed in the future.

Keywords: Dental implant, osseointegration, surface properties, titanium, human histology.

INTRODUCTION

Dental implants have been shown to be highly successful in several clinical indications with high survival and success rates [1, 2]. Pre-clinical studies have been carried out evaluate different implant macro- and micro-topographies under different loading conditions, different bone qualities and quantities [3, 4]. All these studies are extremely valuable; they are, however, few evidence quality and the results obtained from these studies could be transposed to a human situation [5, 6]. Accordingly, it is very important to evaluate retrieved implants from human jaws.

Dental implants can be removed due to biological and technical problems, e.g. mobility, dental implant fracture, peri-implant diseases, peri-implant bone resorption, and infection. They can also be obtained for other reasons, unrestorable prosthetics, misalignment, inability of an implant to meet changed prosthetic needs, psychological reasons, pain,

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dysesthesia, not optimal position from aesthetic and hygienic point of view, or can be retrieved at autopsy [6]. In all these latter cases, the retrieved implants continued to have an excellent bone anchorage. Dental implants retrieved from humans' jaws, in particular micro-implants specially designed for this end, can also be obtained as part of a research protocol approved by an Ethical Committee [5, 6]. Also, in these cases the bone anchorage is still present. The careful evaluation of all these different types of dental implants can be extremely useful to help in understanding the failure modalities or the reactions of both soft and hard the peri-implant tissues. In this review, the main focus will be on dental implants with different implant surface characteristics, on implants inserted in osteoporotic patients, on implants retrieved from smokers and on the peri-implant soft tissues.

IMPLANT SURFACE CHARACTERISTICS: THE INCOMPLETE PARAMETER

Implant surface characteristics plays an important function in several peri-implant cellular and molecular mechanisms [5-7]. In the past years, many dental surfaces alterations, such as anodization, discrete calcium-phosphate crystal deposition, coatings with biological molecules and chemical modification sandblasting, acid-etching and grit-blasting, have been developed, in order to improve the quality of various chemical modification and/or specific micro- and nano- topographies [7, 8].

When an implant is placed into a bone site, a cascade of biological events is initiated. The surface is first covered with blood cells and fibrin, and there is a recruitment and migration of osteogenic cells to the implant surface. Then, new bone formation takes place, which results in the formation of a mineralized interfacial matrix, followed by a bone remodeling process. During these complex phases, the healing/remodeling process is in fact dependent on equilibrium between bone anabolism and catabolism at the bone/implant interface [7]. It is important to emphasize, on the other hand, that adapted surface characteristics of an implant are not the only requirement to obtain a long-lasting implant anchorage. Many parameters are impacting the process of osseointegration; the implant material, surface characteristics and the implant design are major parameters, but but bone tissue volume, surgical technique and load conditions of the implant-supported restorations are also related to the implant long-term success. The percentage of BIC may be employed to evaluate the stability of an implant, but it remains a limited instrument as values higher than 50% appear to be satisfactory and are very common. Torque removal force (RTV) has been used to describe the anchorage of an implant to the bone, and the higher the value, the greater the biomechanical strength of the bone-implant interface. As previously mentioned, data obtained from studies performed in human jaws are more reliable than the findings obtained in studies performed in animals or *in vitro*. Some studies can, however, be performed using an animal model, e.g. RTV evaluations of implants with different macro-and microgeometries, and *in vitro* studies can be useful in helping to understand the biological response of different types of cultured cells in contact with implant topographies.

Dental implant topography should be very accurately defined both at the chemical and morphological levels [7-9]. The chemical characteristics are often associated with the biochemical interlocking of the bone/implant interface (ionic chelation particularly) and the morphological characteristics are in general associated with the biomechanical interlocking of the bone/implant interface, particularly at the microscale [7-13]. All characteristics (particularly chemical modifications and structures at the nanoscale) have also a direct impact on healing and osteogenic cells during the osseointegration process [14]. The surface chemical composition is core material and chemical modification. The dental implant topography is characterized at the micro- (roughness, pores, and particles) and nanoscale (roughness, patterning, tubes, particles, smooth) [7]. The global architecture (homogeneity, cracks, fractal architecture) of a surface is also important to consider [15].

A recent series of papers [9-13] have properly been organized in the range of several implant surface groups to allow better standardization using protocols of analysis and terminology, and a standardized characterization code. The Implant Surface Identification (ISI) standard system for the morphological and chemical characterizations of implant surfaces topographies characterized and established the respective Identification (ID) Card and code of several dental implant [9-13].

The implant surface topography production processes require a tremendous preparation as well as defined parameters of each chemical and physical procedures can be altered to find a useful implant surface topography. However, a plethora of implant surface topography compositions could be regrouped by their main specific patterns.

The re-arrangement of these 5 implant surfaces can be made using the definition of production to compare and understand the main patterns of each technology. There are three main logical concepts of production of an implant surface topography: modification of the core material characteristics, carving of the core material by subtraction or chemical coating of the core material.

These aforementioned papers [9-13] organized the implant surface topographies in four main groups of production: Group 1 - Implant surface topographies prepared through modification of the core material characteristics, mostly the alteration of the titanium metallurgy through anodization or titanium-plasma spraying (TPS); Group 2 - Implant surface topographies prepared through a subtractive processing to carve the surface morphology. Therefore two other subgroups were can be defined: group 2A gathered all surfaces produced through a subtractive sandblasting and acid-etching (SLA type); group 2B gathered Implant surface topographies prepared through all other subtractive methods such as Resorbable Blasting Media (RBM), Dual Acid- Etching (DAE) or Subtractive Impregnation Micro-Nanotexturization (SIMN); Group 3 - Implant surface topographies prepared through chemical coating of the core material, being defined two subgroups: Group 3A produced by subtraction and finally covered with a nanometric coating of Ca, CaP or Na-based nanocrystals; Group 3B implants covered with a micrometric thick layer of hydroxyapatite (mostly Plasma-Sprayed Hydroxyapatite PSHA) or other forms of CaP (Ion-

Beam Assisted Deposition IBAD, brushite coating, and others), therefore becoming the core material of the surface, and Group 4-gathered all surfaces designed specifically for the collar cervical area of the dental implant.

This collar cervical area aims to promote a better stability of both hard and soft peri-implant tissues. This group is based on the concept of specific use of these surfaces designed for the peri-implant cervical implant/bone/ soft tissue interface, and not strictly for the osseointegration itself.

In order to review the current literature about dental implants retrieved from human jaws, it is not possible to use accurately a so detailed definition of surfaces (such as ISI). Indeed, in most articles, the only data available about the examined retrieved implants are the model of implant and very general information about its process of production [7]. Studies with detailed analysis of implants prior to a test in humans are very rare, and anyway the lack of extended characterization of implant surfaces in published studies is still a major issue in this field [7, 15]. For this reason, it was decided to review the literature about implants retrieved from human jaws by identifying them through their general process of production, and not by their real chemical and morphological characteristics. Therefore, the term "implant surface topography" refers in this publication to the general aspect and type of the surface, based on its industrial method of production. The current literature does not allow integrating more detailed parameters at this time.

IMPLANT SURFACE TOPOGRAPHIES: AVAILABLE DATA ON PERI-IMPLANT HUMAN TISSUE

Machined Surfaces

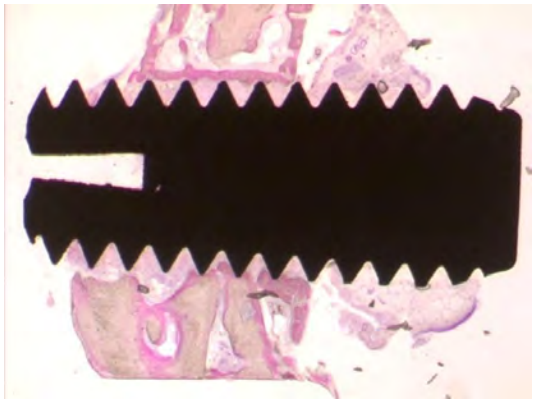
This surface, also called "turned" or "smooth", and microscopic observation under Scanning Electron Microscopy (SEM) revealed the presence of a slight roughness due to the grooves and ridges produced during the turning process, was the most commonly used in the past. The main characteristic of the machined surface is the bone growth pattern characterized by "distance osteogenesis", i.e. bone growth toward the implant surface (implantopetal kind of bone growth) (Fig. **1**).

Sandblasted Surfaces

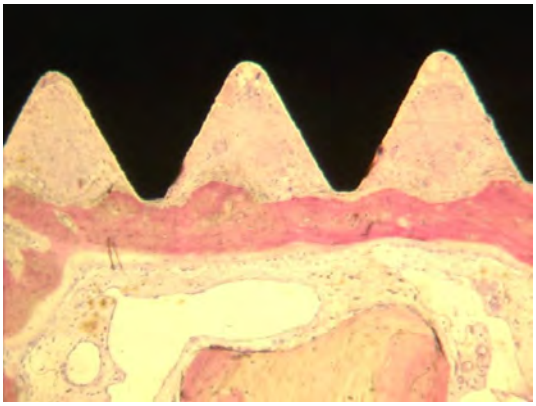
Implant surface topography is produced by treating the commercially pure titanium implant with a spray of air and abrasive material (aluminum oxide -Al_2O_3 or titanium oxide-TiO_2) during a specific period of time and controlled pressure.

The large variability 7 in surface appearance under scanning electron microscopy of several implant surface topography is due to the different techniques employed in the blasting procedure. The sandblasted surfaces have shown, in *in vitro* studies, a higher adhesion, proliferation, and differentiation of osteoblasts. Higher bone-to-implant contact (BIC) values were found in histological studies that compared blasted and turned surfaces [16, 17].

Blasting procedures leave, however, blasting residual particles on the surface of the topography, and this fact modify the bone healing process. Previous studies suggest that aluminum ions could jeopardize bone formation by a possible competitive action to calcium, while others studies speculate that histological features did not support the hypothesis that residual aluminum oxide particles on the implant topography could affect the osseointegration. The bone tissue growth pattern around blasted, rough surfaces is called "contact osteogenesis", i.e. the osteoblasts start depositing osteoid matrix directly on the implant surface topography ("implantofugal type of growth"). This biological event could produce an earlier and a higher quantity of peri-implant bone at the dental implant interface (Fig. **2**).



(**A**)



(**B**)

Fig. (1). A) Machined implant retrieved from the mandible. Trabecular bone is present around the implant (acid fuchsin-toluidine blue 40X). **B**) There are some gaps between pristine bone and newly-formed bone suggesting and indirect osteogenesis (acid fuchsin-toluidine blue 100X).

Plasma Sprayed Surfaces

These surfaces have been used in orthopedics since many decades. These implants were produced by spraying heat on the titanium base, which resulted in a implant surface topography with unspecific sized and shaped valleys and peaks, pores and cavities with an increase of the implant surface area by 6 to 10 times. This surface topography, in which it was possible to observe the formation of bone into the coating, improved the implant fixation in bone, by a biomechanical interlock [18]. One disadvantage of this type of surface could be the detachment of titanium particles from the coating after implant insertion. The implications of this occurrence were, however, not clear (Fig. **3**).

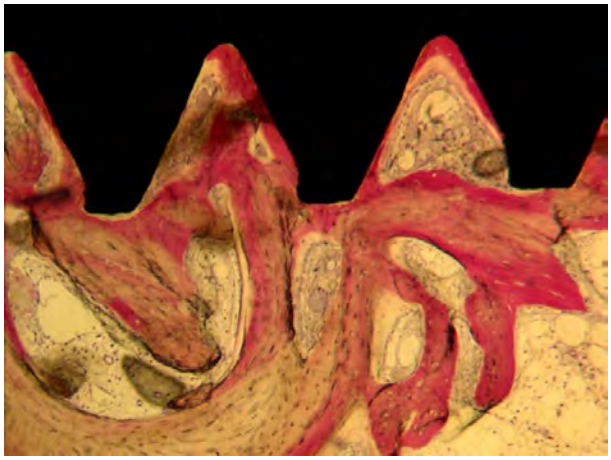


Fig. (2). Sandblasted implant retrieved from the maxilla. Histologic ground section of sandblasted surface presenting reversal line distinguish newly-formed bone and pristine bone (acid fuchsin-toluidine blue 100X).

