UNIVERSIDADE SÃO FRANCISCO
Programa de Pós-Graduação *Stricto Sensu* em Ciências da Saúde

CARLOS HENRIQUE ALVES

ASPECTOS MICROBIOLÓGICOS PARA O SUCESSO A LONGO PRAZO DE IMPLANTES DENTAIS

Bragança Paulista
2022

CARLOS HENRIQUE ALVES - R.A. 001202010035

ASPECTOS MICROBIOLÓGICOS PARA O SUCESSO A LONGO PRAZO DE IMPLANTES DENTAIS

Dissertação apresentada ao Programa de Pós-graduação Stricto Sensu em Ciências da Saúde da Universidade São Francisco, como requisito parcial para obtenção do Título de Mestre em Ciências da Saúde.

Área de Concentração: Biologia Celular e Molecular.

Orientadora Profa Dra Raquel Girardello.

Bragança Paulista
2022

WU 640
A478a

Alves, Carlos Henrique
Aspectos microbiológicos para o sucesso a longo prazo de implantes dentais / Carlos Henrique Alves. – Bragança Paulista, 2022.
75 p.

Dissertação (Mestrado) – Programa de Pós-Graduação *Stricto Sensu* em Ciências da Saúde da Universidade São Francisco.
Orientação de: Raquel Girardello.

1. Implantes odontológicos. 2. Peri-implantite. 3. Infecção. 4. Cicatrizadores. 5. Microbiota oral. I. Girardello, Raquel. II. Título.

Sistema de Bibliotecas da Universidade São Francisco – USF
Ficha catalográfica elaborada por: Denise Isabel Arten / CRB-8/5823

Educando
para a paz

ALVES, Carlos Henrique. "Aspectos microbiológicos para o sucesso a longo prazo de implantes dentais". Dissertação defendida e aprovada no programa de Pós-Graduação *Stricto Sensu* em Ciências da Saúde da Universidade São Francisco em 11 de fevereiro de 2022 pela Banca examinadora constituída pelos professores:

Profa. Dra. Raquel Girardello - Orientadora e Presidente
Universidade São Francisco

Prof. Dr. Fabio Valverde Rodrigues Bastos Neto (por videoconferência)
Universidade Cruzeiro do Sul

Profa. Dra. Michelle Darrieux Sampaio Bertoncini
Universidade São Francisco

Profa. Dra. Thais Parisotto Ulmer
Universidade São Francisco

Dedicatória

Dedico este manuscrito à minha família e aos meus amigos que sempre estiveram presentes em minha vida, motivando e participando de minhas conquistas. Dedico especialmente ao meu pai (*in memoriam*) que sempre acreditou em mim em todos os momentos.

Agradecimentos

Agradeço minha orientadora Profa. Dra. Raquel Girardello por toda orientação, carinho, eficiência e dedicação, sendo um exemplo profissional e uma referência contínua.

Agradeço às Profs. Dra. Thais Parisotto e Dra. Michelle Darrieux, pelo apoio e auxílio na redação dos artigos que compõe este documento.

Agradeço a todos os professores da Pós-graduação da Universidade São Francisco por todo conhecimento transmitido, fundamentais para minha formação.

Agradeço aos membros do laboratório de Microbiologia da Universidade São Francisco pela colaboração na execução deste trabalho, em especial á Natália Rocha.

Agradeço aos meus familiares pelo apoio incondicional.

Agradeço à André Cândido pelo suporte técnico de informática e aos meus amigos pelo apoio. Em especial à Aderbal Cunha Junior.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento: 88887.495991/2020-00. Agradeço à CAPES pelo suporte necessário para que este trabalho fosse realizado.

Epígrafe

“Quem estudou latim se lembra que a palavra "feliz" é “felix”, que significa também "fértil". Felicidade é sinônimo de fertilidade. Fertilidade não é apenas gerar outras pessoas. Fertilidade é impedir que a vida cesse na sua múltipla condição. Fertilidade é dificultar a desertificação dos nossos sonhos. Fertilidade é fazer com que não haja a esterilização do nosso futuro. Ser feliz é sentir-se fértil”. (Mário Sérgio Cortella)

RESUMO

A Implantodontia vem se modificando ao longo do tempo, visando qualidade e longevidade. O conhecimento da microbiota é importante para a compreensão da interação microbiológica natural, patógenos, fungos e consequentemente a formação de biofilme. Uma das maiores causas de perda de implantes odontológicos é a peri-implantite. Este manuscrito tem como objetivo descrever os aspectos microbiológicos envolvidos no sucesso ou insucesso de um implante dentário sendo composto de uma revisão de literatura sobre peri-implantite e análise epidemiológica de pacientes de uma universidade nos últimos 4 anos, na tentativa de justificar a descrição clínica de infecção após colocação de cicatrizador em implantes odontológicos. Os resultados laboratoriais mostraram um aumento expressivo na concentração de Firmicutes e Bacteroidetes na região de língua e bochecha e nenhuma alteração quanto ao número de bactérias totais, após o uso de antibiótico e antisséptico. Além disso, foi detectado um número alto de *Streptococcus mutans* previamente ao procedimento cirúrgico na região de língua e bochecha, porém mínima concentração da mesma bactéria no tecido gengival, na região de instalação do implante. Sugerimos que essa concentração menor de *S. mutans* pode ter facilitado a colonização por outro microrganismo, agindo como um facilitador para instalação de possíveis patógenos resistentes e consequente infecção. O esmagamento e posterior necrose do tecido gengival no momento da instalação do cicatrizador, pode ser outra justificativa, além da possibilidade de resíduos alimentares terem causado infecção *in loco*. Na análise de prontuários, não foi identificado nenhum caso de paciente que apresentou infecção após a colocação do cicatrizador nos últimos 4 anos, sendo, portanto, um caso de infecção raro. Dessa forma, nossas observações alertam para processos infecciosos, após o sucesso inicial do implante. Alterações na microbiota oral do paciente detectadas previamente ao procedimento cirúrgico, sugerem uma vulnerabilidade no momento de reabertura para colocação do cicatrizador e que, associado a um processo inflamatório gerado pelo procedimento, pode ter facilitado o desenvolvimento da infecção após sua colocação. O uso de antibióticos e antissépticos levou a uma alteração dos principais filos que compõe a microbiota oral e, também pode ser um fator seletivo para patógenos que podem se alojar no local do implante e posteriormente gerar uma nova infeção, causando peri-implantite e possível perda do implante.

Descritores: implante dentário. peri-implantite. infecção. microbiota

ABSTRACT

Implantology has been changing over time, aiming for quality and longevity. The knowledge of the microbiota is important for understanding the natural microbiological interaction, pathogens, fungi and, consequently, biofilm formation. One of the biggest causes of dental implant loss is peri-implantitis. This manuscript aims to describe the microbiological aspects involved in the success or failure of a dental implant and it consists of a literature review on peri-implantitis and epidemiological analysis of university patients in the last 4 years, trying to justify the clinical description of infection after placement of a healing cap in dental implants. The laboratory results showed a significant increase in the concentration of Firmicutes and Bacteroidetes in the tongue and cheek region, and no change in the number of total bacteria, after antibiotic and antiseptic usage. We have detected a high charge of *Streptococcus mutans* prior to the surgical procedure in the tongue and cheek region, but a minimal concentration of the same bacteria in the implant installation region, where a higher concentration was expected. We suggest that it is one of the factors that, associated with the use of antibiotics, may have been a facilitator for the installation of possible resistant pathogens and consequent infection. The crushing and subsequent necrosis of the gingival tissue at the time of installation of the healing device can be another reason, in addition to the possibility that food residues might have caused an infection *in loco*. In the four years patient`s records evaluation, infection was not detected after healing cap installation. So, dentistry should be aware of infectious processes, even after the implant is successful. Previous alterations in the patient's oral microbiota suggest a vulnerability at the time of reopening for placement of the healing cap, and which, associated with an inflammatory process generated by the procedure, may have facilitated the development of the infection after its placement. In addition, the use of antibiotics may select pathogen strains that can lodge in the implant site and, later, lead to a new infection, causing peri-implantitis and implant loss.

Keywords: *dental implantation. peri-implantitis. infection. microbiota*

Lista de Símbolos e Abreviações

HE	Implante tipo hexágono externo
HI	Implante tipo hexágono interno
CM	Implante tipo Cone Morse
EG	Epitélio gengival
EJI	Epitélio juncional peri-implantar
TCG	Tecido conjuntivo gengival
O	Osso alveolar

Lista de Gráficos e Figuras

SUMÁRIO

1. Introdução

Histórico da Implantodontia

A reabilitação oral através de implantes é um desafio e, há décadas, foi iniciado com tentativas obtendo-se acertos e erros ao longo do tempo. Civilizações egípcias e sul-americanas antigas fabricavam implantes para substituição de dentes. Estes implantes eram feitos de dentes de animais ou marfim. Contudo, há dúvidas quanto à sua funcionalidade e longevidade na época (1,2). Segundo BLOCK (3), recentemente, implantes intraósseos foram produzidos com superfície rugosa, sem causar lesões às estruturas ósseas adjacentes e com projeções funcionais a longo prazo. Em meados de 1938, dispositivos metálicos começaram a ser implantados em diversas partes do corpo. Cobalto-cromo-molibdênio foi utilizado em um design de implante rosqueado e instalado em alvéolo, coberto com osso autógeno logo após a extração dental (4,5). Esse implante, foi considerado bem-sucedido em termos de funcionalidade. Apesar disso, de acordo com SCHNITMAN (6), somente no "Congresso de Consenso de Implantes Dentários" em 1978, uma variedade de medidas foi apresentada como referência para implantes dentários; e acordou-se que o trabalho de Per-Ingvar Branemark seria o padrão estabelecido para avaliação de implantes. Em 1982, Branemark apresentou em conferência um trabalho de 15 anos, com excelente embasamento científico e um acompanhamento clínico de longo prazo, onde a eficiência na instalação dos implantes foi mensurada através de perda óssea, baseada em radiografias, saúde gengival, funcionalidade e conforto do paciente (7,8). Suas referências são seguidas até os dias de hoje.

De acordo com BASTOS-NETO (9), existem diversas técnicas cirúrgicas possíveis, sistemas anti-rotacionais, carregamentos tardios ou imediatos, tratamentos de superfície de implante e tipos de prótese que podem levar a dificuldades no planejamento, portanto, toda prótese implanto suportada deve começar pelo final, ou seja, primeiramente planeja-se a prótese desejada e depois, por consequência, a correta posição dos implantes (Figura 1). Segundo o autor, modelos montados em articulador, enceramento diagnóstico, exames radiográficos, relação espacial entre os arcos, comprimento do espaço protético, comprimento e altura das futuras coroas, além de necessidade de gengiva artificial e posição e número de implantes, são dados e manobras fundamentais para um bom planejamento de tratamento.

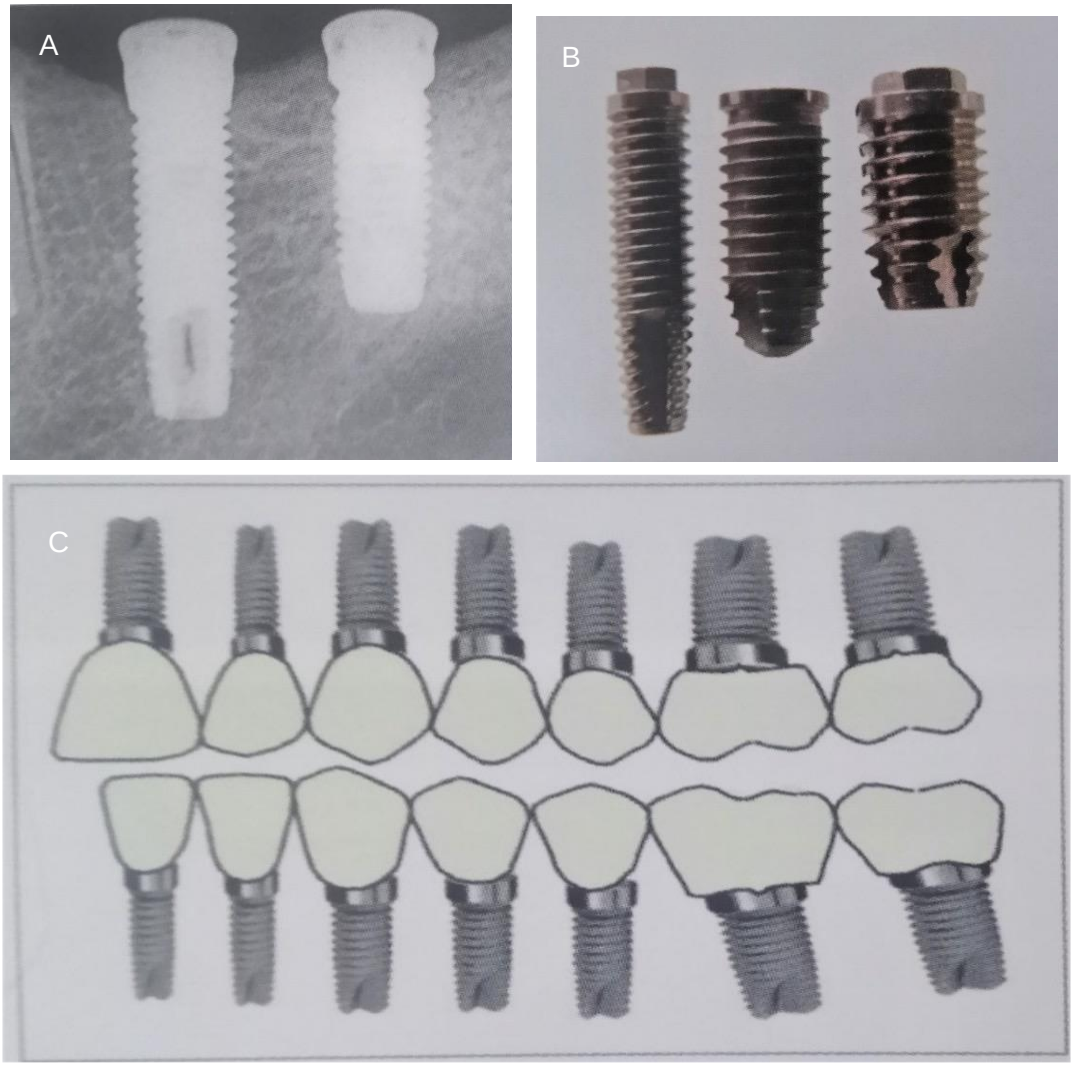


FIGURA 1: Diferentes tipos e tamanhos de implantes dentários. **A** e **B**: Indicam exemplos de implantes de tamanhos e diâmetros diferentes. **C**: Indicação de diâmetros de implantes para cada elemento dental. Fonte: BASTOS NETO (9).

Biomateriais

Segundo NIINOMI (10), biomateriais podem ser definidos como qualquer material, natural ou sintético, que possa ser utilizado por qualquer período, que possa interagir com o sistema biológico, com o objetivo de manter ou melhorar a qualidade de vida do indivíduo. Estudos vêm demonstrando, ao longo dos anos, que o Titânio é o material mais biocompatível para implantes

dentários. Segundo SABINO (11), o titânio e suas ligas são os biomateriais mais utilizados na ortopedia e implantes dentários.

A superfície do biomaterial é de extrema importância para propiciar uma melhor osteocondutividade e, portanto, um melhor processo de osteointegração. Contudo, apesar das propriedades biológicas e mecânicas observadas no titânio, seus compostos ativos antimicrobianos não são suficientes para evitar a colonização por microrganismos, portanto, a infecção microbiana ainda permanece a principal causa da perda de implantes (12).

Ao longo dos anos foi se observada a necessidade da busca de melhores processos de osteointegração associados à superfície dos biomateriais. É comum a utilização de soluções alcalinas e soluções de vidro bioativado, individualmente ou em conjunto, no tratamento de implantes de titânio (13). A utilização em conjunto constitui um método de modificação combinada, que apresenta uma das mais altas performances, tanto mecanicamente quanto biologicamente, em comparação com métodos individuais das superfícies estudadas. SABINO (11) afirma que o tratamento com manganês e solução de vidro bioativo aplicados nos feixes tubulares do titânio, demonstraram habilidade em aumentar a capacidade osteogênica, sendo sugerido para implantes dentários.

Zircônia é descrito na literatura como um material para substituição do Titânio. Segundo BORGES (14), implantes instalados, produzidos a base de zircônia, apresentam alto índice de sucesso nos implantes instalados e um desenvolvimento clínico muito satisfatório, dentro de uma avaliação a curto prazo, em comparação com os implantes de titânio. Por outro lado, AFRASHTEHFAR (15) afirma que, apesar da zircônia ser um bom material, estudos realizados apresentam resultados analisados em períodos curtos, o que torna necessário estudos a longo prazo para uma melhor avaliação do biomaterial.