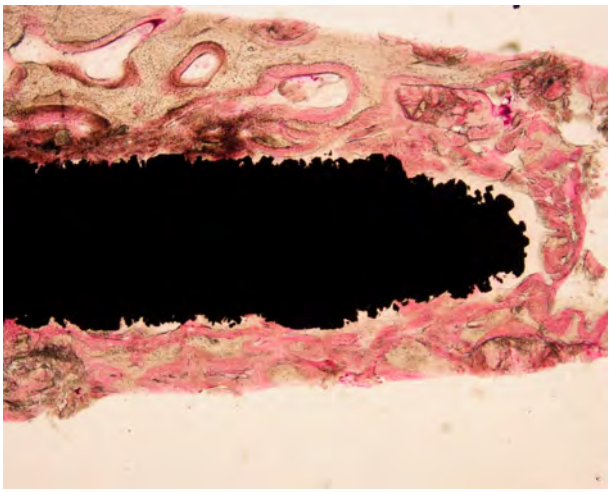


Fig. (3). Plasma-sprayed surfaced implant retrieved from the mandible. It is possible to observe bone tissue in close contact with the implant surface and small marrow spaces in proximity of the surface (acid fuchsin-toluidine blue 40X).

ACID-ETCHED SURFACES

These were introduced to modify the implant surfaces without the residues found after the blasting procedures, to have a uniform implant surface topography treatment, and to control the loss of metallic substance. Baths using chlorides (HCl), sulfuric (H_2SO_4), hydrofluoric (HF), and nitric (HNO3) acids, in different combinations, have been used. The acid-etching process was affected by the acid used, by the bath temperature, and by the etching time. The bone growth pattern was "contact osteogenesis" [19] (Fig. **4**).

SANDBLASTED AND ACID ETCHED SURFACES

They are acquired with a combined prepare of blasting (to produce a macro- texture) followed by acid-etching (to find a final micro-texture).

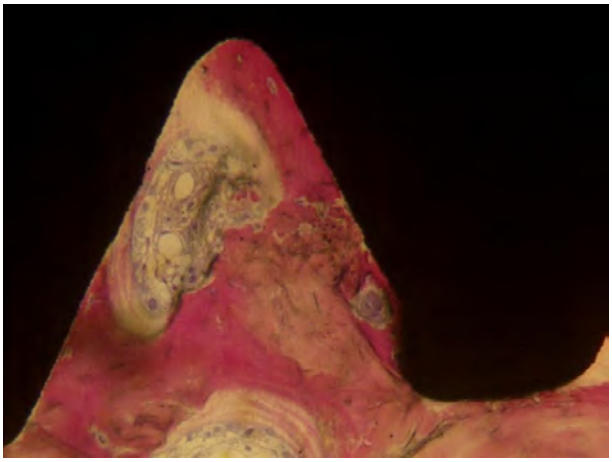


Fig. (4). Acid-etched surfaced implant retrieved from the mandible. Small marrow spaces can be observed in close proximity of the implant surface (acid fuchsin-toluidine blue 100X).

Sandblasted and acid etched implants results in a higher BIC at earlier time points [16, 17] compared toplasma-sprayed- coated dental implants. Sandblasted and acid-etched surfaces showed high osteoconductive properties and capabilities to induce cell proliferation (Figs. **5**).

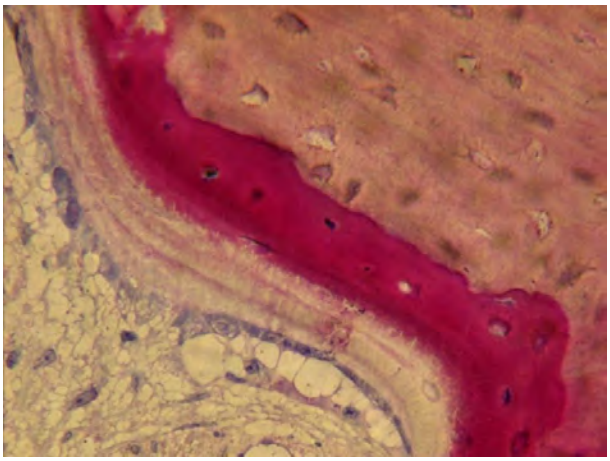


Fig. (5). Acid-etched surfaced implant retrieved from the mandible. Osteoblasts can be observed in the vicinity of implant surface. Osteoid matrix is present (acid fuchsin-toluidine blue 200X).

ANODIZED SURFACES

Anodized or anodic surfaces were obtained by modifying the structure of the superficial oxide layer of the implant surface without depositing grit particles. Anodized surfaces were produced by applying a voltage on the titanium specimen immersed in an electrolyte. The resultant surface presented micro-pores of variable diameters (Fig. **6**) that increase the bone anchorage at early phases of bone healing [20, 21].

ZIRCONIA

Zirconium oxide (ZrO_2) is used in Oral Implantology for its biocompatibility, esthetics (its color is similar to tooth),

and mechanical properties. ZrO_2 implants are biocompatible, bioinert, radiopaque, and present a high resistance to corrosion, flexion and fracture. ZrO_2 implants have been reported to show a bone and soft tissue contact similar to that seen around titanium implants. ZrO_2 can be used to produce an entire implant, or as a coating surface. Recent data obtained on human bone depicted a similar range between BIC of sandblasted acid etched surface and zirconia (Fig. **7**).

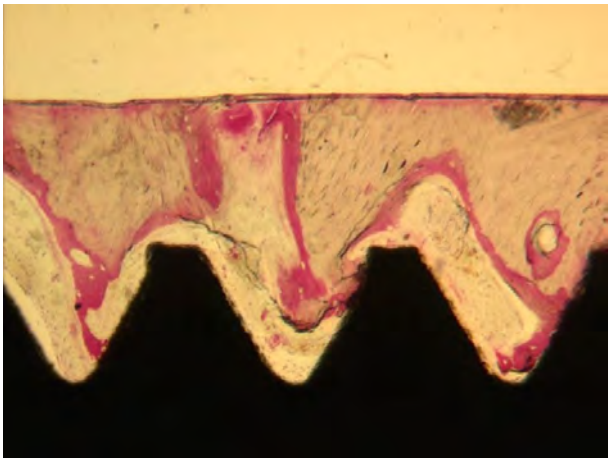


Fig. (6). Anodized surfaced implant retrieved from the maxilla. A osteoblast rim can be observed in the vicinity of implant surface. Osteoid matrix is present (acid fuchsin-toluidine blue 40X).

Fig. (7). Zirconia implant retrieved from mandible. Note the contact of the newly-formed bone with the untread zirconia surface after 60 days of unloade period (Stevenel's blue and alizarin red, 12x).

BIOCERAMIC MOLECULAR IMPREGNATION

Surface properties in the nanometer scale could modulate the properties of the protein layer adhesion in our tissues, the nanoscale structure of the extra-celullar matrix allows an essential and natural web of nanofibers to support cells and show an instructive background to guide their behavior.

Physical and bioceramic incorporation implant surface treatments at nanometer scale have shown higher means of BIC [19] and torque values compared with rough implant surface topography at micrometer scale.

The application of nanotechnology for the alteration of texture and chemistry in implant topography could result in varied cell behavior, ranging from alterations in adhesion, orientation, mobility and surface antigen display of the pre-osteogenic and osteogenic cells. Complementary, nanoscale characteristics may also influence the adsorption and conformation of integrin binding proteins, changing the availability of binding sites and modify integrin signaling (Fig. **8**).

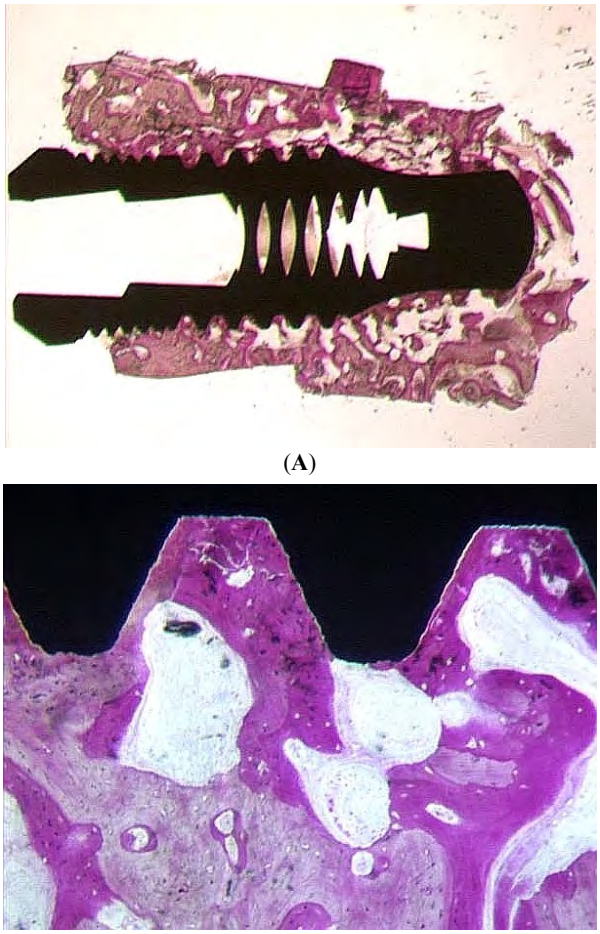


Fig. (8). A) Histologic ground section of a biomolecular impregnated implant surface retrieved from the manbile (Basic fuchsin and toluidine blue staining, original magnification x12); **B**) Newly-formed bone with connecting bridges between the new bone trabeculae and the implant surface (Basic fuchsin and toluidine blue staining, original magnification x40).

DIRECT LASER METAL SINTERING (DLMS) IMPLANT SURFACE

Previous investigations have shown that direct laser metal sintering (DLMS) produces structures with complex geometry that allow better osteconductive properties [22]. DLMS implant surface presented a similar cell density to that on rough implant surface but lower than on smooth surfaces. Moreover, it was shown that dental implants obtained through DLMS were better adapted to the elastic properties of bone tissue. DLMS implant topography not only minimizes stress-shielding effects but also improves implants

long-term success10 rates with higher BIC compared those obtained in machined surfaces [23-27].

These evaluations also suggested that DLMS technique is a less expensive method for preparing dental implants from commercially pure titanium or alloys (Figs. **9**, **10**).

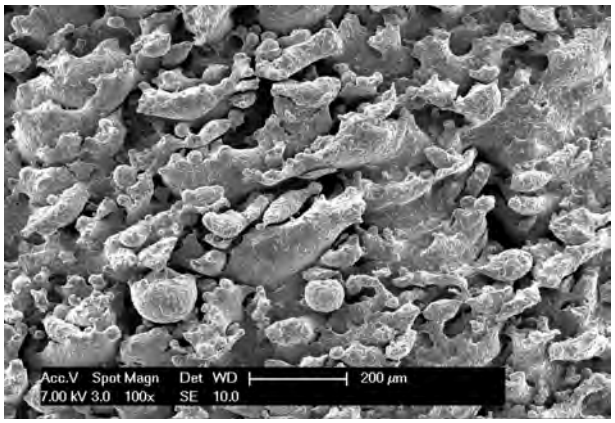


Fig. (9). Scanning electron microphotograph of the direct laser fabrication surface.

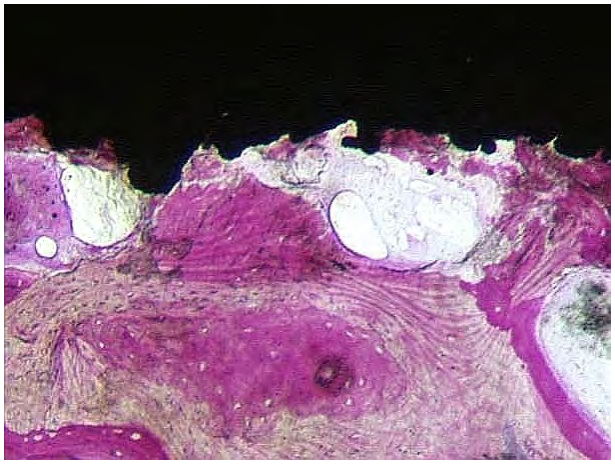


Fig. (10). Histological ground section of the direct laser fabrication micro-implant surface after 2 months of healing showing the newly-formed bone at early maturing stages. There are connecting bridges between the new bone trabeculae and the implant surface (Basic fuchsin and toluidine blue staining, original x40 magnification).