Atualmente, diversos filmes de nanopartículas metálicas vêm sendo desenvolvidos e estudados para promover a redução do crescimento de bactérias patogênicas. O objetivo é não somente combater infecções, mas também as prevenir. Além disso, estudos *in vitro* foram desenvolvidos, onde implantes dentários foram revestidos com depósito de peptídeos antimicrobianos em suas superfícies, no intuito de inibir a colonização de bactérias e,

consequentemente, o desenvolvimento de peri-implantite. A técnica preventiva através dos peptídeos antimicrobianos naturais, prevê a capacidade de destruir bactérias Gram positivas e negativas diretamente. Essa estratégia de revestimento de superfície, principalmente na região transmucosa, facilita a adesão das células epiteliais gengivais e células de tecidos de conexão. Estas células produzem os peptídeos antimicrobianos naturais e podem selar o tecido mole prevenindo o acesso da bactéria à região apical do implante ósseo integrado (16). No entanto, estudos de biossegurança para esta técnica ainda são necessários.

A literatura mostra, não somente a preocupação com a superfície do biomaterial, mas também a aderência de bactérias junto aos implantes. Segundo CHAI (17), inflamação e infecção são as maiores causas de perdas de implantes. Segundo o autor, disseleneto de molibdênio ($MoSe_2$) foi sintetizado e aplicado no revestimento do titânio durante seu tratamento, sugerindo resultados significativos quanto ao aumento das propriedades antibacterianas, *in vitro* e *in vivo*, contra *Streptococcus mutans.*

Microbiota Oral e Formação de Biofilme

Segundo SEDGHI et al. (18), a cavidade oral pré-natal é considerada estéril até o nascimento, porém após o nascimento a microbiota oral do bebê é semelhante à da mãe, onde sugere-se ser derivada da transmissão hematogênica ou intrauterina da mãe. Após o nascimento, a colonização bacteriana ocorre através da interação com pessoas, dieta, ou mesmo transmissão vertical da mãe. Segundo o autor, com a erupção dos dentes decíduos, inicia-se um processo de expansão de nichos microbianos na cavidade oral, proporcionando uma maior diversidade microbiana, que tendem a se estabilizar ao longo do crescimento até a fase adulta. A partir da fase adulta a microbiota passa a ser moldada por fatores genéticos e ambientais, como dieta, estresse, práticas de higiene oral, consumo de álcool e tabagismo. Com o envelhecimento, a diversidade da microbiota vai diminuindo e verificamos que a filogenia e as assinaturas funcionais da microbiota oral estão ligadas a doenças dentais e periodontais, além de doenças sistêmicas como diabetes, Alzheimer, doenças cardiovasculares e cânceres (Figura 2).

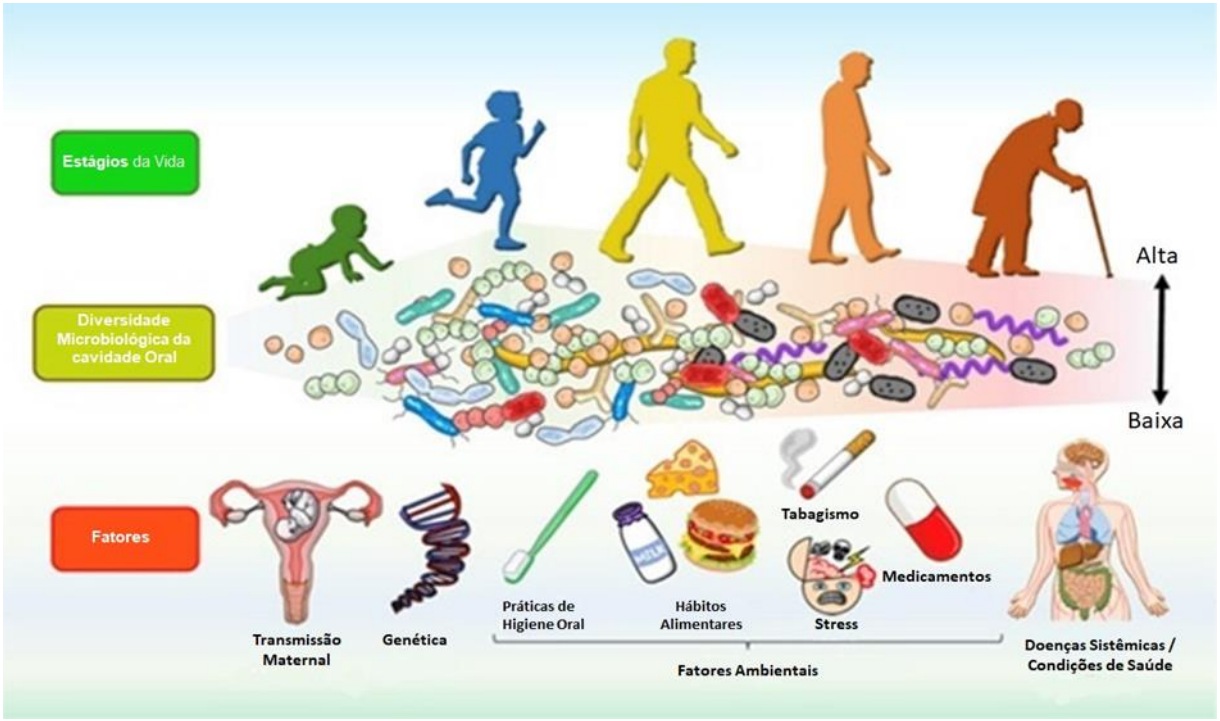


FIGURA 2. Ilustração descrevendo a diversidade da microbiota oral, levando em consideração estágios da vida, do nascimento à fase adulta e fatores biológicos e ambientais envolvidos nesse processo. Fonte: adaptado de SEDGHI et al. (18).

A microbiota oral apresenta uma ampla diversidade de bactérias, fungos e vírus, onde mais de 700 espécies de bactérias já foram identificadas (19). Segundo KUMAR et al. (20), as bactérias encontradas na cavidade oral possuem um importante papel na promoção de saúde bucal, além da etiologia das doenças da boca. Dentro dessa diversidade bacteriana existem aquelas espécies responsáveis pela ação preventiva contra as bactérias patogênicas. Segundo NOBBS et al. (21), bactérias comensais podem destruir patógenos e, provavelmente, parte do mecanismo preventivo seja a competição por nutrientes e sítios de aderência. Contudo, uma modificação na composição da microbiota com alteração de espécies Gram-positivas para um maior número de Gram negativas, associado ao acúmulo de biofilme, podem levar a doenças como periodontite, gengivite ou mesmo a cárie (22).

De acordo com BRITO (23), a maior parte das doenças ligadas à estrutura dentária e aos tecidos da cavidade oral, são causadas por biofilme microbiano. Segundo Hojo et al. (24), biofilmes dentários são caracterizados pela heterogeneidade estrutural, superfície de aderência, complexas

interações entre espécies e por uma matriz extracelular de substâncias poliméricas. Sua formação se inicia por meio de bactérias planctônicas que se aderem fisicamente e diretamente às superfícies da cavidade oral ou se conectam indiretamente às células de outras bactérias que já haviam colonizado a região. A adesão é seguida pela formação de uma matriz polissacarídica que mantém a integridade da comunidade microbiana, impedindo a ação do sistema imune e de substâncias antimicrobianas (25).

É importante ressaltar que a microbiota oral é, também, composta por uma quantidade significativa de fungos. De acordo com GHANNOUM et al. (26), foi constatado mais de 100 espécies diferentes de fungos na cavidade oral.

YODA et al. (27) afirma que, em todo o processo de instalação de um implante, estamos sujeitos a possíveis contaminações e infecções. Até mesmo após um longo período da instalação dos implantes, o paciente pode ser acometido por infecção e/ou inflamação e, possivelmente, ocorrer a perda do implante. Episódios de peri-implantite são grande parte das causas de perda de implantes.

Peri-implantite e composição do biofilme

Peri-implantite pode ser definido como um processo de inflamação ou infecção sobre implantes dentários, que se inicia na inflamação da mucosa ou gengiva, denominada peri-mucosite que, por sua vez, pode progredir à peri-implantite, levando à perda óssea parcial ou total, seguida pela perda do implante (28). No entanto, nem todos os casos de peri-implantite se comportam da mesma forma, sendo que em alguns casos não existe a formação de tecido granulado ao redor do implante, mas sim tecido fibroso. Por outro lado, em alguns casos é observada perda óssea sem a presença prévia de sinais de infecção, causado por fatores genéticos ou fisiológicos; isso pode ocorrer em implantes instalados na região anterior de maxila. A perda óssea inicial expõe o implante intraósseo, que se torna um local propício para adesão bacteriana e formação de biofilme que, por sua vez, pode dar início a um processo de peri-implantite.

É importante levar em consideração que a correta higienização oral, que deve ser executada diariamente pelo paciente, é fundamental para evitar-se o acúmulo de placas bacterianas e por sua

vez formação de biofilme. Esse fator promove contaminação *in loco* e futuramente infecções que possivelmente influenciarão no comprometimento do implante (29).

Existe uma diferença de espécies que compõem a microbiota em implantes dentários sadios e implantes com peri-implantite. Quando um implante é instalado entre dentes naturais, as bactérias que estão formando biofilme do dente estão muito próximas ao implante e podem ser a origem de um biofilme no implante. Segundo LASSERRE et al. (30), é bem aceito na literatura que o biofilme dental tem uma participação na iniciação e progressão das doenças periodontais e peri-implantite, porém detalhes sobre o mecanismo e patogênese ainda necessitam de estudos. Além disso, apesar do processo de formação de biofilme ser o mesmo, existe uma diferença significativa entre as espécies encontradas em um biofilme em tecido dentário e em um biofilme formado em um implante (Figura 3). Microrganismos como *P. gingivalis, Tannerella forsythia* e *Treponema denticola* são comumente encontrados em maior quantidade, formando biofilme em episódios de peri-implantite. Outros microrganismos patogênicos já foram descritos também, como *Prevotella intermedia* e *Fusobacterium nucleatum, Aggregatibacter actinomycetemcomitans* e *Eikenella corrodens* (31,32,33). Além disso, diferenças ecológicas foram encontradas entre as comunidades comparando processos de peri-implantite com periodontite, tendo sido encontrada maior diversidade bacteriana em peri-implantite. O microbioma da peri-implantite se apresenta de forma mais heterogênea, com maior complexidade e composta por espécies Gram-negativas não cultiváveis (33,34,35).

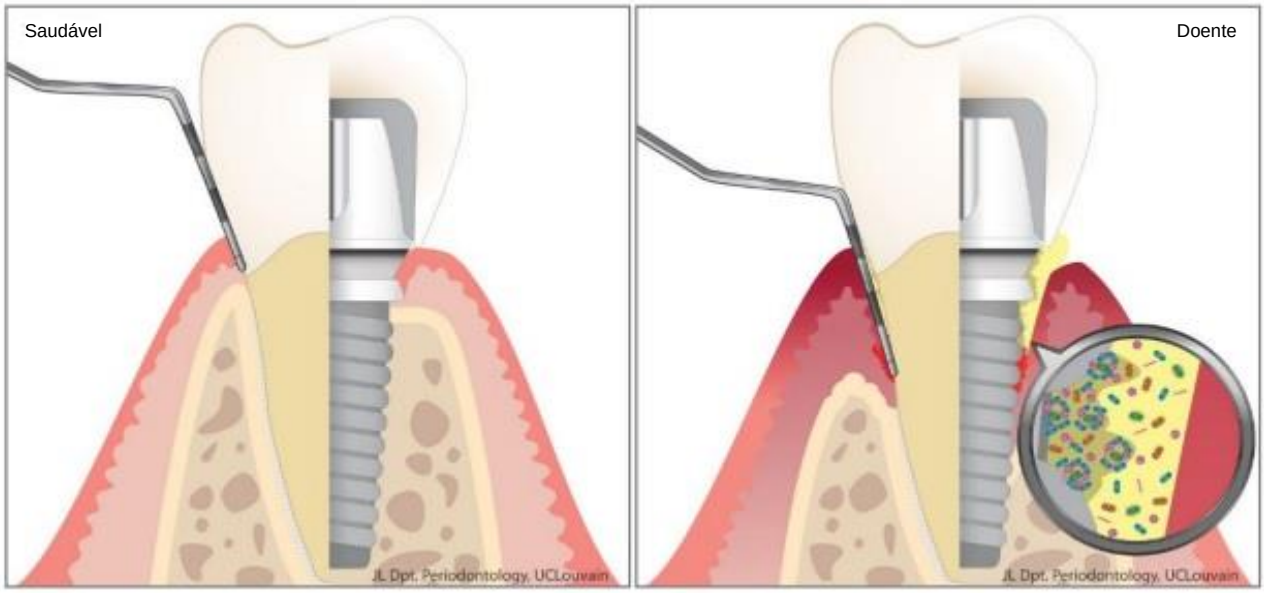


FIGURA 3. Ilustração exibindo implante saudável (à esquerda) e descolamento gengival e formação de biofilme em implante dental (à direita). LASSERRE et al. (30)

Probióticos

O uso de probióticos tem sido sugerido na literatura como profilaxia, substituindo o uso de antibióticos e antissépticos, para redução da disbiose causada pelos últimos. Os probióticos agem tanto no desenvolvimento quanto na estabilidade da microbiota alterando a colonização de patógenos e auxiliando na estimulação do sistema imunológico do indivíduo (36). Probióticos compostos por *Lactobacillus* spp. têm sido avaliados para uso oral, com o intuito de reduzir processos inflamatórios e diminuição da disbiose da microbiota oral (37,38). *Streptococcus salivarius* também tem sido utilizado na composição de probióticos, apresentando boa atividade contra formação de biofilme na superfície de implantes (39).

MULLA (36), demonstrou importante papel de probiótico composto por *Lactobacillus reuteri* contra patógenos comumente encontrados em peri-implantite, como *Porphyromonas gingivalis, Prevotella intermedia, Streptococcus salivaris e Staphylococcus aureus.*

De acordo com FLICHY (37), a colonização por determinadas espécies de bactérias evita um processo de disbiose e, por consequência, leva a uma diminuição nos níveis de citocinas. Dessa forma, a administração de probióticos pode ser uma boa alternativa para o tratamento e prevenção de peri-mucosites e peri-implantitis.

Comorbidades e outros fatores que predispõe a peri-implantite

Comorbidades pré-existentes como diabetes ou hipertensão arterial são citados como fatores de risco para perda de implantes, ou mesmo hábitos do paciente como tabagismo, podem auxiliar no comprometimento da vida útil dos implantes instalados (40,41). De acordo com LUO et al. (42), nos Estados Unidos, pacientes com diabetes perdem o dobro de dentes do que pacientes sem esta comorbidade. Isso significa que a procura e necessidade de restauração oral através de implantes para este perfil de pacientes é grande e um risco maior é associado devido à suscetibilidade à infecção, baixa cicatrização ou outras complicações (43).

LIU et al. (44) defende em seus trabalhos que a diabetes mellitus é um dos fatores de alto risco para periodontite e perda de implantes, pois normalmente o sucesso de um implante depende tanto de uma boa osteo-integração quanto à interface dos tecidos moles que circundam o pescoço do implante, servindo de barreira para invasão de patógenos. De acordo com esses autores, a aderência e proliferação de fibroblastos sobre superfícies de titânio puro foram inibidas pelo aumento da concentração de glicose.

Segundo WANG et al. (45), pacientes com doenças cardiovasculares apresentam aumento significativo das taxas de peri-implantite de níveis moderado a severo. A dentição natural apresenta respostas neuromusculares que tem a função de proteger os dentes, o periodonto e o osso de forças oclusais em excesso. O ligamento periodontal transmite ao osso as forças que incidem sobre os dentes (46). O ligamento periodontal é formado por fibras colágenas que se inserem no cemento e no osso alveolar, denominadas fibras de Sharpey, atuando na absorção de choque e na dissipação dos esforços oclusais (9). Os implantes osteointegrados são justapostos ao tecido ósseo e não possuem os ligamentos periodontais. A falta destes ligamentos é compensada por outros organismos perceptores como estruturas nervosas na articulação temporomandibular, músculos, tendões e osso. Ainda assim, a sensibilidade tátil é reduzida em relação aos dentes naturais (47).

Implantes: Osteointegração e cicatrizadores

Uma variedade de sistemas e uma multiplicidade de formas, diâmetros e comprimentos destes implantes estão disponíveis comercialmente. De forma geral, os implantes são classificados como HE (hexágono externo), HI (hexágono externo) e CM (cone morse). De acordo com

BASTOS-NETO (9), existe a necessidade de uma análise minuciosa da relação entre diâmetro, comprimento e inclinação dos implantes para que se tenha um procedimento bem-sucedido.

A metodologia de implante dentário segue um fluxograma, conforme descrito na Figura 4. Quando a osteo-integração do implante se dá de forma satisfatória, o paciente é encaminhado para fase de confecção da prótese. Esse período de osteo-integração varia, conforme o local do implante, sendo 4 meses após a instalação de implantes na mandíbula e 5 meses após instalação de implantes na maxila (9).

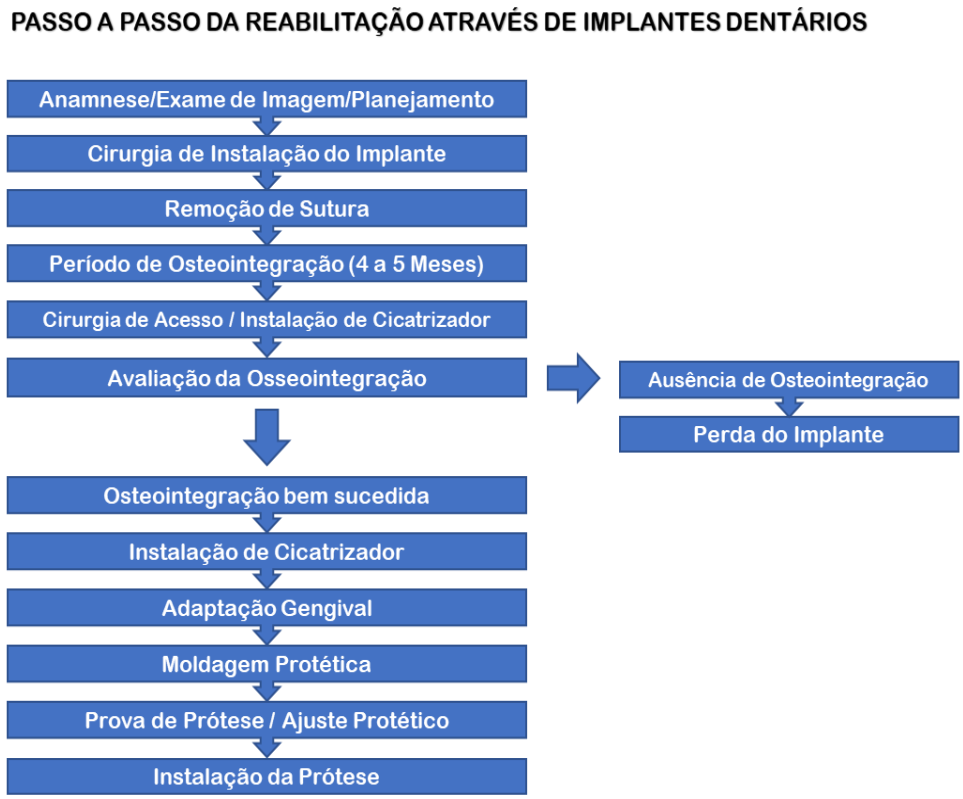


FIGURA 4. Fluxograma dos procedimentos para instalação de implante dentário. Após a colocação do implante, caso a osteointegração seja satisfatória, o procedimento continua até a instalação da prótese. Caso a osteointegração não se dê de forma satisfatória, ocorre a perda do implante e possível reinicialização do processo.

Após a osteointegração, executa-se a reabertura da região implantada através de uma pequena incisão, onde será posicionado um cicatrizador. Esse cicatrizador tem a função de promover o alojamento do tecido gengival ao seu redor (Figura 5A e 5B), formando um túnel gengival que irá proporcionar, futuramente, o perfeito encaixe da futura prótese dentro desta gengiva formada. Na Figura 5C, observamos o perfeito encaixe do cicatrizador no implante, de modo a permitir o acondicionamento dos tecidos ao seu redor.

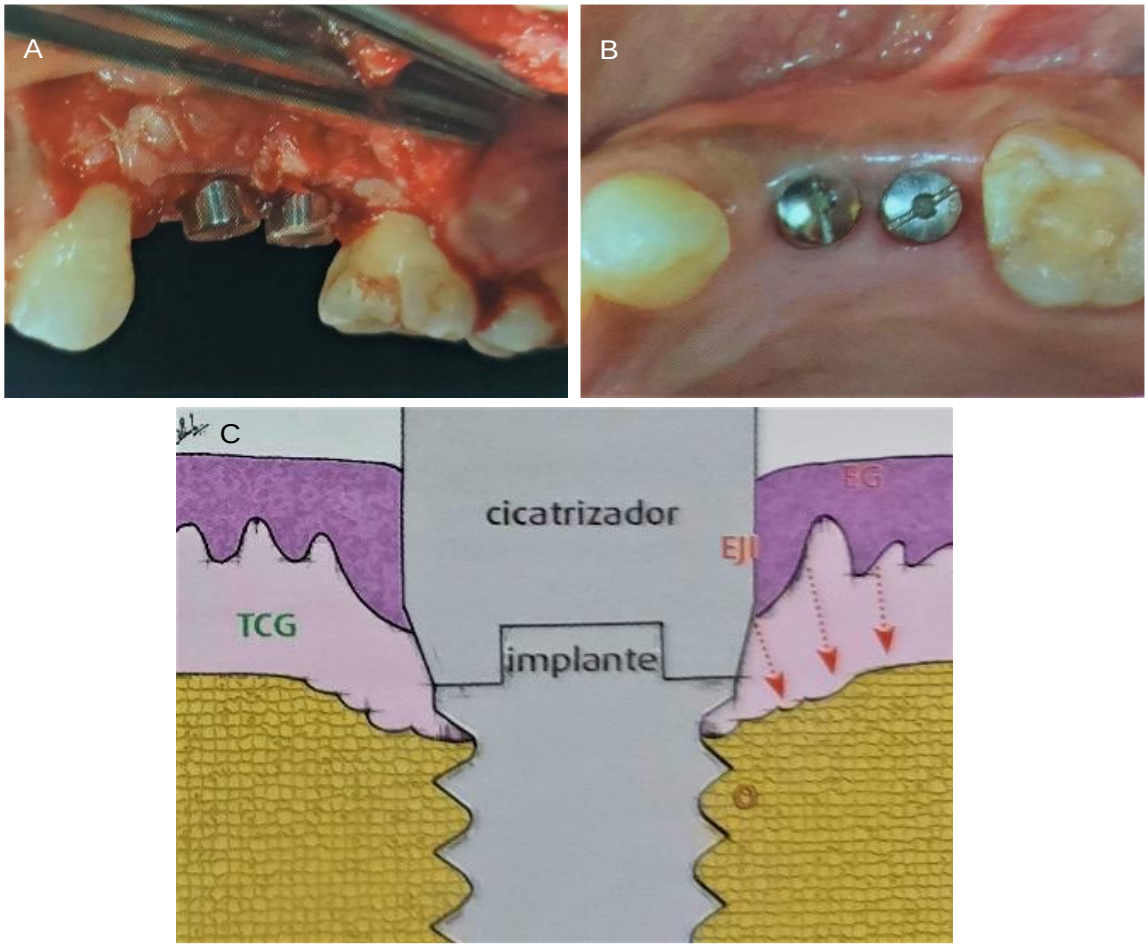


FIGURA 5. Ilustração do procedimento de instalação do cicatrizador, após a osteointegração, para posterior instalação da prótese dentária. **A:** Instalação de cicatrizadores juntamente com procedimento de enxerto. **B:** Imagens de cicatrizadores instalados após período de cicatrização. **C:** Figura esquemática referente a acoplagem do cicatrizador ao implante dentário. EG = epitélio gengival; EJI = epitélio juncional peri-implantar; TCG = tecido conjuntivo gengival; O = osso alveolar. Fonte: BASTOS-NETO (9) e CONSOLARO et al. (48).

Em geral, ao longo da vida útil do implante instalado, devido a fatores biológicos, falta de higiene, falhas de planejamento ou procedimento, ou ausência de acompanhamento profissional, o paciente pode desenvolver peri-mucosite e posterior peri-implantite, ocorrendo a perda óssea promovendo a exposição de espiras do implante para a microbiota oral, facilitando a formação de biofilme.

Dessa forma, torna-se importante o estudo quanto à segurança e sobre fatores biológicos e microbiológicos envolvidos no protocolo da implantodontia, para compreensão do fluxo microbiológico complexo que norteia a cavidade oral no pré-operatório, trans-operatório e pós-operatório. Este conhecimento adquirido, pode auxiliar não somente no tratamento da peri-implantite, mas também na sua prevenção, evitando assim a perda do implante dentário.

2. Objetivos

2.1 Objetivo Geral

Descrever os aspectos microbiológicos envolvidos no sucesso ou insucesso de um implante dentário.

2.2 Objetivos Específicos

- Realizar uma revisão de literatura para descrever os aspetos microbiológicos envolvidos nos processos de implante dentário, visando a obtenção de sucesso a longo prazo;

- Descrever um caso de infecção após colocação de cicatrizador em um implante dentário;

- Quantificar as bactérias que compõe a microbiota oral para estabelecer o padrão observado no episódio de infecção pós-cicatrizador;

- Realizar uma análise epidemiológica de pacientes submetidos à implantes, dos últimos 4 anos, em uma clínica-escola, para verificar a ocorrência de processos infecciosos.

3. CAPÍTULO 1: (submetido)

MICROBIOLOGICAL ASPECTS FOR LONG-TERM SUCCESS OF DENTAL IMPLANTS

Este capítulo inclui uma revisão de literatura sobre a influência dos microrganismos orais associados a fatores como composição do implante, resposta do sistema imune e fatores de risco do hospedeiro, no desenvolvimento de peri-mucosite, peri-implantite e/ou perda óssea e que podem levar a perda de implantes. O estudo descreve o perfil da microbiota oral associada com indivíduos saudáveis e com tecidos infectados, que pode levar a uma redução no tempo de sucesso de um implante dentário. Este artigo foi submetido à revista "Journal of Oral Microbiology" (Anexo I).

MICROBIOLOGICAL ASPECTS FOR LONG-TERM SUCCESS OF DENTAL IMPLANTS

Carlos Henrique Alves[1], Natália Conceição Rocha[1], Fábio Bastos[2], Alexandre Augusto Albigiante Palazzi[1], Michelle Darrieux[1], Thais Manzano Parisotto[1], Raquel Girardello[1#]

[1]Laboratório de Microbiologia Molecular e Clínica, Programa de Pós-Graduação em Ciências da Saúde, Universidade São Francisco, Bragança Paulista, Brazil
[2]Universidade Cruzeiro do Sul, São Paulo, Brazil.

Keywords: oral microbiome, peri-implantitis, dental implants, biofilm, oral diseases
Running title: Microbiology from dental implant

[#]Corresponding Author: Raquel Girardello, phD
Address: 218, São Francisco Ave., Bragança Paulista, São Paulo, Brazil. Zip code: # 12916900.
e-mail: raquel.girardello@usf.edu.br

ABSTRACT

In the last decades, the ortho-aesthetic-functional rehabilitation had a significant advance with the advent of Implantology. Despite the success in Implantology surgeries, there is a percentage of failures due to *in loco* infections, through bacterial proliferation, presence of fungi and biofilm formation, originating peri-implantitis. In this sense, studies have been developed since then, seeking answers to many questions that remain unknown. Thus, the present work aims to discuss peri-implantitis and the oral microbiome of individuals submitted to dental implants who developed peri-implantitis. This study is a literature review about success and failure of dental implants from microbiological perspectives.

1. Introduction

In the last decades, significant advances have occurred through research related to Implantology. According to Albrektsson et al. (2005), even though Branemark osteointegration system was not successful, it generated a great amount of data that led to significant improvements in implant techniques. But it was at the turn of the millennium that a great revolution took place when the surgical and biomaterials areas brought greater clarity and the desired safety in the procedures performed. The rehabilitation of missing teeth has been present in history for a long time, but not long ago, intraosseous implants started to be installed in rough surfaces, causing no trauma to the adjacent bone and the prosthesis projected for longevity intentions (Block, 2018). Due to the progress made in the aesthetic-functional area of implants, there is an increase in the demand for these procedures as a tool for oral rehabilitation. According to Thoma et al. (2017), the reasons for this success are studies in surface treatments, implant design, material and techniques, which have increased the demand for treatments involving dental implants. In addition, the improvement of oral microbiome knowledge has enabled solutions to problems such as biofilm formation and selection of pathogenic bacteria, to prevent infections. This literature review aims to describe the factors involved in the implant success from microbiological and host immunity perspectives.

Oral microbiome and biofilm formation

The Human Oral Microbiome Database (www.homd.org) includes, in the adult individual, 772 prokaryotic species, where 70% are cultivable species, and 30% belong to the uncultivable class of microorganisms. Out of 70% culturable species, 57% has already been named. Recently, the microbiome sequencing techniques have become more accessible, allowing identification of non-cultivable species.

Within the oral cavity, a plethora of species colonization occurs, according to the area or even the local environmental conditions, and the microorganisms are placed according to their metabolic needs. Mager et al. (2003) affirm that the salivary microbiome is composed of a mixture of all microbial sites. Although there is an overlap of all species in all oral bacterial sites, we see species

of *Streptococcus* spp., *Gemella* spp., *Granulicatella* spp., *Neisseria* spp. and *Prevotella* spp. more frequently in the saliva. On other hand, it appears that bacteria that are located on the hard palate are not primarily the same as those present on the tongue. According to Segata et al. (2012), *Rothia* spp. typically colonize the tongue or tooth surfaces, *Simonsieur* spp. colonizes only the hard palate, *Streptococcus salivarius* colonizes the tongue, and *Treponema* spp. is typically restricted to the gingival and subgingival cleft.

Bacteria demonstrate specificity in their colonization, both in terms of the positioning of their sites and in the relationship between them. According to Kolenbrander (2000), they are guided through adhesin binding receptors. Some receptors come from salivary proteins and through the affinity between bacteria, we have the origin of the biofilm (Figure 1). Issues such as adherence and functionality are some of the factors that help the bacterial complex to form the most efficient biofilm. According to Valm et al. (2011), receptors are derivatives from salivary components such as proteins rich in proline and serine, which are submitted to changes in conformation when adhered to surfaces such as the tooth where there is a specific interaction with a strong affinity.

Bacteria have long been implicated in biofilm formation in the oral cavity; however recent studies have revealed that the presence of fungi plays a significant role in biofilm formation. According to Mazari et al. (2018), several fungal species, including *Candida albicans*, *C. guilliermondii*, *C. glabrata*, *Rhodotorula* spp. and *Trichosporon* spp. were identified in dental chairs and waterlines. The author mentions that most yeasts identified had the ability to form biofilms and presented resistance to the antifungal agents applied in the study. Premamalini et al. (2018) demonstrated that diverse *Trichosporon* species are also able to form biofilm, including *T. asahii*, *T. asteroides*, *T. cutaneum* and *T. mucoides*.

The investigation of the oral microbiota associated with pathogenesis is necessary to identify probable causes and seek relevant solutions so that patients submitted to implants do not lose functionality, whether esthetic or bio-mechanical, of the installed implants caused by accumulation of biofilm and consequent loss of peri-implant bone support.

Oral microbiome of peri-implantitis and peri-implant mucositis

Periimplantitis is a biofilm that occurs around dental implant tissues, causing inflammation in the peri-implant followed by a progressive loss of the supporting bone (Berglundh et al., 2018; Figure 2). When compared with healthy implants those progressing to peri-implantitis presented a diversity of bacterial species such as *Tannerela forsythia, Porphiromonas gingivalis, Aggregatibacter actinomycetemcomitans, Prevotella intermedia* and *Streptococcus salivaris* (Persson & Renvert, 2016; Mulla et al., 2021). *P. gingivalis, Treponema denticola* and *Tannerella forsythia* are described as higher proportion of peri-implantitis samples (Shibli et al., 2008; Mulla et al., 2021). Some studies also indicated the presence of pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa,* acting in an opportunistic way in the infectious process, and fungi and viruses are also part of this biofilm complex (Mombelli & Décaillet, 2011; Schwarz et al., 2018). A longitudinal study performed by Jiang et al. (2021) demonstrated that Firmicutes phylum increased during the maturation of peri-implant plaque, and a decrease of *Neisseria* spp. and *Porphyromonas* spp. detection was observed after periimplantitis establishment.

Berglundh et al. (2018) mentions that pre-clinical and clinical studies have clearly shown the accumulation of biofilm maturation in implants, where initially the peri-mucositis appears and then the peri-implantitis can develop later. According to Lang et al. (2011), peri-implant mucositis resembles gingivitis in natural teeth; it is defined as an inflammation of the soft tissues (i.e., peri-implant mucosa) around the implant and presents clinically with bleeding upon simple probing. The author also mentions that, through peri-implant mucositis, peri-implantitis can develop or not. There are etiological similarities between the biofilm found in periodontitis and peri-implantitis; however, in the second, differentiated bacteria were also found. According to Klinge et al. (2018), in terms of clinical appearance and pathogenesis, it is accepted that both have a common etiology: oral dysbiotic biofilm. Polymeri et al. (2021), observed in their studies that the predominant bacteria in peri-implantitis comparing with healthy implants are *Fusobacterium* spp. *and Treponema* spp.; however, in peri-mucositis it was mostly colonized by *Rothia* spp. and *Streptococcus* spp. Belibasakis et al. (2021) conclude that peri-mucositis is more associated with common periodontal pathogens, while peri-implantitis harbor differentially abundant communities. According to the author, partially edentulous patients were colonized by *Fusobacterium* spp., spp.

and *Rothia* spp., and fully edentulous mostly *Veillonella* spp. and *Streptococcus* spp. A study from Ghensi et al. (2020) suggest that *Fusobacterium nucleatum* appears to be the first species that has increased abundance along the mucositis–peri-implantitis axis and results in stronger peri-implantitis at later stages of the disease.