DENTAL IMPLANTS RETRIEVED FROM OSTEOPOROSIS SUBJECTS

Osteoporosis is a systemic disease that affects the quality of bones so that it may become susceptible to fracture. While pre-clinical evaluations have shown the negative impact of osteoporosis on osseointegration, no clinical studies showed a clear association between implant failure and osteoporosis [28]. The mechanism by which osteoporosis acted on peri-implant bone was based on the decrease in bone volume and BIC, consequently reducing bone tissue to support dental implants. However, in studies in humans [29-32], BIC was found to be the same for both osteoporosis and non- osteoporosis subjects, suggesting that that osteoporosis might not present an absolute contra-indication for dental implant placement, at least, after osseointegration has been achieved (Figs. **11**, **12**).

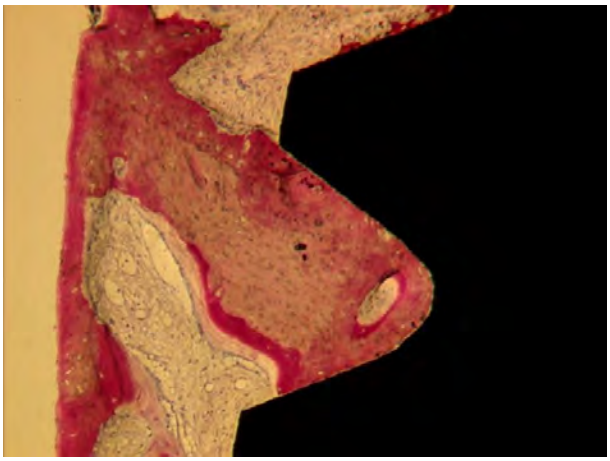


Fig. (11). Implant retrieved in patients with osteoporosis. Mature cortical bone with no remodelling areas (Basic fuchsin and toluidine blue staining, original 100X magnification).

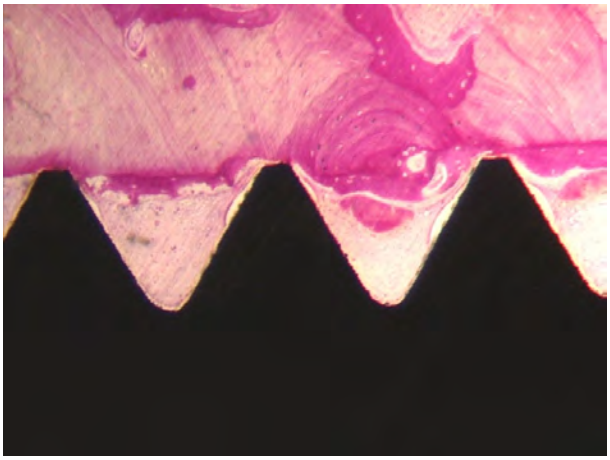


Fig. (12). Implant retrieved in patients with osteoporosis. No osteoblastic activity can be detected. A gap is present at the bone-implant interface. (Basic fuchsin and toluidine blue staining, original magnification x40).

DENTAL IMPLANTS RETRIEVED FROM SMOKERS

The influence of smoking on bone tissue has been evaluated in a several histologic animal models. The majority of these studies agree that smoking caused a detrimental effect on bone healing, bone-to-implant contact and bone mineral density [33]. Cigarette smoking delays the normal bone healing tissue process by a mechanism that inhibits proliferation of precursor cells. Smoking could release over 4,000 toxins with a potential to jeopardize the bone tissue healing. Carbon monoxide, hydrogen cyanide, aldehydes, benzenes, nitrosamines, and nicotine impact essential processes of bone healing. Nicotine was a potent vasoconstrictor that not only inhibited the 11 proliferation of fibroblasts, red blood cells and macrophages, but also the nutrient delivery and blood flow to the surgical site.

Carbon monoxide reduce the oxygen carrying capacity of red blood cells, while hydrogen cyanide lead to hypoxia. In human retrieved specimens, BIC% was found to be significantly lower in smokers [34-36]. A tendency toward slower wound repair has been suggested. Moreover, cigarette smoking reduced the rate of bone formation and increased the rate of bone destruction in post-menopausal women. Cigarette smoking seemed to suppress osteoprotegerin and could contribute towards the reduce the bone healing. (Figs. **13**, **14**).

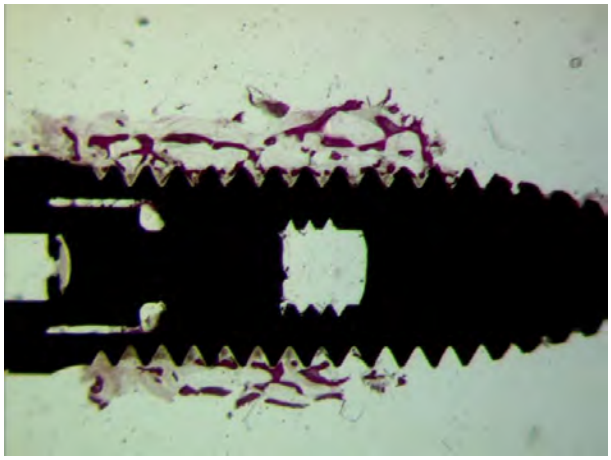


Fig. (13). Histological ground section of a implant retrieved after 8 weeks of healing from a posterior maxilla of a smoker with newly-formed bone showing early maturing and remodelling stages. (Basic fuchsin and toluidine blue staining, original 12x magnification).

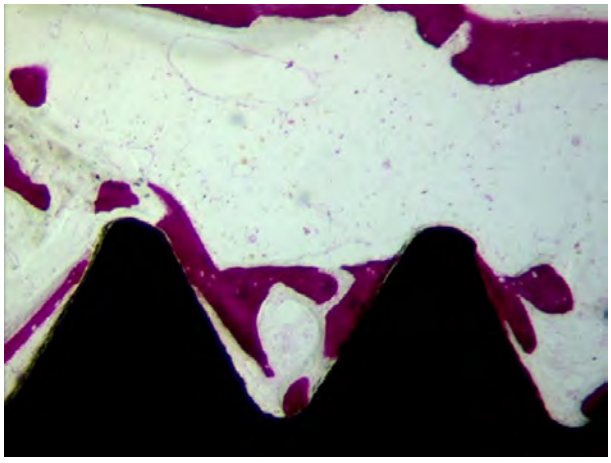


Fig. (14). Higher power view of the lateral area in the section shown in (A). The newly formed bone tissue shows areas of direct contact with the oxidized implant surface, although in some areas there are also a lack of connecting bridges between the new bone and the implant surface (Basic fuchsin and toluidine blue staining, original 100x magnification).

PERI-IMPLANT SOFT TISSUES

Current knowledge of the histological and histomorphometrical features of the supracrestal peri-implant soft tissues was constituted, for the most part, by data obtained on studies in dogs or non-human primates. The distance from the peri-implant soft tissue margin to the alveolar bone was called biological width (BW). Around teeth the BW had a constant vertical dimension providing the gingival esthetics, and was composed by sulcus depth,junctional epithelium, and connective tissue attachment. The sealing of peri-implant mucosa played a pivotal function in the protection of the underlying bone tissue from the invasion of oral bacteria. Around implants, the supracrestal soft tissues had many similarities to the dentogingival tissues around teeth and were composed by an epithelium and a connective tissue. In human retrieved specimens [37], the sulcular epithelium (SE) was composed of about 4-5 layers of parakeratinized epithelial cells and had a length of about 1.2-1.3 mm. The junctional epithelium (JE) presents 3-4 layers of epithelial cells and had a length between 1.0 to 1.5 mm.

Connective tissue attachment had a width of 400-800 µm. Collagen fibers, in form of bundles, perpendicularly oriented to the abutment surface, up to a distance of 200 µm from the surface, where they became parallel running in several directions. Some areas depicted collagen fibers bundles perpendicularly oriented or obliquely to the section plane. In the area neighboring the abutment surface, the CT contained a few blood vessels, and dense collagen fibers, oriented parallel to the longitudinal axis of the abutment, were present.

Collagen fibers showed a 3 -dimensional network around the abutment. This differentiated network of fibers may have a clinical relevance as a sealing defense of the underlying bone. The similarities between the dimension of thehuman peri-implant soft tissues and those described around teeth suggest that the peri-implant BW can be physiologically formed and stable over time (Fig. **15**).

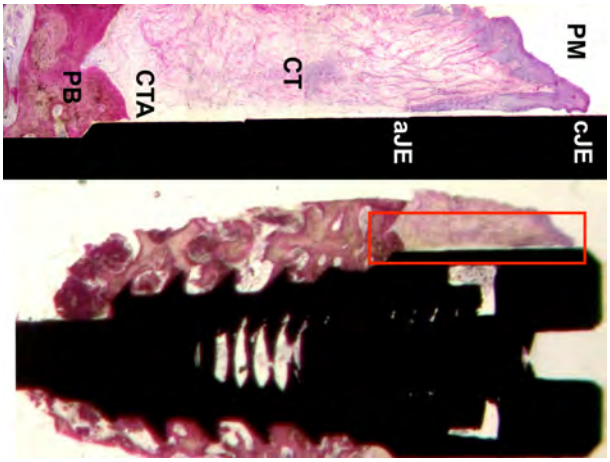


Fig. (15). Two-piece implant retrieved from posterior mandible (Basic fuchsin and toluidine blue staining, original 12x magnification). Detail of the biological width of the 2-piece implant: aJE - apical portion of junctional epithelium, cJE – coronal portion of junctional epithelium; PM – peri-implant margin; CT – connective tissue; CTA connective tissue attachment; PB – pristine bone (Basic fuchsin and toluidine blue staining, original 100x magnification).

CONCLUSION

Despite the extended use of dental implants in daily clinical practice, the exact mechanisms of their integration

remain rarely investigated in humans, for various practical reasons. Data collected from retrieved implants in human jaws are therefore a very important source of information. For this review article, available information from retrieved implants in human jaws were interesting, but remained very general and difficult to interpret, as dental implant surfaces are very rarely characterized in details, particularly in studies using retrieved implants. Various general surface topographies have their own osseointegration patterns observable in these retrieved implants in human, and it would be interesting to understand how each exact characteristic (chemical, morphological) of a surface is impacting osseointegration in this human model. In the future, a better definition of the surface characteristics should help to link more accurately surface patterns to specific clinical observations, in order to obtain more complete observations. Finally, studies with implants retrieved from human jaws should be completed and better developed in the future, to become an even more valuable source of information for our understanding.

CONFLICT OF INTEREST

Like most specialists in the implant surface field of research, the authors of this article are currently involved in studies with various dental implant companies. However, authors have no conflict of interest to report related to this review article.