Besides, the literature shows cases of failure in the procedure of implants caused by peri-implantitis. According to Zahang et al. (2021), both, peri-implantitis and peri-mucositis, were identified in patients with non-closed crown edges, loose crown-retained screws, loose abutment screws and broken abutment screws, considered risky reasons associated with the implantation time, and implant position. Schwarz et al. (2018) mentions also about prosthesis not well structured positioned causing difficulties for the appropriate hygiene leading to biofilm formation and a future peri-implantitis. In a study from Bäumer et al. (2020), affirms that the influencing factor for implant loss is the implant type, while the reasons with statistical significance for peri-mucositis are wider implant diameter and modified plaque index, while in periimplantitis, the reasons are wider implant diameter and number of implants per patient. According to the author, in the periimplantitis infectious process, there is a presence of bacteria that participates in this process causing bone loss, and consequently the loss of the implant installed. The authors consider the knowledge of the oral microbiome based on a phylogenetic database to be important to identify the pre- and post-surgical bacterial niche and develop studies for prevention or even prophylactic measures to reduce the loss of dental implants caused by peri-implantitis.

Zahng et al. (2021) mentions that peri-implantitis and peri-mucositis occurred at lower rates in the Straumann system, while the Osstem system had higher occurrences. The author also says that there was a significantly higher incidence in the anterior maxilla area, and that the peri-implantitis increase is due to factors such as the implant system used, implantation time, implant position and restoration factors. In addition, Jansson et al. (2021) described difference in peri-implantitis and peri-mucositis incidence in the implant surface. The implant regions with highest incidence of moderate/severe peri-implantitis, and consequent bone loss, were mandibular incisor/canine and maxillary incisor/canine; while the occurrence of peri-mucositis was in the regions of maxillary molar, maxillary incisor/canine, and mandibular premolar.

Despite the high incidence of peri-implantitis and peri-mucositis, Thöne-Mügling et al. (2021) suggests the diagnosis of hard and soft tissue by ultrasonography might be a promising tool in implant dentistry, especially for being inexpensive, non-invasive, and painless. The author mentions the importance of the ultrasonography image information, provided for the patients with dental implants affected by soft tissue recession and/or crestal bone loss.

Dental implant composition and biofilm formation

The implant composition is extremely important in the osteointegration process. On the other hand, their composition is also responsible for greater or lesser bacterial adhesion and consequent biofilm production. Bacterial infections are a common cause of implant failure and, when developed around an implant, are difficult to treat, occasionally involving the tooth removal to control the infectious process of the tissue (Matter et al., 2021).

Titanium has been used for dental implants since the 1960s and is one of the most biocompatible materials, biologically inert and has a high resistance to corrosion because of the spontaneous formation of a titanium oxide (TiO2) film on its surface, which separates the metal from its environment, providing bio security against local infections (Zhu, 2004). A layer of apatite hydroxide is applied to the implant surface to obtain better osteo conductivity, what will help in the osteointegration process (Ogle, 2015). In addition, it has shown the ability to promote osteointegration. So, titanium is still the most widely used dental implant material. However, insufficient soft tissue integration and vulnerability to biofilm are described for this implant composition (Craig et al., 1999). An *in vitro* study from Souza et al. (2020), revealed that titanium products, especially ions, have potential to change the microbiological composition of biofilms formed on titanium surfaces. The authors affirm that the presence of titanium products around dental implants may contribute to oral microbiota dysbiosis and, in consequence, to peri-implantitis development.

The zirconium dioxide (zirconia) has earned its place to substitute titanium in implant manufacturing due to its excellent biocompatibility, tissue integration inducing a low degree of bone resorption, and low affinity to bacterial plaque, besides its biomechanical properties (Covacci et al., 1999; Sennerby et al., 2005; Al-Radha et al., 2012). However, according to Sanon (2015),

the main concern about this material due to technical failures occurred like fracture of the material, which is the reason of titanium remains the best material; so, the association of both titanium and zirconium dioxide material is one option to avoid this effect. According to Tang et al. (2021), modifying the surface of zirconia with a TiO_2 coating might be favorable for osteogenic effects. In addition, Jo et al. (2021) showed that, when the surface of titanium implants is coated with zirconia via atomic layer deposition, the level of *S. mutans* and *P. gingivalis* adhesion is reduced, regardless of the presence of zirconia crystal phases deposited on the surface.

Ceramic-based alternatives are promising to avoid metal surfaces use; however, according to Matter et al. (2021) the processing and shaping them is demanding, and thus accessible design options are limited. These authors develop nanostructured implant coatings based on such multi-metal oxide nanohybrid building blocks tailored to both hard and soft tissue healing. Ceria has proven anti-inflammatory and antimicrobial properties and their association with bioglass demonstrate a reduction of methicillin-resistant *S. aureus* (MRSA) growth.

Currently, nanostructures have been suggested as solution for better osteointegration e success of implants. Nanostructured implants have an increased surface area, allowing for better adsorption of proteins and improved attachment of cells to the implant. To improve the implant surface porosity, roughness, and wettability, and to incorporate bioactive components, a wide spectrum of nanocoating techniques have been used on dental implants (Matter et al., 2021).

Associated to increased osteoblast proliferation, the titanium nanoparticles surface appears demonstrate low bacterial adhesion and low biofilm maturation of pathogenic species *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia*, after 30 days of exposition, compared with other surfaces composition (Camargo et al., 2021). Another study from Bright et al. (2021) showed a decrease in pathogenic species *Sthapylococcus aureus* and *Pseudomonas aeruginosa* viability in the 2 μm layer furthest from the nanostructured surface from titanium nanostructured Ti6Al4V.

According to the Heo et al. (2016), gold nanoparticles have been successfully tested immobilizing titanium implant surfaces as osteo inductive agent for fast osseointegration. The effectiveness *in vitro* and *in vivo* were investigated and, in both experiences, they ensure that gold

nanoparticles can be useful as osteo-integration, inducing dental implants to form osteo interface also helping the maintenance of a new bone formation as well. Baumer et al. (2020) affirms on her studies that miR-335-5p/Lipidoid nanoparticles coated on titanium surfaces has shown a transfection efficiency, cell adhesion, proliferation, and osteogenic activity of the bone-implant interface, therefore it might be used as a new methodology to improve the osteogenic capacity of biomedical titanium implants. Kim et al. (2021), mentions that a lot of nanostructures of TiO_2 have been reported and electrospinning has been an efficient practical technique with low cost and high efficiency. Diverse parameters in the process can be controlled, even the molecular weight, concentration of the polymers, deionized water, fluid velocity and others.

Polyetheretherketone (PEEK) is a polymer used for diverse applications in dentistry including dental implants due to their mechanical and physical properties be like bone and dentin (Najeeb et al., 2016). A study from D'Ercole et al. (2020) demonstrate that wettability and nano-roughness of PEEK can significantly affect the CFUs and biofilm biomass from *Streptococcus oralis*, demonstrating bactericidal and/or anti-adhesive effect. In addition, a carbon fiber-reinforced PEEK (CFR-PEEK) has been evaluated to replace titanium in manufacture of implants. According to Tamarakar et al. (2021), the amount of stress generated within the bone in the case of the CFR PEEK implants with different restorative crowns was smaller in comparison with the titanium implants in oblique loading. This could help reduce lateral stress on implants as well as crestal bone loss. No microbiological studies were performed for this structure until now, to verify the biofilm formation.

Recent studies bring new probable future solutions to rehabilitate missing teeth. According to Galler et al. (2011), stem cells have been studied for teeth regeneration. Many successful engineering initiatives related not only to the whole tooth regeneration but also for enamel, the dentin-pulp complex and periodontal ligament have been developed. This task might enable regenerative development for the Dentistry in the future.

Finally, regardless of the composition of the implant, manufacturing failures are responsible for less osseointegration and increased bacterial colonization, leading to an increase in peri-implantitis rates. According to Belibasakis et al. (2021), biocorrosion of the implant, abutment

loosening, prosthetic screen loosening, milled abutment, or prosthetic screw, the cemented crown may affect microbial colonization and disease progression.

Pre-existing periodontal profiles and comorbidities

Most of the patients who seek dental implants are around 40 years old, being many of them edentulous or partially edentulous. There are also specific profiles such accidents which cause tooth loss. Elderly patients have a history of chronic periodontal diseases, where there is an alteration of the tissues that guide the dental element, promoting bone loss and, in the future, tooth loss (Ogle, 2007). Guillaume (2016) affirms the patient goes thru a tooth loss process where the modifications in the periodontal ligaments result from both the loss of bone volume, dental mobility and finally the loss of the dental element over an average period.

It is important to note that the periodontal ligaments lost in the extraction procedure are irretrievable, the ligaments once removed they can´t recover themselves and after the installation of dental implants, tissue formation and/or adaptation will occur, but never substituting the ligaments. According to Schroeder et al. (1981), the mucosa around the implants also forms a seal which is compared to the junctional epithelium. This peri-implant junctional epithelium is composed of three types of epitheliums: peri-implant epithelium, sulcular peri-implant epithelium and oral epithelium (Figure 2). Morphologically, the sulcular peri-implant epithelium is composed of a thin layer of cells 3 and 4, and has immunoglobulins, neutrophils, lymphocytes, and plasma cells, in a wide intracellular space, where together they protect the underlying tissue from harmful exogenous factors (Ikeda et al., 2000).

Despite the formation of this protective seal, the epithelia formed around the dental implant are more fragile than the tissues that surround the dental element, reminding that it does not have the ligaments. Due to this characteristic, similar bacteria present in periodontitis can enter and cause peri-implantitis. According to Berglundh et al. (1992), this might happen due to the immune system of the peri-implant tissue being inferior at the defense site compared to periodontal tissue.

Previous diseases and biological conditions are described as risk factors associated with peri-implantitis development; however, different authors disagree in this affirmation. According to Renvert & Polyzois (2015), poor hygiene, smoking, type II diabetes, and poorly made prostheses

on implants, are risk indicators for the appearance of peri-implant mucositis. In the other hand, studies do not present smoking as a high-risk factor for triggering peri-implantitis or, do not support the fact that diabetes might be a risk as well (Marrone et al., 2013; Daubert et al., 2015; Derks et al., 2016; Rokn et al., 2017). Krebs et al. (2019) affirms that diabetes mellitus, smoking and regular maintenance did not show considerable influence on the prevalence of peri-implant diseases. In addition, studies have been done about patients who use antiretroviral therapy in relation to peri-implantitis and peri-mucositis development, such as patients living with HIV. Vidal et al. (2021), concluded that well-controlled HIV infected individuals are eligible to undergo implant installation even using a high active retrovirus.

Host-pathogen relationship in peri-implantitis

Peri-implantitis is a multifactorial condition resulting from an imbalanced interaction between the pathogen and the host immune response (Saremi et al., 2021). The injury of peri-implant tissue causes an inflammatory response mediated by activation of innate immune cells such as macrophages, dendritic cells, mast cells, and neutrophils. The neutrophils promote the release of pro-inflammatory cytokines IL-1 and TNF-α, which in turn activate the osteolytic and inflammatory tissue damage observed in peri-implantitis (Baseri et al., 2020). According to Hashim et al. (2021), defects in the number or efficacy of neutrophils predisposes individuals to development of periodontal disease. Paradoxically, neutrophil activity, as part of a deregulated inflammatory response, appears to be a crucial element in the destructive disease process. These authors demonstrate that the absence of CXCR-2 neutrophil receptor in gingival tissue significant was associated with changes in the local microbiome, resulting in an increase of periodontal disease. This effect demonstrates the significant role of neutrophils in balancing the oral microbiota. Furthermore, the presence of active CXCR-2 neutrophil receptors was able to reestablish the gingival tissue microbiota.

A machine learning-assisted immune profile was designed by Wang et al. (2021), where patients at low risk of developing peri-implantitis exhibited elevated M1/M2-like macrophage ratios and lower B-cell infiltration. The low-risk immune profile was characterized by enhanced complement signaling and higher levels of Th1 and Th17 cytokines. In addition, *Fusobacterium nucleatum* and *Prevotella intermedia* were significantly enriched in high-risk individuals,

compared with the low-risk group. According to Baseri et al. (2020), macrophages might have a binary role in directing the implant failure or success, depending on their phenotype. M2 macrophages could lead to osseointegration and effective wound healing, while the M1 macrophages could exacerbate the inflammatory process and accelerate osteolysis leading to dental implant failure.

Recently, Saremi et al. (2021) evaluated the influence of immune gene polymorphisms on the development of peri-implantitis and revealed that allele and genotype frequencies of IL-10 ─ 819 C/T, IL-10 ─ 592 C/A, and IL-1ß + 3954 C/T, significantly differed between diseased and healthy patients, indicating that these specific gene polymorphisms may play a role in the pathogenesis of peri-implantitis.

Another factor that has been linked to inflammation and tissue damage is the excess of cement on the tooth crown. According to Tatullo et al. (2017), the excess of cement on the tooth crown is associated with bacterial accumulation. It also promotes tissue inflammation since it is recognized as a foreign body by the host immune system. The immune activation and increased bacterial rates are associated with histological changes in the tooth and lead to lower survival of local osteoblasts.

Li et al. (2021) demonstrates that a fine tuning of osteoclast-osteoblast balance is required for a perfect synchronization of bone resorption and formation, to maintain efficient bone remodeling and bone homeostasis. By contrast, activation of the inflammasome during bacterial infection may lead to bacterial spreading or even an uncontrolled bone destruction, which is quite common in periodontitis, periapical periodontitis, peri-implantitis and other related conditions. This uncontrolled inflammasome activity may cause alveolar osteolysis by activated macrophages, monocytes, neutrophils, and adaptive immune cells like T helper 17 cells. This whole immune response causes an increase in osteoclasts and concomitant decrease of osteoblasts. In addition, osteocytes play an important role in alveolar bone loss, since they respond to inflammatory changes by secreting molecules that affect bone resorption and formation, causing bone loss (Huang et al., 2020).

The presence of different biomarkers of implant diseases has been discussed in the literature. Hentenaar et al. (2021) compared biomarkers levels in peri-implant crevicular fluid (PICF) of healthy implants and patients with peri-implantitis; the latter showed higher levels of IL-1β and MMP-8 in PICF compared to healthy implants. Matrix metalloproteinase (MMP-8, also called collagenase-2 or neutrophil collagenase) is the main proteolytic enzyme responsible for periodontal and peri-implant soft and hard tissue degeneration (APD) (Kinane, 2000). The collagenase group of proteases is the most relevant in periodontal disease (Sorsa, 2006; Bernasconi, 2015). The proteases activity is inhibited by endogenous tissue inhibitors; therefore, alterations in these molecule levels often results in irreversible periodontal and peri-implant degradation.

Probiotic therapy for peri-implantitis

The current antibiotic resistance rates associated with microbiota dysbiosis caused by antibiotic and antiseptic usage, probiotics have been suggested as option for peri-implantitis treatment. Probiotics are defined as living and viable microorganisms which, when administered in adequate quantities, confer benefits to the organism (Lauritano et al., 2019). Probiotics have been considered a safe and useful tool, since they reduce the immunogenicity of microbiotas by improve the balance of the host microorganisms, inhibiting pathogens. In addition, the host balance result in immune homeostasis, and consequent decreasing of proinflammatory cytokines (Mulla et al., 2021).

Lactobacillus species have been used for years to balance gut and vaginal microbiotas, and currently is suggested to oral microbiota too. In a study from Kõll-Klais et al. (2005) the most prevalent *Lactobacillus* species in oral microbiota from healthy persons were *L. gasseri* and *L. fermentum*, while in chronic periodontitis patients, *L. plantarum* was more frequent. In *in vitro* tests, *Lactobacillus* spp. was able to inhibit the pathogenic bacteria *S. mutans*, *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia*. Strongest antimicrobial activity was associated with *L. paracasei*, *L. plantarum*, *L. rhamnosus*, and *L. salivarius*.

L. reuteri-based probiotics are used for gut microbiota balance, and they were suggested for oral microbiota to peri-implantitis treatment. Two studies showed low decreasing of peri-implantitis rate, however, there was a reduction in the number of periodontal and peri-implant

causing species, as *P. gingivalis* (Galofré et al., 2018; Lauritano et al., 2019). On the other hand, Flichy-Fernández et al. (2015) demonstrated that, after treatment with the probiotic *L. reuteri* in patients with implants presenting mucositis, the clinical parameters improved, and the cytokine levels decreased, suggesting a preventing role of *L. reuteri*-based probiotics. Arbildo-Vegas et al. (2020) demonstrated in a systematic review that, though not always achieving significance, there was a difference in the depth of the probing in the treatment of peri-implantitis, when using *L. reuteri* probiotic, which can be clinically effective in terms of pocket depth reduction in this treatment. Peña et al. (2019) demonstrated related results, affirming that the administration of probiotics, the clinical variables, except for probing pocket depth, slightly and progressively increased up to 3 months of follow-up, but without reaching baseline levels. From a microbiological point of view, no major alterations of the subgingival microflora were recorded at different time points between groups during the study.

Together, the results observed in the studies until now demonstrated that the *L. reuteri*-based probiotics appears to be not so effective solution for peri-implantitis diseases; however, more studies evaluating other probiotics composition need to be performed to make available an associated therapy and reduction of antiseptic and antibiotic usage.

A novel proposal to *S. salivarius*-based probiotic in reduction of biofilm formation in implant was demonstrated by Vacca et al. (2020). The authors demonstrated an interaction between bacteriocin produced by *S. salivarius* inhibiting the quorum-sensing signals and reducing the *S. intermedius* biofilm production in titanium implant surface; so, this probiotic could be considered in non-surgical therapy to prevent biofilm-related implant diseases. On the other hand, a study from Martorano-Fernandes et al. (2020) suggest that the use of *S. salivarius* as a probiotic would be ineffective in peri-implant disease treatment, when caused by *C. albicans* pathogen.

There is a lack of diversity about probiotic composition for peri-implantitis therapy, so, more studies are necessary to be invested in an adjuvant and non-medicated solution, to avoid as much as possible the imbalance of the oral microbiota.

2. Conclusion

Understanding the specificity of the oral microbiome and its complexity is a difficult but necessary task to successfully prevent oral infections. A diversity of factors may contribute to periimplantitis, such as poor hygiene, prostheses not well manufactured, smoking and diabetes. It would be useful to have a careful previous investigation based on the patient´s behavior and his history. The increase of dental implant surgeries brought up many biological complications derived from these procedures. The occurrence of the peri-implantitis development in surgeries performed, causes support bone loss leading to loss of the implant. Many studies on the oral microbiome have been developed, but many doubts remain. The proposal to prevent the loss of implants due to peri-implantitis refers to the microbiome involved in this process. The identification of such microbiome, as well as their quantification, are necessary for future behavioral analysis of these colonization processes, their conveniences in the creation of biofilms and responsibility within the infectious process associated to correct procedures. The procedures developed during the implant installation, such as implant system, implant position, patient education and plaque control should be carefully planned. This way, we would be fighting not only against the development of the infectious process, but also collaborating in preventive actions against peri-implantitis.

Legends of Figures

- **Figure 1**: Schematic illustration referring to diversity of species found into a biofilm community formation in Periimplantitis. Each color indicates the bacteria specie biofilm formation based on its metabolic needs. **B1:***Pseudomonas aeruginosa* **B2:** *Aggregatibacter actinomycetemcomitans* **B3:***Prevotella Intermedia* **B4:** *Fusobacterium* spp.; **B5:** *Treponema denticola* **B6:** *Porphyromonas* spp. **B7:** *Tannerela forsythia* **B8:** *Neisseria* spp. **B9:** *Streptococcus salivarius*. **B10***: Staphylococcus aureus*

- **Figure 2**: Peri-implantitis causing bone loss and exposition of dental implant. **A.** Functional differences about to the soft tissue adhesion on natural teeth (left) and their functions when adhered to a successful implant surface (right). **B.** Schematic illustration of bone loss around implant; **C**: Image of a real case of peri-implantitis and consequent bone loss. The asterisks

correspond to bone loss and the white arrow correspond to implant exposition for bacterial colonization, after bone loss.

Conflicts of interest

The authors declare no conflicts of interest.

Author contribution

CHA contributed with study design, conduction of experiments, data analysis, and writing of the manuscript. NCR and AAAP contributed for conduction of experiments, FB contribute with revision of the manuscript, MD and TMP contributed with data analysis, writing and revision of the manuscript, and RG contributed with study design, conduction of experiments, data analysis, writing and revision of the manuscript.

Acknowledgments

We would like to thank National Council for Scientific and Technological Development (CNPq), Ministry of Science and Technology, Brazil; The São Paulo State Research Foundation (FAPESP); and Universidade São Francisco, for supporting in our researchers.

REFERENCES

Albrektsson T, Wennerberg A. The impact of oral implants - past and future, 1966-2042. J Can Dent Assoc. 2005 May;71(5):327.

Al-Radha AS, Dymock D, Younes C, O'Sullivan D. Surface properties of titanium and zirconia dental implant materials and their effect on bacterial adhesion. J Dent. 2012;40(2):146–153. 2011.12.006.

Arbildo-Vega HI, Panda S, Bal A, Mohanty R, Rendón-Alvarado A, Das AC, Cruzado-Oliva FH, Infantes-Ruíz ED, Manfredi B, Vásquez-Rodrigo H, Mortellaro C, Giacomello MS, Parrini M, Greco Lucchina A, Del Fabbro M. Clinical effectiveness of *Lactobacillus reuteri* in the treatment of peri-implant diseases: a systematic review and meta-analysis. J Biol Regul Homeost Agents. 2021 Mar-Apr;35(2 Suppl. 1):79-88.

Baseri M, Radmand F, Hamedi R, Yousefi M, Kafil HS. Immunological Aspects of Dental Implant Rejection. Biomed Res Int. 2020 Dec 9; 2020:7279509.

Bäumer A, Toekan S, Saure D, Körner G. Survival and success of implants in a private periodontal practice: a 10-year retrospective study. BMC Oral Health. 2020 Mar 30;20(1):92

Belibasakis GN, Manoil D. Microbial Community-Driven Etiopathogenesis of Peri-Implantitis. J Dent Res. 2021 Jan;100(1):21-28.

Berglundh T, Lindhe J, Marinello C, Ericsson I, Liljenberg B. Soft tissue reaction to de novo plaque formation on implants and teeth. An experimental study in the dog. Clin Oral Implants Res. 1992 Mar;3(1):1-8.

Berglundh T, Armitage G, Araujo MG, Avila-Ortiz G, Blanco J, Camargo PM, Chen S, Cochran D, Derks J, Figuero E, Hämmerle CHF, Heitz-Mayfield LJA, Huynh-Ba G, Iacono V, Koo KT, Lambert F, McCauley L, Quirynen M, Renvert S, Salvi GE, Schwarz F, Tarnow D, Tomasi C, Wang HL, Zitzmann N. Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Clin Periodontol. 2018 Jun;45 Suppl 20: S286-S291.

Bernasconi L, Ramenzoni LL, Al-Majid A, Tini GM, Graber SM, Schmidlin PR, Irani S. Elevated Matrix Metalloproteinase Levels in Bronchi Infected with Periodontopathogenic Bacteria. PLoS One. 2015 Dec 14;10(12): e0144461.

Block MS. Dental Implants: The Last 100 Years. J Oral Maxillofac Surg. 2018 Jan;76(1):11-26.

Bright R, Hayles A, Fernandes D, Visalakshan RM, Ninan N, Palms D, Burzava A, Barker D, Brown T, Vasilev K. In Vitro Bactericidal Efficacy of Nanostructured Ti6Al4V Surfaces is Bacterial Load Dependent. ACS Appl Mater Interfaces. 2021 Aug 18;13(32):38007-38017.

Camargo SEA, Xia X, Fares C, Ren F, Hsu SM, Budei D, Aravindraja C, Kesavalu L, Esquivel-Upshaw JF. Nanostructured Surfaces to Promote Osteoblast Proliferation and Minimize Bacterial Adhesion on Titanium. Materials (Basel). 2021 Aug 4;14(16):4357.

Covacci V, Bruzzese N, Maccauro G, Andreassi C, Ricci GA, Piconi C, Marmo E, Burger W, Cittadini A. *In vitro* evaluation of the mutagenic and carcinogenic power of high purity zirconia ceramic. Biomaterials. 1999 Feb;20(4):371-6.

Craig RG, LeGeros RZ. Early events associated with periodontal connective tissue attachment formation on titanium and hydroxyapatite surfaces. J Biomed Mater Res. 1999 Dec 15;47(4):585-94. (19991215)47:4<585: aid-jbm16>3.0.co;2-o.

Daubert DM, Weinstein BF, Bordin S, Leroux BG, Flemming TF. Prevalence and predictive factors for peri-implant disease and implant failure: a cross-sectional analysis. J Periodontol. 2015 Mar;86(3):337-47.

D'Ercole S, Cellini L, Pilato S, Di Lodovico S, Iezzi G, Piattelli A, Petrini M. Material characterization and Streptococcus oralis adhesion on Polyetheretherketone (PEEK) and titanium surfaces used in implantology. J Mater Sci Mater Med. 2020 Sep 28;31(10):84

Derks J, Schaller D, Håkansson J, Wennström JL, Tomasi C, Berglundh T. Effectiveness of Implant Therapy Analyzed in a Swedish Population: Prevalence of Peri-implantitis. J Dent Res. 2016 Jan;95(1):43-9.

Flichy-Fernández AJ, Ata-Ali J, Alegre-Domingo T, Candel-Martí E, Ata-Ali F, Palacio JR, Peñarrocha-Diago M. The effect of orally administered probiotic Lactobacillus reuteri-containing

tablets in peri-implant mucositis: a double-blind randomized controlled trial. J Periodontal Res. 2015 Dec;50(6):775-85.

Galler KM, D'Souza RN. Tissue engineering approaches for regenerative dentistry. Regen Med. 2011 Jan;6(1):111-24.

Galofré M, Palao D, Vicario M, Nart J, Violant D. Clinical and microbiological evaluation of the effect of Lactobacillus reuteri in the treatment of mucositis and peri-implantitis: A triple-blind randomized clinical trial. J Periodontal Res. 2018 Jun;53(3):378-390.

Ghensi P, Manghi P, Zolfo M, Armanini F, Pasolli E, Bolzan M, Bertelle A, Dell'Acqua F, Dellasega E, Waldner R, Tessarolo F, Tomasi C, Segata N. Strong oral plaque microbiome signatures for dental implant diseases identified by strain-resolution metagenomics. NPJ Biofilms Microbiomes. 2020 Oct 30;6(1):47.

Guillaume B. Dental implants: A review. Morphologie. 2016 Dec;100(331):189-198.

Hashim A, Alsam A, Payne MA, Aduse-Opoku J, Curtis MA, Joseph S. Loss of neutrophil homing to the periodontal tissues modulates the composition and disease potential of the oral microbiota. Infect Immun. 2021 Sep 7: IAI0030921.

Heo DN, Ko WK, Lee HR, Lee SJ, Lee D, Um SH, Lee JH, Woo YH, Zhang LG, Lee DW, Kwon IK. Titanium dental implants surface-immobilized with gold nanoparticles as osteoinductive agents for rapid osseointegration. J Colloid Interface Sci. 2016 May 1; 469:129-137.

Hentenaar DFM, De Waal YCM, Vissink A, Van Winkelhoff AJ, Meijer HJA, Liefers SC, Kroese FGM, Raghoebar GM. Biomarker levels in peri-implant crevicular fluid of healthy implants, untreated and non-surgically treated implants with peri-implantitis. J Clin Periodontol. 2021 Apr;48(4):590-601.

Huang X, Xie M, Xie Y, Mei F, Lu X, Li X, Chen L. The roles of osteocytes in alveolar bone destruction in periodontitis. J Transl Med. 2020 Dec 11;18(1):479.

Ikeda H, Yamaza T, Yoshinari M, Ohsaki Y, Ayukawa Y, Kido MA, Inoue T, Shimono M, Koyano K, Tanaka T. Ultrastructural and immunoelectron microscopic studies of the peri-implant epithelium-implant (Ti-6Al-4V) interface of rat maxilla. J Periodontol. 2000 Jun;71(6):961-73.

Jansson L, Guan T, Modin C, Buhlin K. Radiographic peri-implant bone loss after a function time up to 15 years. Acta Odontol Scand. 2021 Jul 30:1-7.

Jiang Q, Yu Y, Xu R, Zhang Z, Liang C, Sun H, Deng F, Yu X. The temporal shift of peri-implant microbiota during the biofilm formation and maturation in a canine model. Microb Pathog. 2021 Sep; 158:105100.

Jo Y, Kim YT, Cho H, Ji MK, Heo J, Lim HP. Atomic Layer Deposition of ZrO_2 on Titanium Inhibits Bacterial Adhesion and Enhances Osteoblast Viability. Int J Nanomedicine. 2021 Feb 24; 16:1509-1523.

Kim WT, Park DC, Yang WH, Cho CH, Choi WY. Effects of Electrospinning Parameters on the Microstructure of PVP/TiO_2 Nanofibers. Nanomaterials (Basel). 2021 Jun 20;11(6):1616.

Kinane DF. Regulators of tissue destruction and homeostasis as diagnostic aids in periodontology. Periodontol 2000. 2000 Oct; 24:215-25.

Klinge B, Klinge A, Bertl K, Stavropoulos A. Peri-implant diseases. Eur J Oral Sci. 2018 Oct;126 Suppl 1:88-94. doi: 10.1111/eos.12529.

Kolenbrander PE. Oral microbial communities: biofilms, interactions, and genetic systems. Annu Rev Microbiol. 2000; 54:413-37.

Kõll-Klais P, Mändar R, Leibur E, Marcotte H, Hammarström L, Mikelsaar M. Oral lactobacilli in chronic periodontitis and periodontal health: species composition and antimicrobial activity. Oral Microbiol Immunol. 2005 Dec;20(6):354-61.

Krebs M, Kesar N, Begić A, von Krockow N, Nentwig GH, Weigl P. Incidence and prevalence of peri-implantitis and peri-implant mucositis 17 to 23 (18.9) years postimplant placement. Clin Implant Dent Relat Res. 2019 Dec;21(6):1116-1123.

Lang NP, Berglundh T; Working Group 4 of Seventh European Workshop on Periodontology. Periimplant diseases: where are we now? --Consensus of the Seventh European Workshop on Periodontology. J Clin Periodontol. 2011 Mar;38 Suppl 11:178-81.

Lauritano D, Carinci F, Palmieri A, Cura F, Caruso S, Candotto V. Reuterinos® as adjuvant for peri-implant treatment: A pilot study. Int J Immunopathol Pharmacol. 2019 Jan-Dec; 33:2058738419827745.

Li Y, Ling J, Jiang Q. Inflammasomes in Alveolar Bone Loss. Front Immunol. 2021 Jun 9; 12:691013.

Mager DL, Ximenez-Fyvie LA, Haffajee AD, Socransky SS. Distribution of selected bacterial species on intraoral surfaces. J Clin Periodontol. 2003 Jul;30(7):644-54. doi: 10.1034/j.1600-051x.2003.00376. x.

Marrone A, Lasserre J, Bercy P, Brecx MC. Prevalence and risk factors for peri-implant disease in Belgian adults. Clin Oral Implants Res. 2013 Aug;24(8):934-40. doi: 10.1111/j.1600-0501.2012.02476. x.

Martorano-Fernandes L, Rodrigues NC, de Souza Borges MH, Cavalcanti YW, de Almeida LFD. Interkingdom interaction between C. albicans and S. salivarius on titanium surfaces. BMC Oral Health. 2020 Dec 1;20(1):349.

Matter MT, Maliqi L, Keevend K, Guimond S, Ng J, Armagan E, Rottmar M, Herrmann IK. One-Step Synthesis of Versatile Antimicrobial Nano-Architected Implant Coatings for Hard and Soft Tissue Healing. ACS Appl Mater Interfaces. 2021 Jul 21;13(28):33300-33310.

Mazari W, Boucherit-Otmani Z, El Haci IA, Ilahi A, Boucherit K. Risk assessment for the spread of Candida sp. in dental chair unit waterlines using molecular techniques. Int Dent J. 2018 Dec;68(6):386-392.

Mombelli A, Décaillet F. The characteristics of biofilms in peri-implant disease. J Clin Periodontol. 2011 Mar;38 Suppl 11:203-13. doi: 10.1111/j.1600-051X.2010.01666. x.

Mulla M, Mulla M, Hegde S, Koshy AV. In vitro assessment of the effect of probiotic lactobacillus reuteri on peri-implantitis microflora. BMC Oral Health. 2021 Aug 19;21(1):408.

Najeeb S, Zafar MS, Khurshid Z, Siddiqui F. Applications of polyetheretherketone (PEEK) in oral implantology and prosthodontics. J Prosthodont Res. 2016 Jan;60(1):12-9.

Ogle F, Naharro M, Carlsson GE. What are the prevalence and incidence of tooth loss in the adult and elderly population in Europe? Clin Oral Implants Res. 2007;18(suppl 3):2-14.

Ogle OE. Implant surface material, design, and osseointegration. Dent Clin North Am. 2015 Apr;59(2):505-20.