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ORIGINAL ARTICLE

Early bone formation around immediately loaded implants with nanostructured calcium-incorporated and machined surface: a randomized, controlled histologic and histomorphometric study in the human posterior maxilla

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Abstract
Objective The aim of this randomized, controlled histologic/histomorphometric study was to compare the early bone formation around immediately loaded implants with nanostructured calcium-incorporated (NCI) and machined (MA) surface, placed in the human posterior maxilla.
Materials and methods Fifteen fully edentulous patients (six males; nine females; mean age 57.9 ± 6.7 years) were selected for this study. Each patient was installed with two temporary transmucosal implants, with different surfaces: one NCI (*test*) and one MA (*control*) implant. All temporary implants were placed in the posterior maxilla, according to a split-mouth design, to help to support an interim complete maxillary denture. After 8 weeks, all temporary transmucosal implants were retrieved for histologic/histomorphometric evaluation. The bone-to-implant contact (BIC%) and the bone density (BD%) were calculated. The Wilcoxon matched-pairs signed-rank test was used to evaluate differences (BIC%, BD%) between the surfaces. The level of significance was set at 0.05.
Results Eight weeks after placement, 24 clinically stable implants (12 *test*, 12 *control*) were subjected to histologic/histomorphometric evaluation. In the MA implants, the histomorphometric evaluation revealed a mean BIC(±SD)% and BD(±SD)% of 21.2(±4.9)% and 29.8(±7.8)%, respectively. In the NCI implants, the histomorphometric analysis revealed a mean BIC(±SD)% and BD(±SD)% of 39.7(±8.7)% and 34.6(±7.2)%, respectively. A statistically significant difference was found between the two surfaces with regard to BIC% ($p < 0.001$), while no significant difference was found with regard to BD% ($p = 0.09$).
Conclusions The NCI surface seems to increase the peri-implant endosseous healing properties in the native bone of the posterior maxilla, under immediate loading conditions, when compared with the MA surface.
Clinical relevance Under immediate loading conditions in the human posterior maxilla, the nanostructured calcium-incorporated surface has led to better histologic and histomorphometric results than the machined surface; therefore, the clinical use of implants with nanostructured calcium-incorporated surface may be beneficial in the posterior maxilla, under immediate loading protocol.

Keywords Immediate loading · Early bone formation · Implant surface · Human histology

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Introduction

In recent years, immediate loading protocols have become extremely popular in modern oral implantology; in fact, they meet the needs of patients, who ask for a reduction in the number of operating sessions and, therefore, of time/costs of surgical and prosthetic therapy [1–4]. Immediate loading eliminates the need for second-stage surgery and is highly appreciated because it offers immediate comfort, avoiding the inconvenience of temporary removable prostheses during the healing phase [5–7].

In order to load implants immediately, particularly in regions with poor bone quality (such as the posterior maxilla), some authors have recommended to use implants with surfaces that are able to stimulate new bone apposition and can increase the values of the connection between the bone and the implant, reducing the healing time [8–10]. The objective of modern oral implantology is twofold: on the one hand, it aims to obtain satisfactory long-term bone–implant integration (achieving a direct bone-to-implant connection on most of the implant surface) [8, 9]; on the other hand, it aims to reduce the healing time, in order to proceed as soon as possible with functionalization of the implant [3, 4, 8, 10].

The study of the implant–surface interface is key, and the introduction of surfaces with specific microtopographical features (sandblasted, acid etched, sandblasted/acid-etched surfaces) designed to stimulate the apposition of new bone tissue has already allowed clinicians to obtain excellent results [11, 12].

More recently, the focus has shifted to the nanotopography of the implant surfaces [13, 14]. In fact, the nanotopography of moderately rough implant surfaces seems to promote osteogenesis, increase the ratio of bone-to-implant contact, and increase the mechanical strength of the bone to the implant at the interface [14, 15].

Since titanium and its alloys exhibit bone-bonding bioactivity when a certain kind of thin ceramic layer is grown on their surface via simple chemical and heat treatments [14], various nanostructured calcium-incorporated implant surfaces have been introduced [8, 15]. Among these, there are surfaces treated with discrete crystal deposition of calcium phosphates [16, 17], surfaces obtained through ion-beam assisted deposition of calcium ions [18–20], and surfaces enriched with calcium ions through hydrothermal methods [21].

Human histological studies are certainly the best way to study the bone healing on the implant surfaces [22–25]. Although several studies have shown that the clinical use of implants with nanostructured calcium-incorporated surfaces can ensure high survival and rate success, at least in the short term [26–29], little is known about the early bone response to nanostructured calcium-incorporated implants in humans. In fact, only a few histologic and histomorphometric studies have addressed this topic [30–32]. Most of these studies were based on few samples, retrieved from the posterior maxilla of different subjects after an unloaded healing period [30–32]; to our knowledge, no human histological and histomorphometric studies on immediately loaded nanostructured calcium-incorporated implants are currently available in the literature.

Hence, the aim of the present randomized controlled histologic and histomorphometric study was to compare the early peri-implant endosseous healing properties of immediately loaded nanostructured calcium-incorporated (NCI) implants and machined (MA) implants, placed in the native bone of the posterior maxilla.

Materials and methods

Study design

The present study was designed as a randomized controlled histologic/histomorphometric investigation reporting on immediately loaded temporary transmucosal implants that were placed in the human posterior maxilla and retrieved after a period of 8 weeks. In particular, the study aimed to compare the early bone response to immediately loaded implants with an NCI surface and MA surface, placed in the human posterior maxilla. During a normal surgical procedure for the placement of conventional implants, each enrolled patient also received two temporary transmucosal implants (n = 1 NCI implant: *test*; n = 1 MA implant: *control*), which were inserted in the posterior maxilla, according to a split-mouth design. The temporary transmucosal implants were placed with the aim to support an interim complete maxillary denture, until healing of the conventional implants. After 8 weeks, during the two-stage surgery to uncover the conventional implants, all temporary transmucosal implants were retrieved for histologic/histomorphometric evaluation.

Patient selection

A total of 15 fully edentulous patients (6 males; 9 females; aged between 48 and 69 years, mean age 57.9 ± 6.7 years, median 57, CI 95% 54.6–61.2), referred for oral rehabilitation with dental implants to the Oral Implantology Clinic, Dental Research Division, Guarulhos University, SP, Brazil, were consequently enrolled in the present study. Inclusion criteria were good systemic and oral health and sufficient native bone to place implants of a 3.25-mm diameter and 8-mm length. Exclusion criteria for this study were any systemic condition that could affect bone healing (immunocompromised status, uncontrolled diabetes; radio- or chemotherapy of the head or neck; treatment with oral and/or intravenous aminobisphosphonates), pregnancy, nursing, and smoking. All participants received detailed explanations about the nature of the study and signed a written informed consent form. The Institutional Clinical Research Ethics Committee of

Guarulhos University (CEP #201/03) approved the protocol of the present study, which was conducted according to the principles outlined in the World Medical Association's Declaration of Helsinki on experimentation involving human subjects, as revised in 2008.

Temporary transmucosal implants

The temporary transmucosal implants used in the present study were made of titanium grade 4. All implants were one piece, macroscopically identical (3.0 mm diameter × 6 mm length), but different in the surface treatment. In fact, *test* implants (Anyridge®, Megagen Implant Co., Gyeongbuk, South Korea) had a NCI titanium implant surface (Xpeed®), while the control implants had a conventional MA surface. The *test* implant surface was obtained by modifying an original surface produced by grit-blasting with particles of resorbable calcium phosphate (resorbable blast media, RBM), which was enriched with the calcium using hydrothermal method. In brief, RBM implants were immersed in a mixed solution of 0.2 M sodium hydroxide (NaOH) and 2 mM calcium oxide (CaO) dissolved in deionized water using a Teflon-lined hydrothermal reactor system at 180 °C for 24 h under a water pressure of 1 MPa^2. With this procedure, a nanolayer of Ca^{2+} ions was incorporated onto the RBM surface, giving a calcium titanate ($CaTiO_3$) nanostructure. The NCI implant surface was investigated with scanning electron microscopy (SEM) (Fig. 1). The following standard roughness parameters were measured: Ra (the arithmetic mean of the absolute height of all points), Rq (the square root of the sum of the squared mean difference of all points), and Rt (the difference between the highest and lowest points). The SEM evaluation of NCI surface implants revealed a mean Ra of 1.6 (±0.2) μm, a mean Rq of 2.1 (±0.3) μm, and a mean Rt of 15.7 (±0.2) μm, respectively.

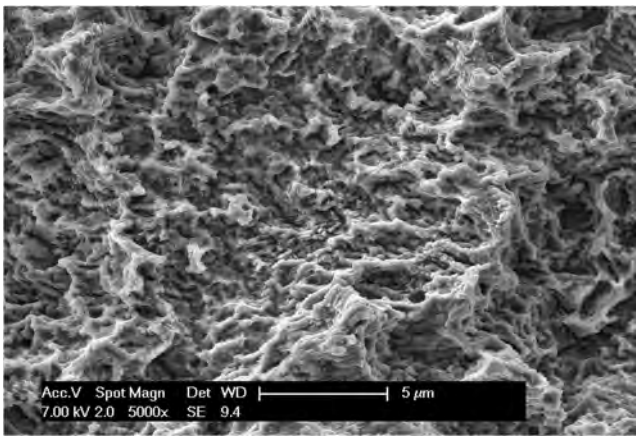

Fig. 1 Nanostructured calcium-incorporated (NCI) implant (*test*). Scanning electron microscopy evaluation revealed a mean Ra of 1.6 (±0.2) μm, a mean Rq of 2.1 (±0.3) μm, and a mean Rt of 15.7 (±0.2) μm, respectively. Magnification ×5000

Surgical protocol

Thirty transmucosal temporary implants ($n = 15$ *test* implants and $n = 15$ *control* implants) were inserted in this study. All implants were placed under aseptic conditions. After local anesthesia, a crestal incision connected with two releasing vertical incisions was made. Mucoperiosteal flaps were raised and conventional implants were inserted, in accordance with the surgical and prosthetic plan prepared for each patient. After placement of the conventional implants, two transmucosal temporary implants ($n = 1$ *test* implant and $n = 1$ *control* implant) were inserted in each patient, according to a split-mouth design. The transitional implants were inserted in the posterior region of the maxilla, among the conventional placed implants. The assignment of *test* and *control* implants (right posterior maxilla or left posterior maxilla) was random, as determined by a coin toss. The temporary implant sites were prepared according to the manufacturer's recommendations, under profuse irrigation with sterile saline. The stability of all implants was checked using a dedicated instrument (Osstell Mentor®, Osstell, Gothenburg, Sweden): if an implant showed insufficient primary stability (implant stability quotient—ISQ <35), it was removed and a backup surgical site had to be prepared. The flaps were then sutured to allow the emergency of the solid abutment of one-piece implants through the mucosa: these implants helped to support the interim maxillary denture during the entire healing period. Immediately after implant surgery, the interim maxillary denture was seated in the patient's mouth and relined intraorally with soft resin. Interim maxillary denture stability, retention, and occlusion were immediately checked. Patients were instructed not to remove the denture for 24 h to minimize swelling. Clindamycin 300 mg (ClindaminC®, Teuto, Anapolis, Goias, Brazil) was administered three times a day for a week, in order to avoid post-surgical infection. Post-operative pain was controlled with 600 mg ibuprofen (Actron®, Bayer Scherig Pharma, Berlin, Germany) every 12 h for 2 days. To enable subjects to control post-operative dental biofilm, 0.12% chlorhexidine rinses (Chlorexidine®; OralB, Boston, MA, USA) were prescribed, twice a day for 14 days. The sutures were removed after 10 days.

Specimen retrieval and histologic/histomorphometric analysis

The interim prosthesis remained connected to the temporary implants for a period of 8 weeks. After this period, during the two-stage surgery to uncover the conventional implants, the transitional fixtures (one *test* and one *control* implant) and the surrounding tissues were retrieved from each patient, using a 4.5-mm-wide trephine bur. During this procedure, which was performed as previously reported [22, 33], great attention was placed and care was taken not to damage the bone–implant interface and to preserve the integrity of the peri-implant tissues. Clinically, mobile temporary implants were not

considered for the histologic/histomorphometric evaluation. The specimens were fixed by immediate immersion at 10% buffered formalin and processed (Precise 1 Automated System®, Assing, Rome, Italy) to obtain thin ground sections, as previously described. The specimens were dehydrated in an ascending series of alcohol rinses and embedded in glycol methacrylate resin (Technovit 7200 VLC®, Kulzer, Wehrheim, Germany). After polymerization, the specimens were sectioned longitudinally along the major axis of the implants with a high-precision diamond disk at about 150 μm and ground down to about 30 μm. Two slides were obtained for each implant. The slides were stained with basic fuchsin and toluidine blue. The specimens were analyzed under a transmitted light microscope (Laborlux S®, Leitz, Wetzlar, Germany) that was connected to a high-resolution video camera (3CCD-JVC KY-F55B®, JVC, Yokohama, Japan) and interfaced to a monitor and a personal computer (Intel Pentium III 1200 MMX®, Intel, Santa Clara, CA, USA). This optical system was associated with a digitizing pad (D-Pad®, Matrix Vision GmbH, Oppenweiler, Germany) and controlled by a software package with image capturing capabilities (Image-Pro Plus® 4.5, Media Cybernetics, Immagini & Computer Snc, Milan, Italy). For the histomorphometric evaluation, the bone-to-implant contact (BIC%), defined as the amount of mineralized bone in direct contact with the implant surface, was measured around all implant surfaces. Finally, the bone density (BD%) in a 500-μm-wide zone lateral to the implant surface was measured bilaterally, as previously reported.

Statistical analysis

All collected data were inserted in a sheet for statistical analysis (Excel 2003®, Microsoft, Redmond, WA, USA). Mean, standard deviation, median, and confidence intervals (CI 95%) of histomorphometric values (BIC%, BD%) were calculated for each implant and then for each group of implants (*test* versus *control* implants). Comparisons of the differences in bone–implant percentage values in both groups were carried out using the non-parametric Wilcoxon test for paired samples. The level of significance was set at 0.05. Results were presented as mean ± standard deviation (SD), and differences at $p < 0.05$ were considered statistically significant. All computations were carried out with a statistical analysis software (SPSS 17.0®, SPSS Inc., Chicago, IL, USA).

Results

Clinical observations

Two months after placement, a total of 30 temporary transmucosal implants ($n = 15$ *test* implants and $n = 15$ *control* implants) were evaluated and retrieved. Five implants (two *test* implants and three *control* implants) in three different patients were clinically unstable and showed no osseointegration, although they did not show any sign of infection. All implants retrieved from these three patients were therefore excluded from the study and were not histologically/histomorphometrically evaluated. The remaining 24 implants retrieved from 12 patients were clinically stable at the time of retrieval and were therefore histologically/histomorphometrically evaluated.

Histologic/histomorphometric evaluation

In the ground sections from the NCI implants (*test*), at low-power magnification, it was possible to see newly formed bone around the implant surface. In a few samples, the implants were almost completely surrounded by newly formed bone (Fig. 2), while in others, mature bone was evident far from the implant surface and bone neoformation between the pre-existing bone and the implant surface (Fig. 3). In the coronal portion, only newly formed bone with a trabecular structure and strongly stained with acid fuchsin and a few areas of osteoid matrix could be observed. In some specimens, new bone on the surface, even in areas far from the pre-existing bone, was present (Fig. 4). In some areas of the middle and apical portions of the implants, the native bone was evident far from the surface and newly formed bone was present on the surface. Wide osteocyte lacunae could be observed and they often were in close vicinity to the implant surface (Fig. 5).

In the MA implants (*control*), at low-power magnification, compact bone with small marrow spaces was present around all the fixtures, but not in contact with their surface. Only in the apical portion of the threads was it possible to see pre-existing bone in contact with the surface, while newly formed bone was evident only in the apical portion of the implants (Fig. 6).

In the NCI implants (*test*), the histomorphometric analysis revealed mean BIC(±SD)% and BD(±SD)% of 39.7(±8.7)% and 34.6(±7.2)%, respectively. The BIC% ranged from 24.6 to 60.9, the median was 39.1, and the confidence interval (95%)

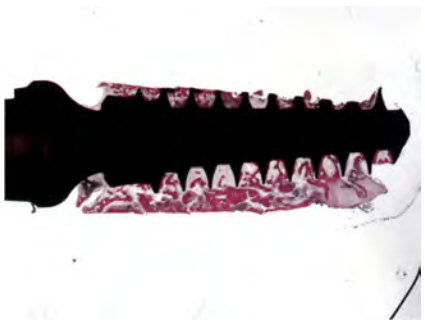

Fig. 2 Nanostructured calcium-incorporated (NCI) implant (*test*). Newly formed trabecular bone surrounded the whole implant perimeter. Acid fuchsin and toluidine blue, magnification ×12

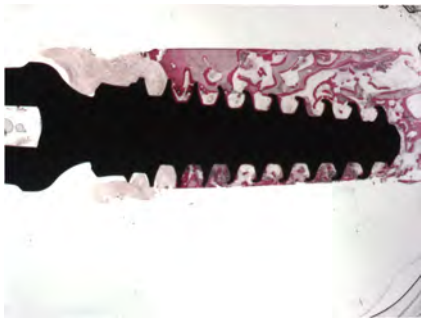

Fig. 3 Nanostructured calcium-incorporated (NCI) implant (*test*). Pre-existing bone far from the implant surface, and newly formed bone close to it were evident. Acid fuchsin and toluidine blue, magnification ×12

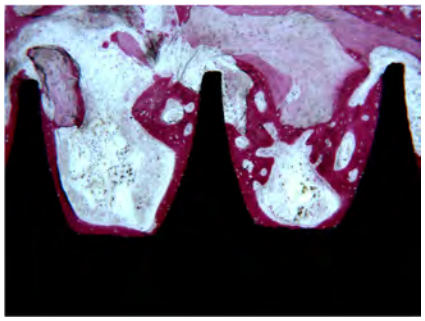

Fig. 5 Nanostructured calcium-incorporated (NCI) implant (*test*). The implant thread was lined by newly formed bone, and an intense osteoblastic activity was still evident. Acid fuchsin and toluidine blue, magnification ×40

was 34.8–44.7. The BD% ranged from 19.0 to 45.0, the median was 33.4, and the confidence interval (95%) was 30.5–38.7.

In the MA implants (*control*), the histomorphometric evaluation revealed mean BIC(±SD)% and BD(±SD)% of 21.2(±4.9)% and 29.8(±7.8)%, respectively. The BIC% ranged from 12.5 to 34.5, the median was 21.0, and the confidence interval (95%) was 18.4–24.0. The BD% ranged from 19.2 to 44.0, the median was 29.1, and the confidence interval (95%) was 25.4–34.3.

A significant difference was found between the two implant surfaces with regard to BIC% ($p < 0.001$). Although BD% was higher in the *test* group than in the *control* group, this difference was not statistically significant ($p = 0.09$). The histomorphometric results are summarized in Figs. 7 and 8.

Discussion

At present, histologic/histomorphometric assessment is the most accurate method to investigate the bone healing processes and morphological characteristics of the bone–implant interface [22–25].

Unfortunately, only a few studies in the present literature have dealt with histologic/histomorphometric evaluation of human-retrieved NCI implants [30–33]: this is because of ethical issues related to implant retrieval from human subjects.

In a human histologic and histomorphometric study, Goenè and coll. [30] inserted nine pairs of small experimental implants (nine dual acid-etched conditioned with discrete crystal deposition of nanometer-scale crystals of calcium phosphate as the *test* and nine conventional dual acid-etched as the *control*) in the native bone of posterior maxilla. The implants were retrieved with trephine drills after 4 or 8 weeks of unloaded healing, for the purpose of assessing the rate and extent of new bone development through histologic analysis [30]. The mean bone-to-implant contact value for the *test* implants was significantly increased over that of the *control* implants at both time intervals [30]. The authors concluded that the addition of a nanometer-scale calcium phosphate treatment to a dual acid-etched implant surface increased the extent of bone apposition after 4 and 8 weeks of healing [30].

Similar results were obtained by Orsini and coll. [31], who evaluated the bone response to the same nanostructured implant surface, obtained through discrete deposition of nanometer-sized calcium phosphate particles on a dual acid-etched surface. One experimental mini-implant with a novel nanostructured calcium–phosphate added surface (*test*) and one dual acid-etched surface mini-implant (*control*) were placed in the posterior maxilla of 15 patients. After 2 months, the mean BIC(±SD)% was 32.2(±18.5)% and 19.0(±14.2)%

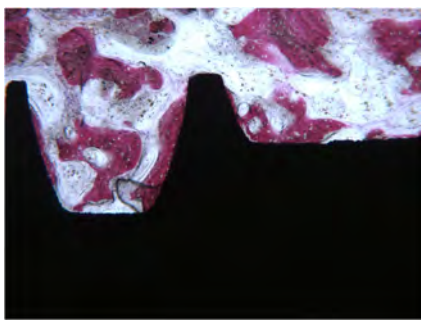

Fig. 4 Nanostructured calcium-incorporated (NCI) implant (*test*). Newly formed trabecular bone around and in contact with the coronal portion of the implant. Acid fuchsin and toluidine blue, magnification ×40

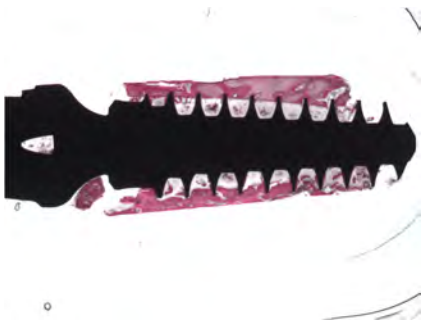

Fig. 6 Machined implant (*control*). Compact bone with small marrow spaces was present around the implant but not in contact with its surface. Acid fuchsin and toluidine blue, magnification ×12

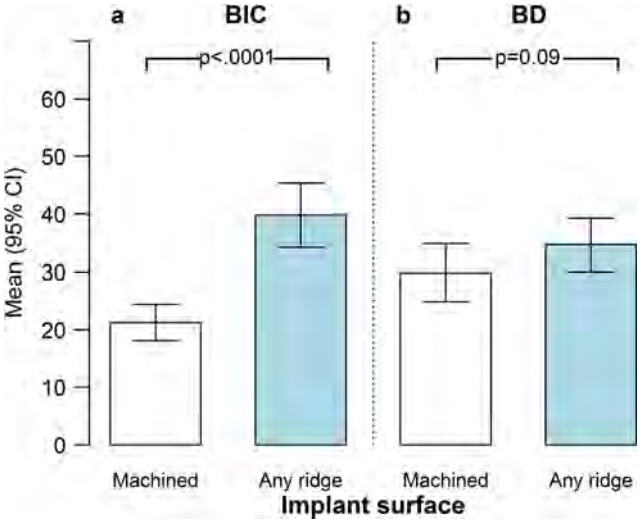

Fig. 7 Histomorphometric results with MA and NCI implants: bone-to-implant contact (*BIC%*) and bone density (*BD%*). In the MA implants, the histomorphometric evaluation revealed mean (±SD) BIC% and BD% of 21.2 (±4.9) and 29.8 (±7.8), respectively. In the NCI implants, the histomorphometric analysis revealed mean (±SD) BIC% and BD% of 39.7 (±8.7) and 34.6 (±7.2), respectively

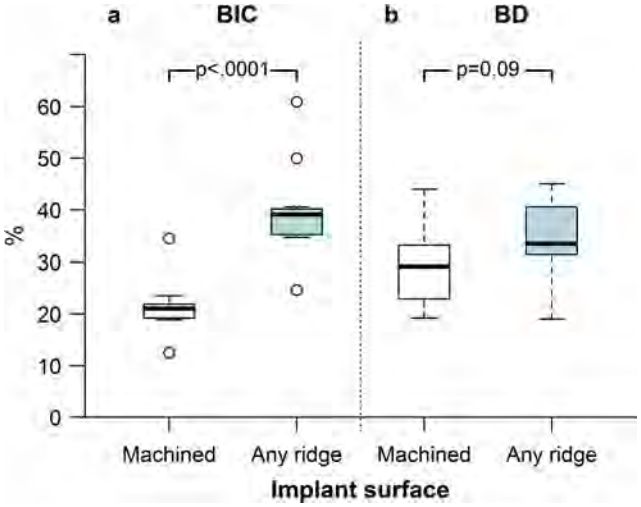

Fig. 8 Histomorphometric results with MA and NCI implants: bone-to-implant contact (*BIC%*) and bone density (*BD%*). A statistically significant difference was found between the two surfaces with regard to BIC% ($p < 0.001$), while no significant difference was found with regard to BD% ($p = 0.09$)

for *test* and *control* implants, respectively: this difference was statistically significant [31]. In the *test* specimens, new bone was tightly contacting the implant surface, with better adaptation to the threads. These results were confirmed by the 3D reconstruction of sections obtained using confocal laser scanning microscopy (CLSM), which showed the intimacy of the contact between the bone and *test* surfaces through the entire thickness of the specimens [31]. The authors concluded that the use of implants with novel nanostructured calcium–phosphate surface may be indicated in areas of poor bone quality [31].