Peña M, Barallat L, Vilarrasa J, Vicario M, Violant D, Nart J. Evaluation of the effect of probiotics in the treatment of peri-implant mucositis: a triple-blind randomized clinical trial. Clin Oral Investig. 2019 Apr;23(4):1673-1683.

Persson GR, Renvert S. Cluster of bacteria associated with peri-implantitis. Clin Implant Dent Relat Res. 2014 Dec;16(6):783-93.

Polymeri A, van der Horst J, Buijs MJ, Zaura E, Wismeijer D, Crielaard W, Loos BG, Laine ML, Brandt BW. Submucosal microbiome of peri-implant sites: A cross-sectional study. J Clin Periodontol. 2021 Sep;48(9):1228-1239.

Premamalini T, Anitha S, Mohanapriya K, Kindo AJ. Evaluation of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide method for assessing biofilm formation in vitro by Trichosporon spp. J Lab Physicians. 2018 Oct-Dec;10(4):380-386.

Renvert S, Polyzois I. Risk indicators for peri-implant mucositis: a systematic literature review. J Clin Periodontol. 2015 Apr;42 Suppl 16: S172-86.

Rokn A, Aslroosta H, Akbari S, Najafi H, Zayeri F, Hashemi K. Prevalence of peri-implantitis in patients not participating in well-designed supportive periodontal treatments: a cross-sectional study. Clin Oral Implants Res. 2017 Mar;28(3):314-319.

Sanon C, Chevalier J, Douillard T, Cattani-Lorente M, Scherrer SS, Gremillard L. A new testing protocol for zirconia dental implants. Dent Mater. 2015 Jan;31(1):15-25.

Saremi L, Shafizadeh M, Esmaeilzadeh E, Ghaffari ME, Mahdavi MH, Amid R, Kadkhodazadeh M. Assessment of IL-10, IL-1ß and TNF-α gene polymorphisms in patients with peri-implantitis and healthy controls. Mol Biol Rep. 2021 Mar;48(3):2285-2290.

Schroeder A, van der Zypen E, Stich H, Sutter F. The reactions of bone, connective tissue, and epithelium to endosteal implants with titanium-sprayed surfaces. J Maxillofac Surg. 1981 Feb;9(1):15-25.

Schwarz F, Derks J, Monje A, Wang HL. Peri-implantitis. J Periodontol. 2018 Jun;89 Suppl 1: S267-S290.

Segata N, Haake SK, Mannon P, Lemon KP, Waldron L, Gevers D, Huttenhower C, Izard J. Composition of the adult digestive tract bacterial microbiome based on seven mouth surfaces, tonsils, throat and stool samples. Genome Biol. 2012 Jun 14;13(6): R42.

Sennerby L, Dasmah A, Larsson B, Iverhed M. Bone tissue responses to surface-modified zirconia implants: a histomorphometric and removal torque study in the rabbit. Clin Implant Dent Relat Res. 2005;7(Suppl 1): S13–20.

Sorsa T, Tjäderhane L, Konttinen YT, Lauhio A, Salo T, Lee HM, Golub LM, Brown DL, Mäntylä P. Matrix metalloproteinases: contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. Ann Med. 2006;38(5):306-21.

Souza JGS, Costa Oliveira BE, Bertolini M, Lima CV, Retamal-Valdes B, de Faveri M, Feres M, Barão VAR. Titanium particles and ions favor dysbiosis in oral biofilms. J Periodontal Res. 2020 Apr;55(2):258-266.

Tamrakar SK, Mishra SK, Chowdhary R, Rao S. Comparative analysis of stress distribution around CFR-PEEK implants and titanium implants with different prosthetic crowns: A finite element analysis. Dent Med Probl. 2021 Jul-Sep;58(3):359-367.

Tang S, Zhang J, Ding N, Zhang Z. Biological activity of titania coating prepared with zirconium oxychloride and titania on zirconia surface. J Mech Behav Biomed Mater. 2021 Nov; 123:104780.

Tatullo M, Marrelli M, Mastrangelo F, Gherlone E. Bone Inflammation, Bone Infection and Dental Implants Failure: Histological and Cytological Aspects Related to Cement Excess. J Bone Jt Infect. 2017 Jan 17;2(2):84-89.

Thoma DS, Sailer I, Ioannidis A, Zwahlen M, Makarov N, Pjetursson BE. A systematic review of the survival and complication rates of resin-bonded fixed dental prostheses after a mean observation period of at least 5 years. Clin Oral Implants Res. 2017 Nov;28(11):1421-1432.

Thöne-Mühling M, Kripfgans OD, Mengel R. Ultrasonography for noninvasive and real-time evaluation of peri-implant soft and hard tissue: a case series. Int J Implant Dent. 2021 Sep 14;7(1):95.

Vacca C, Contu MP, Rossi C, Ferrando ML, Blus C, Szmukler-Moncler S, Scano A, Orrù G. In vitro Interactions between *Streptococcus intermedius* and *Streptococcus salivarius* K12 on a Titanium Cylindrical Surface. Pathogens. 2020 Dec 20;9(12):1069.

Valm AM, Mark Welch JL, Rieken CW, Hasegawa Y, Sogin ML, Oldenbourg R, Dewhirst FE, Borisy GG. Systems-level analysis of microbial community organization through combinatorial labeling and spectral imaging. Proc Natl Acad Sci U S A. 2011 Mar 8;108(10):4152-7.

Vidal F, Peres RV, de Souza RC, Gonçalves C, Pavan P, Gonçalves LS. Dental implants in individuals living with HIV-1: Results from a prospective study in patients undergoing highly active antiretroviral therapy. Spec Care Dentist. 2021 Sep 19.

Wang CW, Hao Y, Di Gianfilippo R, Sugai J, Li J, Gong W, Kornman KS, Wang HL, Kamada N, Xie Y, Giannobile WV, Lei YL. Machine learning-assisted immune profiling stratifies peri-implantitis patients with unique microbial colonization and clinical outcomes. Theranostics. 2021 May 3;11(14):6703-6716.

Zahng TT, Hu XJ, Lin L. [Prevalence of peri-implantitis and peri-implant mucositis within 15 years of implant placement]. Shanghai Kou Qiang Yi Xue. 2021 Jun;30(3):292-296.

Zhu X, Chen J, Scheideler L, Reichl R, Geis-Gerstorfer J. Effects of topography and composition of titanium surface oxides on osteoblast responses. Biomaterials. 2004 Aug;25(18):4087-103.

Anexo I – Carta de submissão da revista "Journal of Oral Microbiology"

GRUPO EDUCACIONAL BOM JESUS

Raquel Girardello <raquel.girardello@usf.edu.br>

Submission received for Journal of Oral Microbiology (Submission ID: 218436074)

ZJOM-peerreview@journals.tandf.co.uk <ZJOM-peerreview@journals.tandf.co.uk> 29 de novembro de 2021 13:00
Para: raquel.girardello@usf.edu.br

Taylor & Francis
Taylor & Francis Group

Dear Raquel Girardello,

Thank you for your submission.

Submission ID	218436074
Manuscript Title	MICROBIOLOGICAL ASPECTS IN LONG-TERM SUCCESS OF DENTAL IMPLANTS
Journal	Journal of Oral Microbiology
Article Publishing Charge (APC)	USD $1287.50 (plus VAT or other local taxes where applicable in your country)

**APC only payable if your article is accepted*

You can check the progress of your submission, and make any requested revisions, on the Author Portal.

Thank you for submitting your work to our journal.
If you have any queries, please get in touch with ZJOM-peerreview@journals.tandf.co.uk.

For any queries relating to your APC, please get in touch with APC@tandf.co.uk

Kind Regards,
Journal of Oral Microbiology Editorial Office

Figure 1

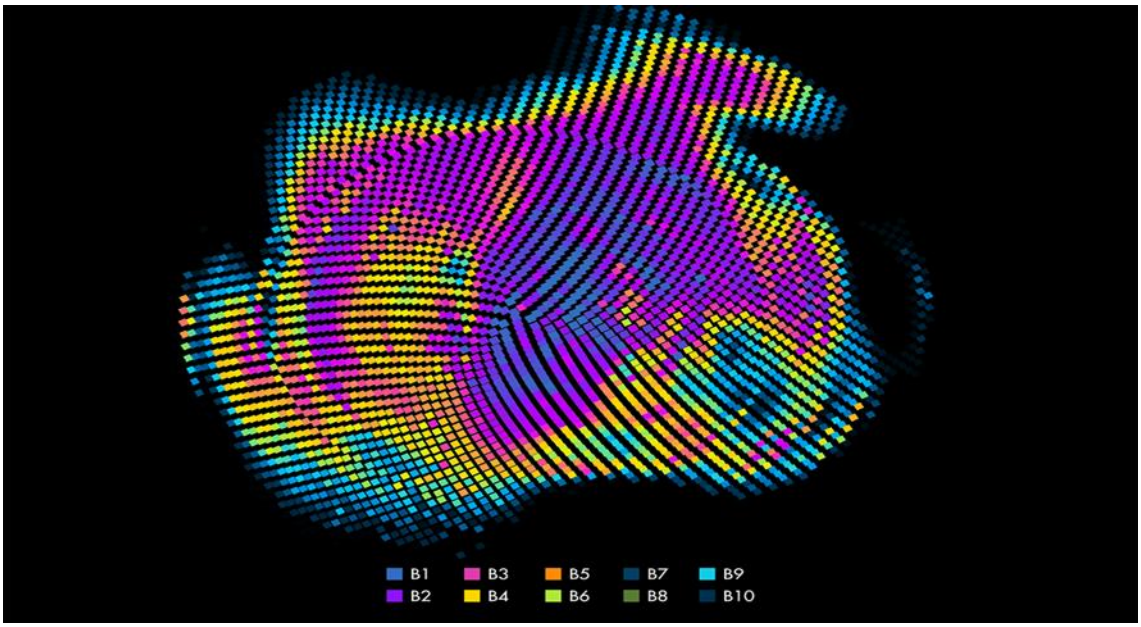
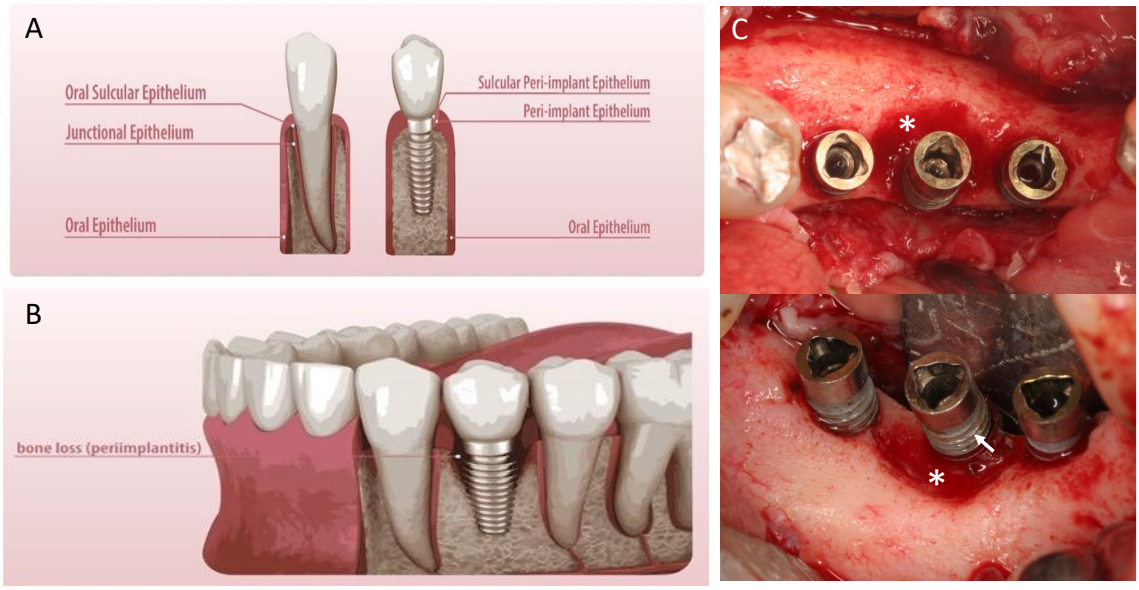


Figure 2


A
Oral Sulcular Epithelium
Junctional Epithelium
Oral Epithelium
Sulcular Peri-implant Epithelium
Peri-implant Epithelium
Oral Epithelium
B
bone loss (periimplantitis)
C

4. CAPÍTULO 2: Em elaboração

GINGIVAL INFECTION AFTER HEALING CAPS PLACEMENT IN DENTAL IMPLANTS: A MICROBIOLOGICAL AND EPIDEMIOLOGICAL STUDY

Este capítulo aborda a obtenção de dados epidemiológicos realizados em revisão de prontuários médicos de pacientes atendidos na Clínica de Implantodontia da Universidade Cruzeiro do Sul em São Paulo, Brasil, de 2017 a 2020. Nesta revisão epidemiológica foram levantados dados sobre quantidade de implantes instalados, tipos de implantes, faixa etária dos pacientes atendidos, bem como sucesso ou insucesso dos implantes instalados. Além disso, também levantados dados sobre comorbidades presentes nos pacientes submetidos a implantes odontológicos.

Além da análise epidemiológica foi realizada a análise das bactérias da cavidade oral de um raro episódio de infecção no local do implante, após a colocação do cicatrizador, em um implante que apresentou boa osteointegração, conforme ilustrado na Figura 6.

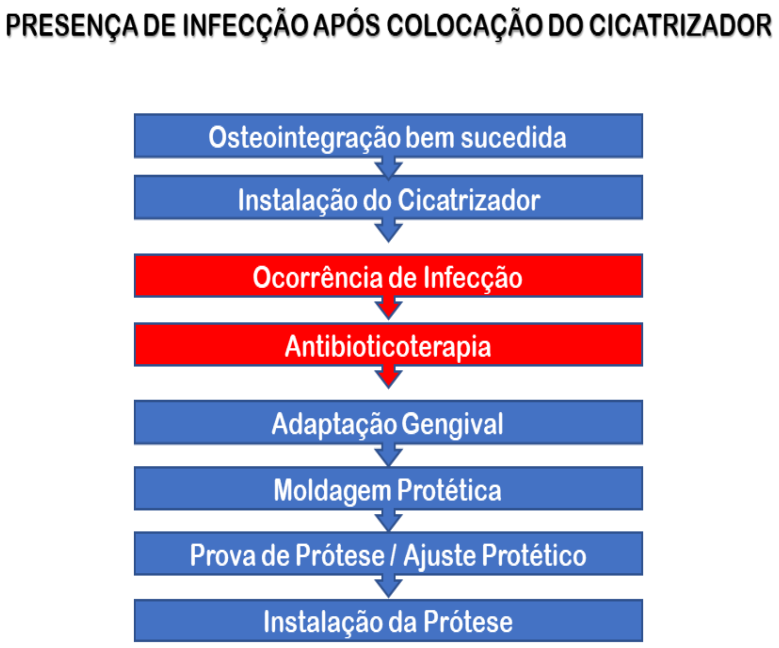


FIGURA 6. Fluxograma utilizado no paciente que apresentou caso de infecção pós colocação de cicatrizador.

GINGIVAL INFECTION AFTER HEALING CAPS PLACEMENT IN DENTAL IMPLANTS: A MICROBIOLOGICAL AND EPIDEMIOLOGICAL STUDY

Carlos Henrique Alves[1#], Kenji Fugito Júnior[2], Karolayne Larissa Russi[1], Natália Conceição Rocha[1], Karina Ferreira Rizzardi[1], Carlos Augusto das Neves[1], Fabio Valverde Rodrigues Bastos Neto[3], Thais Manzano Parisotto[1], Raquel Girardello[1]

[1]Laboratório de Microbiologia Molecular e Clínica, Programa de Pós-Graduação stricto sensu em Ciências da Saúde, Universidade São Francisco, Bragança Paulista, Brazil.

[2]Kenji

[3]Universidade Cruzeiro do Sul, São Paulo, Brazil.

Running Title: Infection after healing-cap installation

Keywords: peri-implantitis; implant; oral microbiota; healing caps

[#]Corresponding Author: Carlos Henrique Alves
Address: 218, São Francisco Ave., Bragança Paulista, São Paulo, Brazil. Zip code: # 12916900.
e-mail: cahe005@hotmail.com

ABSTRACT

The failure of implant procedure is associated with lack of osseointegration of implant and it occurs in the initial phase of protocol, frequently caused by inflammatory or infectious process. Here, we report a patient, without previous comorbidities presenting a gingival tissue infection after healing cap installation. A previous dysbiotic oral microbiota was characterized by qPCR, by detection of elevated load of *S. mutans* in the cheek and tongue surfaces, while these species were not detected in the gingival tissue. In addition, altered oral bacteria quantification was detected after surgery and antimicrobial prophylactic therapy. After literature review and epidemiological data recovered from patients'records in the last four years, reporting of this infection episode was not found.