Finally, Tellemann and coll. [32] inserted two experimental mini-implants (one dual acid-etched implant as the *control* and one dual acid-etched implant conditioned with discrete deposition of nanometer-sized calcium phosphate particles as the *test*) to fixate an iliac crest bone graft to the maxilla of 15 patients. A part of each mini-implant was in contact with the grafted bone and a part extended into the native maxillary bone [32]. After an undisturbed healing period of 3 months, the specimens were harvested for the histological evaluation [32]. At the end of the study, the discrete deposition of nanometer-sized crystal of calcium–phosphate increased the peri-implant endosseous healing properties in the native bone of the maxilla compared with the conventional dual acid-etched surface, with a statistically higher BIC%; however, no significant difference in new bone apposition was reported in the bone graft area [32].

Shibli and coll. [33] evaluated the influence of two different implant surfaces (a bioceramic molecular impregnated surface as the *test* versus a dual acid-etched surface as the *control*) on the BIC% and bone osteocyte density in the human posterior maxilla after 2 months of unloaded healing. Ten patients received two implants (one of each surface) during conventional implant surgery in the posterior maxilla [33]. After an undisturbed healing period of 2 months, the implants and the surrounding tissue were removed for histologic/histomorphometric analysis [33]. Histometric evaluation showed significantly higher BIC% for the *test* compared to the *control* surface. These data suggested that the bioceramic molecular impregnated surface-treated implants positively modulated bone healing at early implantation times compared to the dual acid-etched surface [33].

Although all the aforementioned human studies suggest that treatment with nanometer-sized calcium phosphate particles can promote osseointegration, supporting new bone formation on the implant surface [30–33], still there are no histologic/histomorphometric studies on the immediate loading of NCI implants in humans.

Therefore, the aim of our present randomized, controlled histologic/histomorphometric study was to evaluate the early bone formation around immediately loaded NCI implants placed in the human posterior maxilla and to compare these results with those obtained with macroscopically identical implants with an MA surface. Fifteen fully edentulous patients were installed with two temporary transmucosal implants with different surfaces: one NCI (*test*) and one MA (*control*) implant. All temporary implants were placed in the posterior maxilla, according to a split-mouth design, and were subjected to immediate loading conditions, since they helped to support an interim complete maxillary denture. After 8 weeks, all clinically stable temporary transmucosal implants were retrieved for histologic/histomorphometric evaluation. In the MA implants, the histomorphometric evaluation revealed mean

BIC(±SD)% and BD(±SD)% of 21.2(±4.9)% and 29.8(±7.8)%, respectively. In the NCI implants, the histomorphometric analysis revealed mean BIC(±SD)% and BD(±SD)% of 39.7(±8.7)% and 34.6(±7.2)%, respectively. A statistically significant difference was found between the two surfaces with regard to BIC% ($p < 0.001$), while no significant difference was found with regard to BD% ($p = 0.09$). Hence, the results of our study seem to confirm that the deposition of calcium–phosphate nanoparticles on the implant surface can actually stimulate bone healing in the short-term, even under critical conditions, such as immediate loading in the posterior maxilla [22]. This can represent an important advantage today, in a context in which immediate loading is increasingly demanded by patients and practiced by clinicians [34, 35], as it may contribute to the survival and success of dental implants in the long term [22]. In our present study, in particular, a blasted titanium surface was thermally modified to form a nanostructured calcium-incorporated (NCI) surface [21]. This procedure has the potential to increase the osteoconductivity of endosseous implants at the cellular level. In fact, calcium titanate ($CaTiO_3$) has been shown to promote osteoblast adhesion and proliferation; moreover, increased calcium composition in the outer oxide layer increased protein adsorption onto the titanium surface by ionic bonding at a physiological pH, which subsequently affected cell adhesion [12, 13, 21]. This finally results in a biochemical bone bonding of NCI implants in vivo, as previously reported [13, 21] and confirmed here. Recently, several clinical studies have reported excellent survival and success rates for implants with a surface enriched with calcium ions through hydrothermal methods in different clinical contexts [6, 28, 29, 36, 37].

Our present study has limits, such as the limited number of implants placed and retrieved, as well as the dimensions of the fixtures inserted. In fact, in the present study, we have used implants of reduced dimensions (6.0 mm in height × 3.0 mm in diameter): this may be a limitation because the use of standard length and diameter implants could have led to different results [38]. In a recent systematic review reporting on human histologic/histomorphometric studies, the authors reported that there are differences in BIC% of commercially available and experimental mini-implants; in addition, the authors reported that the implant design, coupled with the anatomical region and the state of loading seem to have an influence on BIC% [38]. In our present study, only patients in whom both implants were clinically stable were considered for the histologic/histomorphometric evaluation. In fact, five implants (two *test* and three *control* implants) in three different patients were clinically unstable and showed no osseointegration: these patients were therefore excluded from the study, and their implants were not considered for the histologic/histomorphometric evaluation. Finally, in the present study, we did not conduct an analysis of the removal torque of implants. The existence of a strong positive correlation between the force necessary for removal of implants and the degree of bone–implant contact has long been known in the scientific literature [39, 40], and the biomechanical findings are often consistent with the histologic/histomorphometric data. For these reasons, it could be interesting to study the removal torque of the implants placed in this study and compare this evidence with that emerging from the histologic/histomorphometric evaluation. For all these reasons, more randomized controlled clinical studies are needed to confirm the evidence emerging from our present histologic/histomorphometric work.

Conclusions

Within the limits of these histologic/histomorphometric data, immediately loaded NCI temporary implants in human posterior maxilla presented statistically significantly higher BIC% compared to MA implants. However, these data must be considered with caution because of the study design and methodology (only stable implants were evaluated). Therefore, additional controlled randomized clinical studies are needed to draw more specific conclusions about the early bone response to NCI implants, when subjected to immediate loading.

Compliance with ethical standards The Institutional Clinical Research Ethics Committee of Guarulhos University (CEP #201/03) approved the protocol of the present study, which was conducted according to the principles outlined in the World Medical Association's Declaration of Helsinki on experimentation involving human subjects, as revised in 2008.

Conflict of interest The authors declare that they have no conflict of interest.

Funding This work has not received any funding.

Ethical approval All procedures involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Research Article

Early Bone Formation around Immediately Loaded Transitional Implants Inserted in the Human Posterior Maxilla: The Effects of Fixture Design and Surface

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Aim. To evaluate the effects of fixture design and surface on the early bone formation around immediately loaded implants inserted in the human posterior maxilla. *Materials and Methods*. Ten totally edentulous subjects received two transitional implants: one tapered implant with knife-edge threads/nanostructured calcium-incorporated surface (test: Anyridge®, Megagen, Gyeongbuk, South Korea) and one cylindrical implant with self-tapping threads/sandblasted surface (control: EZPlus®, Megagen). The implants were placed according to a split-mouth design and immediately loaded to support an interim complete denture; after 8 weeks, they were removed for histologic/histomorphometric analysis. The bone-to-implant contact (BIC%) and the bone density (BD%) were calculated. The Wilcoxon test was used to evaluate the differences. *Results*. With test implants, a mean BIC% and BD% of 35.9 (±9.1) and 31.8 (±7.5) were found. With control implants, a mean BIC% and BD% of 29.9 (±7.6) and 32.5 (±3.9) were found. The mean BIC% was higher with test implants, but this difference was not significant ($p = 0.16$). Similar BD% were found in the two groups ($p = 0.9$). *Conclusions*. In the posterior maxilla, under immediate loading conditions, implants with a knife-edge thread design/nanostructured calcium-incorporated surface seem to increase the peri-implant endosseous healing properties, when compared to implants with self-tapping thread design/sandblasted surface.

1. Introduction

In the last few years, the world of oral implantology and osseointegration has changed radically [1, 2].

In fact, new surgical techniques have been proposed, such as the placement of implants in extraction sockets [3, 4] and new prosthetic protocols, such as immediate [5, 6] or early [7] loading of the implants. These changes have been introduced to meet the modern needs of the patients, who wish to reduce the number of surgical sessions (and consequently the stress of the surgery and postoperative discomfort) and who want to be able to shorten the time of implant and prosthetic treatment [2, 3, 5].

The shortening of the treatment time translates into a reduction of the costs, with additional benefits for the clinician [4, 5].

However, the introduction of these new surgical and prosthetic protocols should not reduce, in the short and long term, the high percentages of survival and success recorded for rehabilitations supported by implants placed using conventional techniques, in fully healed ridges [8] and with delayed prosthetic loading [9, 10]. In fact, an increase in failures could be unacceptable for patients, who are increasingly demanding and would represent a major problem for clinicians [2, 4–6].

To be able to adapt to these new challenging surgical and prosthetic protocols, which continue to spread, while maintaining the high percentages of survival and success obtainable with conventional techniques, the industry has proposed a number of modifications and improvements of the implant macro- and microtopography [11–13].

The macrotopography (implant design) represents a very important element: it is believed that it can contribute significantly to the primary implant stabilisation [10, 11], together with patient-related factors (medical condition, bone quantity, and quality) [14, 15] and the experience and skills of the surgeon [16].

In fact, for the success of the implant therapy, it is well known that the fixture must have adequate stability at the time of positioning [5, 6, 11, 14]. In the absence of such stability, the risk of a failure is particularly high [11, 14]. The primary implant stabilisation is mainly of a mechanical nature, as it is determined mechanically by the interlocking between the threads of the implant and the preexisting bone at the recipient site [11, 14].

This primary stabilisation, however, must be followed by a proper secondary and biological stabilisation, due to the deposition, as fast as possible, of new bone onto the implant surface [7, 12, 13].

In fact, without this there is again the risk of implant failure due to a lack of osseointegration [12, 13]. Histologic studies have provided evidence that there is a period of bone remodeling following implant placement that results in a transient decrease in implant stability [17, 18]; resonance frequency analysis (RFA) evaluation has confirmed this evidence, reporting a drop in implant stability quotient (ISQ) values from the first to the third/fourth week following implant placement [19–21]. This reduction of the primary stability must therefore be balanced by an appropriate secondary stabilisation, determined by the deposition of new bone on the surface [11, 14, 22].

The influence of the macro- and micro/nanostructure of the implant on the success of osseointegration and in particular on the first healing phases of bone is now a subject of great interest for both researchers and clinicians [22]; the best way to assess the influence of design and implant surface on bone healing is certainly the histological and histomorphometric analysis of the interface between bone and implant [23, 24].

However, few studies to date have compared the influence of the macro- and micro/nanostructure of different implant systems on bone healing in humans [23, 25–29]: this is because it is difficult to perform comparative histologic and histomorphometric studies in humans, for ethical reasons.

Most of the studies available are based on a few samples [25] and implants are not subjected to immediate loading [23, 26, 27].

The purpose of this histological and histomorphometric study on humans is therefore to evaluate the early bone healing following the placement of implants with different macro- and microstructural characteristics, when positioned in the posterior maxilla and subjected to immediate loading.

2. Materials and Methods

2.1. Study Design. The present study was designed as a randomised controlled histologic/histomorphometric investigation, reporting on immediately loaded transitional transmucosal implants that were placed in the human posterior maxilla, and retrieved after a period of 8 weeks. In particular, this study aimed to compare the early bone response to tapered implants with knife-edge threads and a nanostructured calcium-incorporated surface (*test*: Anyridge, MegaGen, Gyeongbuk, South Korea) with the bone response to cylindrical implants with self-tapping threads and a sandblasted surface (*control*: EZPlus, MegaGen, Gyeongbuk, South Korea), when placed in the human posterior maxilla and subjected to immediate loading protocol. During a normal surgical procedure for the placement of conventional implants, each enrolled patient also received two transitional transmucosal implants ($n = 1$ *test* implant; and $n = 1$ *control* implant), which were inserted in the posterior maxilla, according to a split-mouth design. The transitional implants were placed with the aim of supporting an interim complete maxillary denture, until healing of the conventional implants. After 8 weeks, during the second-stage surgery to uncover the conventional implants, all transitional implants were retrieved for histologic/histomorphometric evaluation.

2.2. Patient Selection. A total of 10 fully edentulous patients (6 males, 4 females; aged between 46 and 77 years, mean age 61.7 ± 10.7, median 62, CI 95% 55.1–68.3) referred for oral rehabilitation with dental implants to the Oral Implantology Clinic, Dental Research Division, Guarulhos University, SP, Brazil, were consequently enrolled in the present study. The inclusion criteria were good systemic and oral health and sufficient native bone to place implants of 3.0 mm diameter and 6 mm length. The exclusion criteria were pregnancy, nursing, smoking, and any systemic condition that could affect bone healing. All participants received detailed explanations about the nature of the study and signed a written informed consent form. The Institutional Clinical Research Ethics Committee of Guarulhos University (CEP #201/03) approved the protocol of the present study, which was conducted in accordance with the Declaration of Helsinki on experimentation involving human subjects (2008).

2.3. Transitional Transmucosal Implants. The transitional transmucosal implants used in the present study were made of titanium grade 4. All implants were one-piece, 3.0 mm diameter × 6 mm length, but different in the macro- and micro/nanotopography.

The *control* implants (EZPlus, MegaGen, Gyeongbuk, South Korea) were cylindrical and featured a classical macroscopic design with self-tapping threads [22]. These implants were characterised by a surface blasted with particles of resorbable calcium phosphate (resorbable blast media, RBM). The surface was studied with scanning electron microscopy (SEM) (Figure 1) and the following standard roughness parameters were analysed: R_a (the arithmetic mean of the absolute height of all points), R_q (the square root of the sum of the squared mean difference of all points), and R_t (the

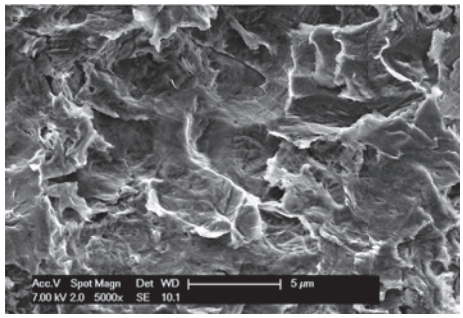

Figure 1: *Control* implant. Scanning electron microscopy of the resorbable blast media surface. Scanning electron microscopy evaluation revealed a mean R_a of 1.56 (±0.08) μm, a mean R_q of 2.11 (±0.13) μm, and a mean R_t of 18.53 (±1.56) μm, respectively. Magnification 5000x.

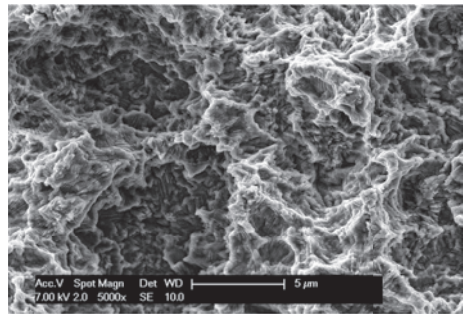

Figure 2: *Test* implant. Scanning electron microscopy of the nanostructured calcium-incorporated surface. Scanning electron microscopy evaluation revealed a mean R_a of 1.63 (±0.22) μm, a mean R_q of 2.16 (±0.30) μm, and a mean R_t of 15.76 (±0.29) μm, respectively. Magnification 5000x.

difference between the highest and the lowest points). The scanning electron microscopy evaluation revealed a mean R_a of 1.56 (±0.08) μm, a mean R_q of 2.11 (±0.13) μm, and a mean R_t of 18.53 (±1.56) μm, respectively.

Conversely, the *test* implants (Anyridge, MegaGen, Gyeongbuk, South Korea) were characterised by a tapered design with knife-edge, thin self-cutting threads [6, 22, 30–32]. The *test* implants had a nanostructured, calcium-incorporated surface (Xpeed®, Megagen Implant Co., Gyeongbuk, South Korea). This surface was obtained by modifying the original grit-blasted surface (resorbable blast media, RBM), which was enriched with calcium using a hydrothermal method. In brief, RBM implants were immersed in a mixed solution of 0.2 M sodium hydroxide (NaOH) and 2 mM calcium oxide (CaO) dissolved in deionized water using a Teflon-lined hydrothermal reactor system at 180°C for 24 h under a water pressure of 1 MPa^2. With this procedure, a nanolayer of Ca^{2+} ions was incorporated onto the RBM surface, giving a $CaTiO_3$ nanostructure [7, 30, 33]. Again, the surface was studied with scanning electron microscopy (SEM) (Figure 2). In this case, the SEM evaluation revealed a mean R_a of 1.63 (±0.22) μm, a mean R_q of 2.16 (±0.30) μm, and a mean R_t of 15.76 (±0.29) μm, respectively.

2.4. Surgical Protocol. Twenty transmucosal transitional implants ($n = 10$ *test* implants and $n = 10$ *control* implants) were inserted in this study. All implants were placed under aseptic conditions. After local anaesthesia, a crestal incision connected with two releasing vertical incisions was made. Mucoperiosteal flaps were raised and conventional implants were inserted, in accordance with the surgical and prosthetic plan prepared for each patient. After placement of the conventional implants, two transitional transmucosal implants ($n = 1$ *test* implant and $n = 1$ *control* implant) were inserted in each patient, according to a split-mouth design. The transitional implants were inserted in the posterior region of the maxilla, among the conventional placed implants. The assignment of *test* and *control* implants (right posterior maxilla or left posterior maxilla) was random, as determined by a coin toss. The implant sites were prepared according to the manufacturer's recommendations, under profuse irrigation with sterile saline. The stability of all the implants was checked using a dedicated instrument (Osstell Mentor®, Osstell, Goteborg, Sweden): if an implant showed insufficient primary stability (implant stability quotient- ISQ <35), it was removed and a backup surgical site had to be prepared. The flaps were then sutured, to allow the emergency of the solid abutment of one-piece implants through the mucosa: these implants helped to support the interim maxillary denture during the entire healing period. Immediately after implant surgery, an interim maxillary denture was seated in the patient's mouth and relined intraorally. The stability of the interim complete denture, its retention, and the occlusion were carefully controlled. Clindamycin 300 mg (ClindaminC®, Teuto, Anapolis, Goias, Brazil) was administered three times a day for one week, to prevent infection. Postoperative pain was controlled with 600 mg ibuprofen (Actron®, Bayer Schering Pharma, Berlin, Germany) every 12 h for 2 days. To enable subjects to control postoperative dental biofilm, 0.12% chlorhexidine mouth rinses (Chlorhexidine®; Oral B, Boston, MA, USA) were prescribed, twice a day for 2 weeks. The sutures were removed after 10 days.

2.5. Specimen Retrieval and Histologic/Histomorphometric Analysis. The interim prosthesis remained connected to the transitional implants for a period of 8 weeks. After this period, during the 2-stage surgery to uncover the conventional implants, all clinically stable transitional fixtures (one *test* and *one* control implants) and the surrounding tissues were retrieved from each patient, using a 4.5-millimeter-wide trephine bur. Clinically mobile temporary implants were not considered for the histologic/histomorphometric evaluation. The specimens were fixed by immediate immersion at 10% buffered formalin and processed (Precise 1 Automated System®, Assing, Rome, Italy) to obtain thin sections, as previously described [23]. The specimens were dehydrated in an ascending series of alcohol rinses and embedded in glycol methacrylate resin (Technovit 7200 VLC®, Kulzer, Wehrheim, Germany). After polymerization, the specimens were cut longitudinally along the major axis of the implants with a high-precision diamond disc at about 150 μm and ground down to about 30 μm. Two slides were obtained for

each implant. The slides were stained with basic fuchsin and toluidine blue. The specimens were studied using a transmitted-light microscope (Laborlux S®, Leitz, Wetzlar, Germany) interfaced with a high-resolution camera (3CCD-JVC KY-F55B®, JVC, Yokohama, Japan) and to a monitor and a personal computer (Intel Pentium III 1200 MMX®, Intel, Santa Clara, CA, USA). The whole system was connected to a digitizing pad (D-Pad®, Matrix Vision GmbH, Oppenweiler, Germany) and controlled by specific software for image capture (Image-Pro Plus® 4.5, Media Cybernetics, Immagini & Computer snc, Milan, Italy). For the histomorphometric evaluation, the bone-to-implant contact (BIC%), defined as the amount of mineralized bone in direct contact with the implant surface, was measured around all implant surfaces. Finally, the bone density (BD%) in a 500 μm wide zone lateral to the implant surface was measured bilaterally, as previously reported.

2.6. Statistical Analysis. The mean, standard deviation, median, and confidence intervals (CI 95%) of histomorphometric values (BIC%, BD%) were calculated for each implant and then for each group of implants (*test* versus *control* implants). Comparisons of the differences in bone-implant percentages values in both groups were carried out using the Wilcoxon matched-pairs signed-rank test. The level of significance was set at 0.05. Results were presented as mean ± standard deviation (SD) and differences at $p < 0.05$ were considered statistically significant. All computations were carried out with a statistical analysis software (SPSS 17.0®, SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Clinical Observations. Two months after placement, a total of 20 transitional transmucosal implants ($n = 10$ *test* implants and $n = 10$ *control* implants) were evaluated and retrieved. Two implants (one *test* implant and one *control* implant, placed in the same patient) were clinically unstable and showed no osseointegration, although they did not show any sign of infection. These two implants were excluded from the study and were not histologically/histomorphometrically evaluated. The remaining 18 implants were clinically stable at the time of retrieval and were therefore histologically/histomorphometrically evaluated.

3.2. Histologic/Histomorphometric Evaluation. In the *test* implants, at low-power magnification, it was possible to see newly formed bone around and in contact with the implant surface. Around the implant collar, soft tissues were present. In the coronal portion, only newly formed bone with a trabecular structure and strongly stained with acid fuchsin could be observed. In the middle and apical portion of the implant, the native bone was evident far from the surface (Figure 3). At higher magnification, in the interthread concavities the newly formed bone was in contact with the implant surface and adapted perfectly to its microirregularities. Native bone in contact with newly formed bone could be seen. Osteoblasts secreting osteoid matrix near the bone-implant interface were found (Figure 4). Wide osteocyte lacunae could be observed often when they were in close vicinity to the implant surface. No inflammatory cell infiltrate was present. The histomorphometric evaluation revealed a BIC% of 35.9 ± 9.1 and a BD% of 31.8 ± 7.5, respectively. The BIC% ranged from 19.2 to 49.9; the median was 38.8; confidence interval (95%) was 29.9–41.8. The BD% ranged from 19.0 to 44.7; the median was 32.4; confidence interval (95%) was 26.9–36.7.

Figure 3: *Test* implant. Newly formed trabecular bone surrounded the whole implant perimeter. (Acid fuchsin and toluidine blue, magnification 12x).

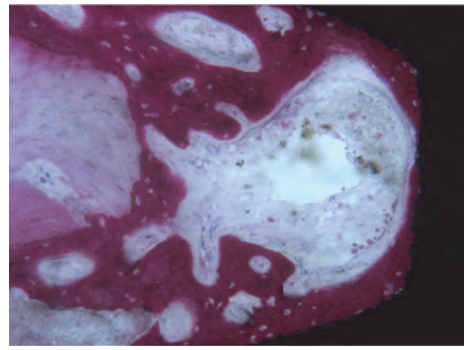

Figure 4: *Test* implant. The implant thread was lined by newly formed bone and an intense osteoblastic activity was still evident. (Acid fuchsin and toluidine blue, magnification 100x).