1. Introduction

Oral rehabilitation through dental implants has become more accessible in the last years. The concern with the implant's lifespan, its adaptation to the bone tissue, the esthetics involved in the process, as well as the constant discoveries of differentiated structures, are understandable and naturally effective progress towards the improvement in the oro-aesthetic-functional rehabilitation of patients (1). Risk factors as smoking patients, bacterial colonization after oral microbiota dysbiosis, and implant texture surface are associated with the unsuccess of the implant. The failure of implant procedure is associated with lack of osseointegration of implant and it occurs in the initial phase of protocol, frequently caused by inflammatory or infectious process (2). Peri-implantitis is defined as inflammatory or infectious process occurring in tissues around dental implants, being results in subsequent progressive loss of supporting bone, which can lead to the implant loss (3,4). ZAHNG et al. (5), observed 45% of peri-mucosite and 10% of peri-implantitis in patients submitted to implant surgeries among 15 years. In this study we presented a patient with success in the osseointegrated implant but presenting an uncommon later infectious process after the healing caps placement.

CASE REPORT

Patient, PFMN, 47 years old, male, healthy, was submitted to single dental implant installation of the lower right first molar. The implant brand used was Dentoflex (switch plataform), size 3.75 mm x 10.0 mm, Morse Cone type.

The entire implant procedure installation was developed as expected, with a 20 N torque, without comorbidities. All necessary biosafety protocols were used. Post-surgical medications prescriptions were amoxicillin 500mg every 8 hours for 7 days, Nimesulide 100 mg every 12 hours, for 7 days, and Sodium Dipyrone 40 drops, every 6 hours, only when feeling pain. Previous prophylactic medications were not prescribed. A “cover” (screw in the form of a cap, sterile, used to protect the implant against external contamination) was installed. The surgical opening was sutured using 4.0 nylon suture thread. Four months after surgery, the patient returned to the dentist office to continue the treatment. In this phase, the surgical site where the implant was installed was

reopened, the cover was removed, and a component called healing cap, “Dentoflex” brand, size 3.0 mm high, was installed.

Three days after the healing cap installation, the patient returned to the office complaining of pain, presence of edema, presence of abscess and periodontal fistula. Infection in the healing cap installation area was detected (Figure 1A and 1B). The radiography performed in this day showed a slight bone lost around the implant, which exposed around 1,5 mm of the implant (Figure 1C). The cap was removed, and the edema was drained. Amoxicillin 500mg 8/8 hours for 7 days and Sodium Dipyrone 40 drops, 6/6 hours for 7 days were administered.

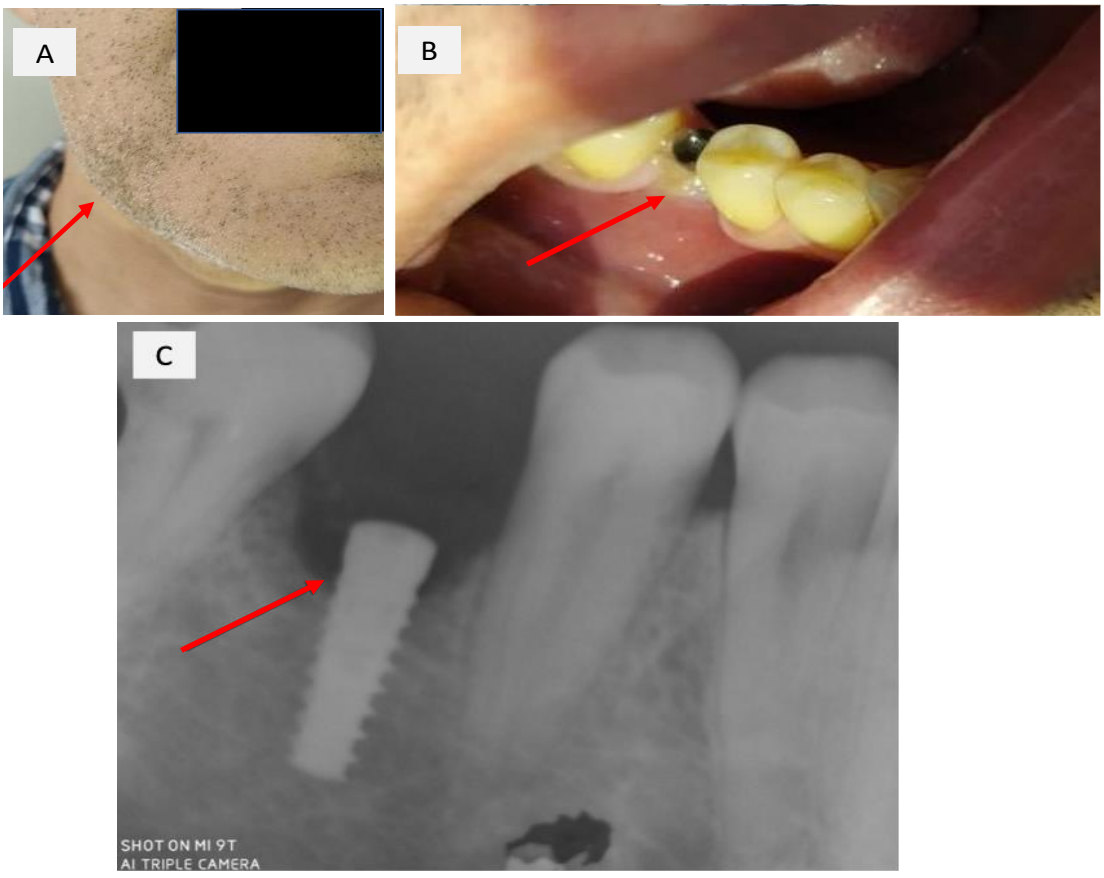


Figure 1. Images obtained at the day the patient sought for attendant reporting infection symptoms. A: Edema caused by infection; B: Periodontal fistula in the gingival tissue; C: Radiography from implant plus healing cap. The arrow indicates the bone loss promoting the exhibition of implant surface.

The patient was asked to return to the office 7 days later for control. The patient had an excellent recovery. One week later the prosthetic phase began. The prosthesis over implant was installed without any apparent problem.

MATERIAL AND METHODS

Ethics Statement

This study was reviewed and approved by Universidade São Francisco research ethical committee. The patients/participants provided their written informed consent to participate in this study.

Samples recover

Oral samples were recovered from tongue and cheek surfaces, and from gingival tissue (implant site) in the patient, in two periods: before the patient had been submitted to implant surgery (T0), and after implant procedure, followed by 7 days of amoxicillin and chlorhexidine prophylactic use (T1). The samples were collected by tissues smears, using ESwab (Copan), and evaluated in research laboratory in the Universidade São Francisco, Bragança Paulista, Brazil, by qPCR analysis.

The samples were selected from a previous study, which did not provide for a sample recover at the healing cap installation phase, so, was not possible to determine the microorganisms from infection period.

Oral microorganisms' quantification

The DNA was extracted by using Microbiome DNA kit (ZymoBiomics, CA, USA), according to manufacture recommendation. The DNA concentration from clinical samples were normalized and submitted to qPCR in a 7300 Real-Time System (Applied Biosystems, Foster City, CA, USA) for total bacteria, Firmicutes phylum, Bacteroidetes phylum, *Streptococcus mutans*, *Lactobacillus* spp. and *Candida* spp., according to protocols previously described. For each sample, 10 mL of the reaction mixture, including 5 μL of SYBR Green Power up (Thermo Fisher Scientific, Carlsbad, CA, USA), 2.9 μL of ultrapure water, 1.5 μL of DNA sample, and 0.3 μL of each primer

were used. Amplification and detection were performed according to the following cycles: 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C, and 1 min at 60°C (6).

Total bacterial DNA extracted from the oral cavity was quantified, based in the 466 pb fragment of conservative sequence of *16S rRNA* gene, using specific primers (F: 5' TCCTACGGGAGGCAGCAGT 3' and R: 5' GGACTACCAGGGTATCTAATCCTGTT 3'), previously described (7). *Escherichia coli* DH5-Alpha was used as control for bacteria quantification standard curve. For Firmicutes phylum quantification, sequences of primers previously described (F: 5′-GGAGYATGTGGTTTAATTCGAAGCA-3′ and R: 5′-AGCTGACGACAACCATGCAC-3′) were used (Guo et al., 2008). *Clostridium perfringens* ATCC 13124 was used as control for Firmicutes standard curve. Forward primer, 5′-GGARCATGTGGTTTAATTCGATGAT-3′ and reverse primer 5′-AGCTGACGACAACCATGCAG-3′ were used to quantify Bacteroidetes phylum bacteria, generating a 126-bp amplicon (8). *Bacteroides fragilis* ATCC 25285 was used as positive control for Bacteroidetes phylum.

Lactobacillus spp. was quantified based in the conservative 126 bp *tuf* gene, using specific primers previously described F: 5'-GAGGCAGCAGTAGGGAATCTTC – 3' and R: 5'-GGCCAGTTACTACCTCTATCCTTCTTC – 3' (9). *Lactobacillus casei* isolate previously characterized in the laboratory was used as control. For *Streptococcus* spp. quantification, a 114 pb fragment of *gtfB* gene was amplified using primers previously described F: 5-GCCTACAGCTCAGAGATGCTATTCT – 3' and R: 5'- GCCATACACCACTCATGAATTGA – 3' (10). *S. mutans* UA159 (ATCC 70061) was used as control to quantification curve. Finally, for *Candida albicans* quantification, previous described primers producing a 229 bp was used: F – 5'- CCTGTTTGAGCGTCRTTT-3'and R: 5'- TCCTCCGCTTATTGATATGC-3' (11). *C. albicans* isolates previously characterized in our laboratory was used as control for curve quantification.

Epidemiological data

Medical records were analyzed, from UNICSUL- Cruzeiro do Sul University, São Paulo, Brazil, at the Implant Specialization Clinic, from patients who had been submitted to implant

surgery, implant brand “Conexão”, from February 2017 to December 2020. This study was previously approved by Ethics Committee of São Francisco University. Statistical analyses were performed by using Qui-square test (Biostat 2.0).

RESULTS

Oral microorganisms’ quantification

The analysis of oral microorganisms’ quantification during the implant surgery procedure evidenced a dysbiosis after 7 days of surgery and prophylaxis with amoxicillin and chlorhexidine (Figure 2). Differences between tongue and cheek tissues, and implant place (gingival tissue) were observed before and after therapy.

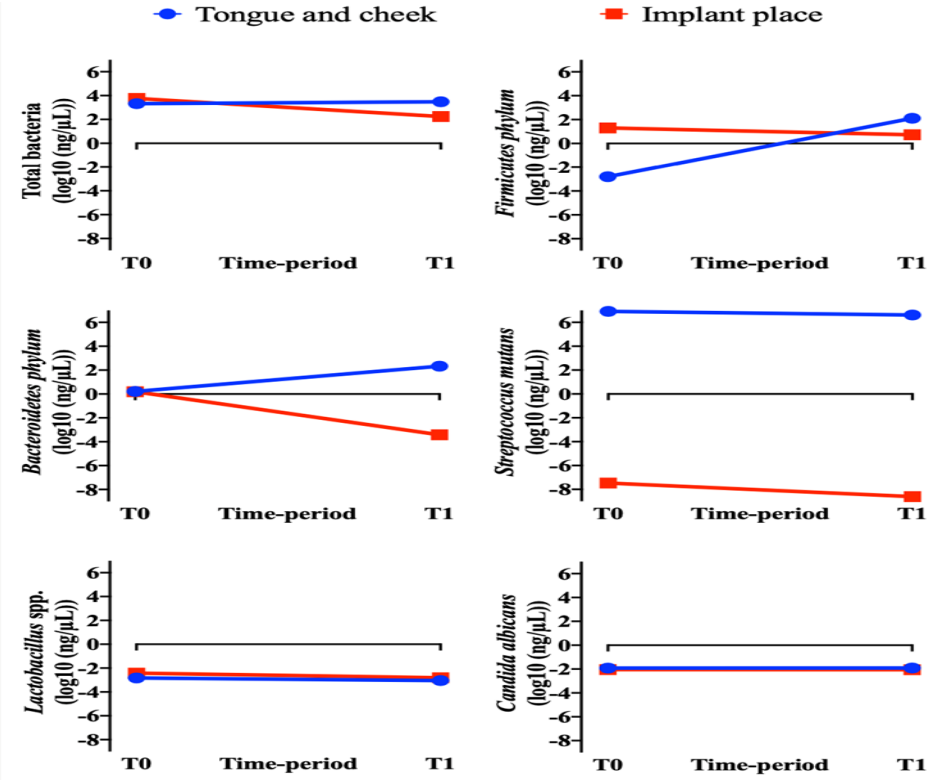


Figure 2. Absolute quantification of oral microorganisms before and after prophylactic therapy using amoxicillin and chlorhexidine mouthwash. The blue lines represent the samples collected from tongue and cheek; The red lines represent the samples collected from gingival tissue in the implant place. The data were expressed in ng/µL and were transformed by log10.

However, no alteration of total bacteria was observed in tongue and cheek tissues. An expressive reduction from 5800,67 ng/µL to 176,24 ng/µL was demonstrated in the gingival tissue. In other hand, increased Firmicutes and Bacteroidetes phylum quantity was observed in the tongue and cheek (from 0,00157 ng/µL to 129,17 ng/µL and from 1,63 ng/µL to 216,19 ng/µL, respectively), but a reduction from 19,77 ng/ µL to 5,40 ng/µL was observed for Firmicutes, and from 216,19 ng/µL to 0,000385 ng/µL for Bacteroidetes in the gingival tissue. Elevated *S. mutans* level was detected in the tongue and cheek sample (8370000 ng/µL) before the surgery. The sample recovered after antimicrobial prophylactic therapy, in the same local, demonstrated a reduction of almost half of the initial level (4120000 ng/µL) to this specie. In addition, *S. mutans* was not detected in gingival tissue (Table 1). For *Lactobacillus* spp. and *C. albicans* microorganisms, low absolute quantity was observed before and after prophylactic therapy.

Table 1. Absolute quantification of oral microorganisms before and after implant surgery followed by amoxicillin and chlorhexidine prophylactic therapy.

Microorganisms group	**Tongue and cheek tissue (ng/µL)[1]**		**Implant place (gingival tissue) (ng/µL)[1]**	
	T0	**T1**	**T0**	**T1**
Total bacteria	2094.92	2989.72	5800.67	176.24
Firmicutes phylum	0.00157	129.17	19.77	5.40
Bacteroidetes phylum	1.63	216.19	1.54	0.000385
Streptococcus spp.	8370000	4120000	0.00000003400	0.00000000248
Lactobacillus spp.	0.00149	0.000902	0.003780	0.009010
C. albicans	0.0119	0.0122	0.001540	0.008790

[1]Absolute quantification performed by qPCR. Data expressed in ng/µL T0: Samples recovered before implant surgery; T1: Samples recovered after implant surgery followed by amoxicillin 500 mg, 8/8 hours, for 7 days and chlorhexidine mouthwash for 3 days.

Patient`s records analysis

In the retrospective four years patients' records analysis, 114 patients (81 women [71.05%] and 33 men [28.95%]) were attended, and 476 implants were installed (Table 2). From 476 implants, 319 (67.0%) were unitary implants installation; 138 (29.0%) were classified as implant supported complete denture (5 implants at the inferior arcade and 6 implants at the superior arcade); and 19 (4.0%) had both protocols in the same patient. The age ranged between 38 and 71 years old and the most implants (unitary implants) were installed in patients between 40 and 50 years old (Figure 3). For overall view, from 476 implants installation, 457 implants (96.01%) were successful, and 19 implants (3.99%) were unsuccessful, due to lack of osseointegration. No infectious diseases were observed in the short term among the patients, over the period evaluated in this study.

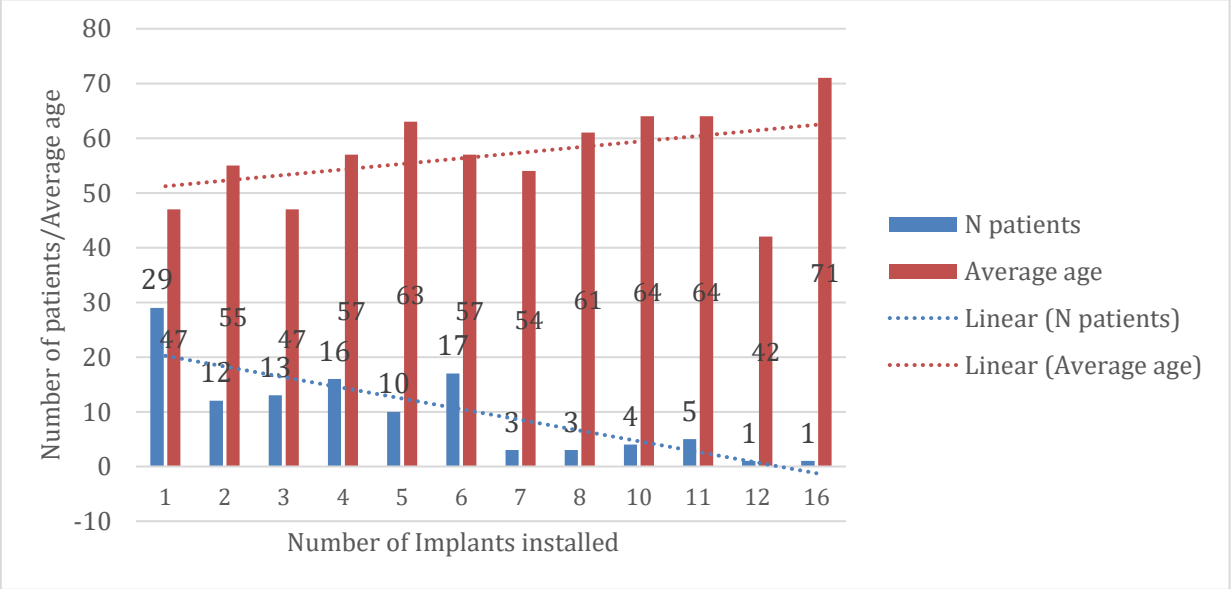


Figure 3. Number of patients and their average age, according to implants quantity distribution.