In the *control* implants, at low-power magnification, trabecular bone with small marrow spaces was mainly present in the coronal portion of the implant, while in the middle portion they tended to be wider (Figure 5). In the apical area, bone tissue was lacking. At higher magnification, newly formed bone tissue could be observed inside the thread concavity with osteocyte lacunae in contact with the surface. Not yet mineralized osteoid matrix could also be seen (Figure 6). The histomorphometric analysis revealed a BIC% of 29.9 ± 7.6 and a BD% of 32.5 ± 3.9, respectively. The BIC% ranged from 20.7 to 35.6; the median was 28.7; confidence interval (95%) was 24.6–35.2. The BD% ranged from 29.0 to 41.1; the median was 32.0; confidence interval (95%) was 29.8–35.2.

Figure 5: *Control* implant. The density of the bone tissue was different along the implant perimeter ranging from a more compact bone in the coronal portion to a very trabecular bone in the apical areas. (Acid fuchsin and toluidine blue, magnification 12x).

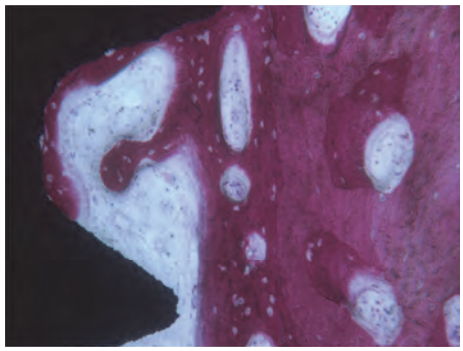

Figure 6: *Control* implant. Part of the implant thread was surrounded by newly formed bone and not yet mineralized osteoid matrix. (Acid fuchsin and toluidine blue, magnification 100x).

Although the mean BIC% was higher in the *test* implants, this difference was not statistically significant ($p = 0.16$). Similar BD% were found in the two groups ($p = 0.9$). The histomorphometric results were summarised in Table 1 and displayed in Figure 7.

4. Discussion

At present, histologic/histomorphometric assessment is the most accurate method to investigate the bone healing processes and morphological characteristics of the bone-implant interface [22–24].

Unfortunately, only a few studies in the present literature have dealt with histologic/histomorphometric evaluation of human-retrieved implants [23, 25–29]; this is because of ethical issues related to implant retrieval from human subjects [23]. For this reason, little is known about the effects of different implant designs and surfaces on the early bone healing around dental implants [25].

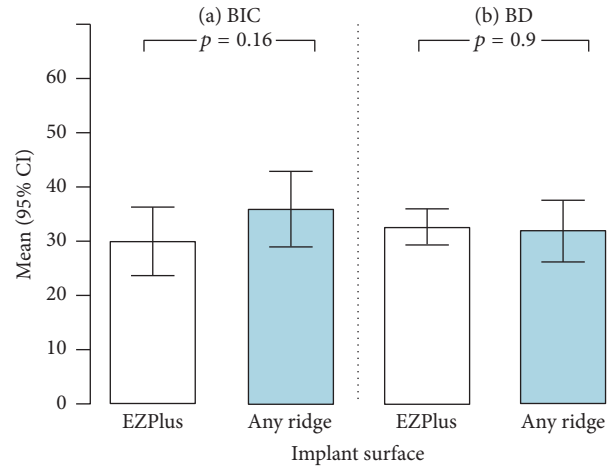

Figure 7: Histomorphometric results with EZPlus and Anyridge implants: bone-to-implant contact (BIC%) and bone density (BD%). In the EZPlus implants, the histomorphometric evaluation revealed mean (±SD) BIC% and BD% of 29.9 (±7.6) and 32.5 (±3.9), respectively. In the Anyridge implants, the histomorphometric analysis revealed mean (±SD) BIC% and BD% of 35.9 (±9.1) and 31.8 (±7.5), respectively.

In a recent systematic review reporting on human histologic/histomorphometric studies, the authors found that the bone-to-implant contact (BIC%) in the lower jaw is higher than in the upper jaw and that the BIC% in the anterior areas is higher than in the posterior areas [25]. In addition, they found that the implant design is a factor capable of affecting the BIC% [25]. In fact, the insertion of mini-implants in the posterior region results in lower outcomes, and differences were detected in the BIC% of standard length/diameter implants and mini-implants [25]. Finally, with regard to the loading protocols, the authors found that conventionally loaded implants had a higher BIC% than immediately loaded implants [25].

In the present randomised and controlled histologic/histomorphometric study, we have assessed the early bone healing of two different implants, under immediate loading in the human posterior maxilla. In particular, we have compared two different implants, with different design and surface, in order to understand which of the two could determine the best histologic and histomorphometric result. Twenty transitional transmucosal fixtures (6 mm length × 3.0 mm diameter) were inserted in the posterior maxilla, 10 *test* implants and 10 *control* implants; all these implants were subjected to immediate loading (as they helped to stabilise an interim complete removable denture) and remained in place for a period of two months, after which they were removed and analysed histologically. *Control* implants were characterised by a conventional macroscopic design, as well as by V-shaped, self-tapping threads, with four cutting edges; the surface of these implants was blasted with calcium phosphate particles (resorbable blast media treatment) and therefore it possessed microtopographic features. *Test* implants featured a novel knife-edge thread design. The surface of the *test* implants represented the development of the previous sandblasted

Table 1: Bone to implant contact (BIC%) and bone density (BD%): means, standard deviations, medians, ranges, and confidence intervals for *test* and *control* implants, respectively.

	Mean	SD	Median	Range	CI 95%	p
BIC%						
Test implants	35.9	9.1	38.8	19.2–49.9	29.9–41.8	0.16
Control implants	29.9	7.6	28.7	20.7–35.6	24.6–35.2	
BD%						
Test implants	31.8	7.5	32.4	19.0–44.7	26.9–36.7	0.9
Control implants	32.5	3.9	32.0	29.0–41.1	29.8–35.2	

surface, as a result of an ultrastructural treatment for superimposition/incorporation of calcium ions: it was therefore a nanostructured surface. Two months after placement and functional loading, the histologic evaluation revealed newly formed bone around and in contact with the surface of both implants; the new bone was formed in the interthread cavities, with osteoblasts secreting osteoid matrix near the bone-implant interface. These positive histologic outcomes were confirmed by the histomorphometric evaluation, with high percentages of bone-to-implant contact with both *test* and *control* implants. The histomorphometric results seemed to favour the *test* implants, for which a mean value (±SD) of BIC% corresponding to 35.9% (±9.1) was obtained; this value was higher than that found in *control* implants, which corresponded to 29.9 (±7.6). The contact between bone and implant values was higher in *test* implants; however, this difference was not statistically significant ($p = 0.16$). The BD% values were instead equivalent in the two groups ($p = 0.9$), with an average value for the *test* fixtures (31.8 ± 7.5) which was similar to that reported for the *control* fixtures (32.5 ± 3.9).

In the present study, the BIC% of the *test* implants (35.9% ± 9.1) was higher than that of the *control* implants (29.9 ± 7.6), although there was no statistically significant difference ($p = 0.16$) between the two groups. This result is not negligible. In fact, in particularly difficult clinical contexts such as the placement of implants in low quality bone areas (posterior maxilla) [6, 7, 10, 18] or in the case of immediate loading protocols [1, 2, 4, 6], it is important to achieve and maintain, in the short and medium term, high percentages of contact between bone and implant. This is because, in the end, high percentages of contact between bone and implant can determine the success, or failure, of the therapy [22].

At the time of positioning, the implant stabilisation is obtained mechanically, through the interlocking between the implant threads and the preexisting bone [10, 11, 14, 22]; however, in the next 3-4 weeks, a partial resorption of the bone tissue involved in this primary stabilisation occurs physiologically [11, 14, 22]. It is therefore necessary to deposit new bone on the implant surface, to counteract this physiological resorption and to avoid the mobilisation (and loss) of the implant [14, 22].

The aim of modern implantology is therefore twofold: on the one hand, it aims to maximise the primary stability at implant placement, through the search for new designs and macrotopographies that enable effective stabilisation and a high bone-to-implant contact [22, 34, 35]; on the other hand, it intends to counteract the physiological fall of stability occurring due to remodeling phenomena, stimulating new bone deposition on the implant, through the use of bioactive surfaces [12, 13, 33, 36].

In the present work, the best result of contact between bone and implant can be due either to the design or the surface of the *test* implants. The novel thread design of *test* implants may, in fact, result in maximum bone-to-implant contact (BIC), maximised compressive force resistance, and minimised shear force production; thereby it has the potential to prevent a drop in stability in the immediate postplacement healing period [22, 34]. At the same time, the novel nanostructured calcium-incorporated surface of *test* implants may stimulate a faster new bone formation onto the implant surface, through increased surface area and increased free energy, as currently reported in the scientific literature [7, 12, 13, 33, 35, 36].

The present histologic/histomorphometric study supports the concept that implants with knife-edge threads and a nanostructured calcium-incorporated surface seem to represent the best choice in the event of clinically challenging situations (such as areas of poor bone quality, or immediate loading protocols), at least when compared with implants with self-tapping threads and a sandblasted surface. Several clinical studies have confirmed that implants with knife-edge threads and a nanostructured calcium-incorporated surface can successfully support different kinds of prosthetic restorations, under different loading protocols, with high survival rates, at least in the short term [6, 7, 30–32, 37].

The present study has limitations. A critical factor for the present study is the fact that we have compared two implant systems that are characterised by different designs (macrotopography) and surfaces (micro/nanotopography); to better assess the effects of micro/nanotopography of the implant surface on early bone healing, it would be more appropriate to compare two macroscopically identical fixtures that differ only in the surface [23, 26–28]. Similarly, to more effectively assess the effects of macrotopography on early bone healing, it would have been more appropriate to compare implants with different thread designs, but characterised by the same surface topography. Another limitation of this study is the number of enrolled patients (10) and positioned implants (20): a larger number of patients and implants would certainly have been preferable, but in the specific case it was not possible to enrol more than 10 patients.

Moreover, in the present work, two fixtures (1 *test* implant and 1 *control* implant, inserted in the same patient) were not clinically stable at the time of removal, due to lack of osseointegration: these fixtures were excluded from histologic and histomorphometric evaluation, and this could be another limitation of our research. Finally, in the present study we have used implants of reduced dimensions (6.0 mm in height × 3.0 mm in diameter): this may be a limitation because the use of standard length and diameter implants could lead to different results, compared to the outcomes found here. If we examine it more carefully, however, the fact that the small implants have been used may even be an advantage of this study: in fact, excellent histologic and histomorphometric results have emerged from the removal, after 2 months of functional loading, of these immediately loaded, short, and narrow fixtures [27, 28], placed in the posterior maxilla. In any case, it would not have been ethically possible here to use implants with a standard length and diameter. Further randomised controlled studies on a larger number of patients are required, in order to confirm the positive findings from this work.

5. Conclusions

In the present histologic/histomorphometric study in the human posterior maxilla, immediately loaded implants with a knife-edge thread design and nanostructured calcium-incorporated surface increased the peri-implant endosseous healing properties, when compared with immediately loaded implants with a self-tapping thread design and sandblasted surface. The present data must be considered with caution because of the study design and methodology (only stable implants were evaluated) and the limited number of patients enrolled and fixtures inserted. Therefore, additional controlled randomised clinical studies are needed to draw more specific conclusions about the early bone response to implants with a knife-edge thread design and a nanostructured calcium-incorporated surface.

Competing Interests

The authors report no conflict of interests for the present study, as they did not receive any financial support for the present histologic/histomorphometric investigation and they do not have any financial interest in the materials presented here.

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4. CONCLUSÃO

Baseado nas observações e resultados histológicos obtidos, conclui-se que o processo de osseointegração foi influenciado pelo tipo de superfície do (microgeometria) e pelo desenho (macrogeometria) dos implantes. Embora os resultados oriundos da avaliação do tecido peri-implantar humano sejam importantes, estudos clínicos avaliando o comportamento longitudinal desses implantes sobre a longevidade e taxas de sucesso das restaurações implantossuportadas são necessários para um melhor entendimento.

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