The Table 2 describes the comorbidities found in the patient in theses analysis. Eighteen (15.78%) patients had at least one comorbidity (10 patients [8.77%] had arterial hypertension; 6 patients [5.26%] had diabetes; and 2 patients [1.75%] were smokers), and 8 patients (7.02%) had more than one comorbidity concomitantly.

Table 2. Number of successful and unsuccessful implants x patient health condition.

Comorbidities*	***Patients N (%)***	***Number of implants***		
		N (%)	***Successful implants***	***Unsuccessful implants***
Arterial hypertension	10 (8.77%)	40 (8.40%)	37 (92.50%)	3 (7.50%)
Diabetes	6 (5.26%)	44 (9.24%)	43 (97.73%)	1 (2.27%)
Smokers	2 (1.75%)	7 (1.47%)	7 (100%)	0 (0%)
Associated comorbidities	8 (7.02%)	47 (9.87%)	45 (95.74%)	2 (4.26%)
No Comorbidities	88 (77.19%)	338 (71.01%)	325 (96.15%)	13 (3.85%)
Total	114 (100%)	476 (100%)	457 (96.01%)	19 (3.99%)

*No comorbidities demonstrated statistically significance for determine the successful, according to implants Qui-square test ($p < 0,05$).

Forty implants (8.40%) were installed on the patients with arterial hypertension (37 were successful [92.50%]; 7 were unsuccessful [7.50%]). Forty-four implants (9.24%) were installed in the patients with diabetes (43 implants were successful [97.73%]; 1 was unsuccessful [2.27%]). Forty-seven (9.87%) implants were installed in patients that had more than one comorbidity concomitantly, where 45 (95.74%) were successful and two were unsuccessful (4.26%), due to lack of osteo integration as well. In these patients' groups, the unsuccessful implants were due to lack of osseointegration, when the prosthesis manufacturing process begins.

Seven implants (1.47%) were installed in two smoker patients and all implants' procedures were successful, with excellent osseointegration and with prosthesis manufacturing processes developed and concluded.

Among the patients that presented no comorbidity, 338 implants were installed (71.01%), being 325 implants (96.15%) successful. Only 13 implants (3.85%) had no osseointegration therefore implant loss.

DISCUSSION

We have observed, by epidemiological data and review literature review, that the infection occurred after the placement of the healing cap, is quite rare. Based on the analysis, we suggest some probable causes for the related infection. During the placement of the healing cap, which is screw made, there a possibility of part of the gingival tissue being smashed between the implant surface and the healing cap. That might have caused necrosis of the tissue and therefore contamination *in loco* by the bacteria leading to infection. When the healing cap is placed, it is necessary a small incision to expose the implant installed. Quite often it is necessary suturing around the healing cap after its placement. We also suggest that after the healing cap placement, food waste might have gotten into the implant region and caused the related infection.

The first day after de surgery, the patient reported no complain at all, having normal food diet, and no medication was prescribed, except analgesic. After 3 days, when the patient had the infection, antibiotic therapy was prescribed. Even though, the antibiotic attacked the infection, we believe that it also contributes to increase the dysbiosis in the patient microbiota (12). It is also interesting remembering that despite of the dysbiosis presented plus the antibiotic therapy afterwards, anaerobic bacterial species might have been installed in the implant local, and they might cause future complications not only to the implant, causing periimplantitis, but also eventual systemic problems for the patient's health (13).

The oral microorganism's quantification evidenced a dysbiosis even before surgery procedure since elevated *S. mutans* load was detected in tongue and cheek samples. After surgery procedure/antimicrobial therapy, variable results were observed comparing different microorganism's groups and between tongue and cheek, and gingival tissues. When total bacteria were evaluated, no difference was observed in the tongue and cheek sample, however, in the gingival tissue was observed an expressive reduction of bacterial level. In a previous study from our group, we observed that the dysbiosis caused by prophylactic therapy associated to surgical procedure is not reversed after a long time from antibiotic and antiseptic use, suggesting that pathogenic microorganisms could be responsible to infections after prolonged time (14).

Despite elevated *S. mutans* load detected in the tongue and cheek samples, low level of this specie was detected in the gingival tissue, even before the implant surgery. According to BAO et al. (15), oral healthy gingival tissue presents more commonly *Streptococcus* species, but, in the gingival disease's tissue, *Streptococcus* spp. are replaced by *Treponema* spp. The *Treponema* species are described as one of the first pathogens in the gingival tissue.

In our study the Firmicutes and Bacteroidetes phylum were increased in the tongue and cheek, but a decrease was observed in the gingival tissue sample, after surgery. This dysbiosis was probably caused by antimicrobial prophylaxis and may contribute to selection of pathogenic species. In addition, HASHIM et al. (16), affirm that the neutrophils had a significant role in the oral microbiota maintenance and periodontal diseases. The authors report that defects in the neutrophils number or efficacy of function predisposes individuals to development of periodontal disease. The inflammatory process produced by healing cap installation associated with previous microbiota dysbiosis could collaborate selecting pathogenic microorganisms and inducing an infectious process. It`s important to highlight that pathogen selected in a disbiotic oral microbiota may also cause extra-buccal infections, as endocarditis, meningitis, and ventilator-associated pneumonia (17). Furthermore, the selection of resistant pathogens in loco may be, in the future, the cause of harmful systemic diseases to the patient.

Associated to dysbiosis in the oral microorganisms, the slight bone loss observed promoted an exhibition of implant surface, which may allow bacterial colonization and biofilm formation in the local.

The patient described in this study had no initial comorbidities. In our patients'records analysis, the patients with arterial hypertension demonstrated a trend to higher proportion of unsuccessful implants, comparing with other comorbidities, including patients with associated comorbidities; despite that, no statistical significance was observed. However, studies reported in this manuscript refer to the period of implant installation up to the prosthetic phase. Long-term clinical follow-up would be necessary to affirm the influence of these comorbidities on the success or failure of implants. All patients had more than 90% of success in their implant surgeries. According to RENVERT & POLYZOIS (18), poor hygiene, smoking, type II diabetes, and poorly made prostheses on implants, are risk indicators for the appearance of peri-implant mucositis. In

the other hand, studies do not present smoking as a high-risk factor for peri-implantitis or, do not support the fact that diabetes might be a risk as well (19,20,21,22). KREBS et al. (23) affirms that diabetes mellitus, smoking and regular maintenance did not show considerable influence on the prevalence of peri-implant diseases. According to systematic review from NASERI et al. (24), there is an increased implant failure only in patients who smoked elevate quantity of cigarettes (>20) per day compared with non-smokers. In addition, no patient had infection detected in the short-term and, no post healing cap installation infection was observed among 476 implants installed.

The results obtained in this study suggest that the determination of an oral microbiota "fingerprint" to determine previous dysbiosis may be important to better clinical management. More studies are necessary to determine the related microbiota referring to the infection process development after implant installation.

2. Conclusion

Considering the clinical aspects and results related to bacterial quantification presented, we concluded that knowledge about oral microbiota is extremely important to warn about possible infections during implant dentistry procedures, not only during the implantation surgery of dental implants, but also at the time of placement of the healer, which is often considered a simple procedure. At this point we may have a possible bacterial contamination leading to infection. The previous bacterial alteration observed suggests that the patient was vulnerable at the time of reopening for placement of the healing cap, but it is important to note that due to this dysbiosis associated with the use of antibiotics, we can obtain resistant pathogen strains that can lodge in the implant site and later, at some point, lead to a new infection, causing peri-implantitis and possible implant loss.

Ethical statement

This study was previously approved by Research Ethical Committee from Universidade São Francisco, Bragança Paulista, Brazil (CAAE number: 22765919.1.0000.5514).

Conflicts of interest

The authors declare no conflicts of interest.

Author contribution

CHA contributed with study design, conduction of experiments, data analysis, and writing of the manuscript. NCR, KLR, KFR and CAN contributed for conduction of experiments, TMP and KFJ contributed with data analysis, and writing and revision of the manuscript, and RG contributed with study design, conduction of experiments, data analysis, and writing and revision of the manuscript.

Acknowledgments

We would like to thank National Council for Scientific and Technological Development (CNPq), Ministry of Science and Technology, Brazil, and by The São Paulo State Research Foundation (FAPESP), for supporting in our researchers.

References

01. Block MS. Dental Implants: The Last 100 Years. J Oral Maxillofac Surg. 2018 Jan;76(1):11-26.

02. Hadadi AA, Mezied MS. Evidence-based analysis of the effect of smoking on osseointegrated implant outcome. Natl J Maxillofac Surg. 2021 May-Aug;12(2):133-138. NJMS_287_20. Epub 2021 Jul 15. PMID: 34483570; PMCID: PMC8386262.

03. Berglundh T. Armitage G, Araújo MG, Avila-Olrtiz G, Blanco J, Camargo PM, Chen S, Cochran D, Derks J, Figuero E, Hammerle CHF, Heitz-Mayfield LJA, Huynh-Ba G, Iacono V, Koo KT, Lambert F, McCauley L, Quirynen M, Renvert S, Salvi GE, Schwarz F, Tarnow D, Tomasi C, Wang HL, Zitzmann N. Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions. J. Clin Periodontol. 2018 Jun;45 Suppl 20: S286-S291.

04. Toledano M, Osorio MT, Vallecillo-Rivas M, Toledano-Osorio M, Rodríguez-Archilla A, Toledano R, Osorio R. Efficacy of local antibiotic therapy in the treatment of peri-implantitis: A systematic review and meta-analysis. J Dent. 2021 Aug 27; 113:103790.

05. Zahng TT, Hu XJ, Lin L. [Prevalence of peri-implantitis and peri-implant mucositis within 15 years of implant placement]. Shanghai Kou Qiang Yi Xue. 2021 Jun;30(3):292-296. Chinese.

06. Rizzardi KF, Indiani CMDSP, Mattos-Graner RO, de Sousa ET, Nobre-Dos-Santos M, Parisotto TM. Firmicutes Levels in the Mouth Reflect the Gut Condition with Respect to Obesity and Early Childhood Caries. Front Cell Infect Microbiol. 2021 May 27; 11:593734.

07. Nadkarni MA, Martin FE, Jacques NA, Hunter N. Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. Microbiology (Reading). 2002 Jan;148(Pt 1):257-266.

08. Guo X, Xia X, Tang R, Zhou J, Zhao H, Wang K. Development of a real-time PCR method for Firmicutes and Bacteroidetes in faces and its application to quantify intestinal population of obese and lean pigs. Lett Appl Microbiol. 2008 Nov;47(5):367-73.

09. Delroisse JM, Boulvin AL, Parmentier I, Dauphin RD, Vandenbol M, Portetelle D. Quantification of Bifidobacterium spp. and Lactobacillus spp. in rat fecal samples by real-time PCR. Microbiol Res. 2008;163(6):663-70.

10. Childers NK, Osgood RC, Hsu KL, Manmontri C, Momeni SS, Mahtani HK, Cutter GR, Ruby JD. Real-time quantitative polymerase chain reaction for enumeration of Streptococcus mutans from oral samples. Eur J Oral Sci. 2011 Dec;119(6):447-54.

11. Rahn S, Schuck A, Kondakci M, Haas R, Neuhausen N, Pfeffer K, Henrich B. A novel comprehensive set of fungal Real time PCR assays (fuPCR) for the detection of fungi in immune ocompromised haematological patients-A pilot study. Int J Med Microbiol. 2016 Dec;306(8):611-623.

12. Swanson BA, Carson MD, Hathaway-Schrader JD, Warner AJ, Kirkpatrick JE, Corker A, Alekseyenko AV, Westwater C, Aguirre JI, Novince CM. Antimicrobial-induced oral dysbiosis exacerbates naturally occurring alveolar bone loss. FASEB J. 2021 Nov;35(11): e22015.

13. Jepsen K, Falk W, Brune F, Fimmers R, Jepsen S, Bekeredjian-Ding I. Prevalence and antibiotic susceptibility trends of periodontal pathogens in the subgingival microbiota of German periodontitis patients: A retrospective surveillance study. J Clin Periodontol. 2021 Sep;48(9):1216-1227.

14. Carlos A. Das Neves, Carlos H. Alves, Natália C. Rocha, Karina Ferreira Rizzardi, Karolyne L. Russi, Alexandre A. Palazzi, Thaís M. Parisotto, Raquel Girardello. Firmicutes dysbiosis after chlorhexidine prophylaxis in healthy patients submitted to impacted lower third molar extraction. Front. Cell. Infect. Microbiol. 2021.

15. Bao K, Li X, Poveda L, Qi W, Selevsek N, Gumus P, Emingil G, Grossmann J, Diaz PI, Hajishengallis G, Bostanci N, Belibasakis GN. Proteome and Microbiome Mapping of Human Gingival Tissue in Health and Disease. Front Cell Infect Microbiol. 2020 Oct 2; 10:588155.

16. Hashim A, Alsam A, Payne MA, Aduse-Opoku J, Curtis MA, Joseph S. Loss of neutrophil homing to the periodontal tissues modulates the composition and disease potential of the oral microbiota. Infect Immun. 2021 Sep 7: IAI0030921.

17. Del Giudice C, Vaia E, Liccardo D, Marzano F, Valletta A, Spagnuolo G, Ferrara N, Rengo C, Cannavo A, Rengo G. Infective Endocarditis: A Focus on Oral Microbiota. Microorganisms. 2021 Jun 4;9(6):1218.

18. Renvert S, Polyzois I. Risk indicators for peri-implant mucositis: a systematic literature review. J Clin Periodontol. 2015 Apr;42 Suppl 16:S172-86.

19. Marrone A, Lasserre J, Bercy P, Brecx MC. Prevalence and risk factors for peri-implant disease in Belgian adults. Clin Oral Implants Res. 2013 Aug;24(8):934-40.

20. Daubert DM, Weinstein BF, Bordin S, Leroux BG, Flemming TF. Prevalence and predictive factors for peri-implant disease and implant failure: a cross-sectional analysis. J Periodontol. 2015 Mar;86(3):337-47.

21. Derks J, Schaller D, Håkansson J, Wennström JL, Tomasi C, Berglundh T. Effectiveness of Implant Therapy Analyzed in a Swedish Population: Prevalence of Peri-implantitis. J Dent Res. 2016 Jan;95(1):43-9.

22. Rokn A, Aslroosta H, Akbari S, Najafi H, Zayeri F, Hashemi K. Prevalence of peri-implantitis in patients not participating in well-designed supportive periodontal treatments: a cross-sectional study. Clin Oral Implants Res. 2017 Mar;28(3):314-319.

23. Krebs M, Kesar N, Begić A, von Krockow N, Nentwig GH, Weigl P. Incidence and prevalence of peri-implantitis and peri-implant mucositis 17 to 23 (18.9) years postimplant placement. Clin Implant Dent Relat Res. 2019 Dec;21(6):1116-1123.

24. Naseri R, Yaghini J, Feizi A. Levels of smoking and dental implants failure: A systemaistic review and (24) meta-analysis. J Clin Periodontol 2020 Apr;47;47(4): 518-528. Epub 2020 Feb 7.

5. Conclusões

- Os profissionais implantodontistas devem ficar atentos a processos infecciosos, mesmo após a completa osteointegração e sucesso do implante.
- No momento da colocação do cicatrizador pode haver uma contaminação bacteriana, levando à infecção futura.
- A alteração na quantificação das bactérias bucais, apresentada pelo paciente, sugere que ele estava vulnerável no momento da reabertura para colocação do cicatrizador e que, associado a um processo inflamatório gerado pelo procedimento, pode ter facilitado o desenvolvimento da infecção pós colocação de cicatrizador.
- Uma alteração da microbiota oral, associada ao uso de antibióticos pode selecionar cepas de patógenos que podem se alojar no local do implante e, posteriormente, levar a uma nova infecção, causando peri-implantite e possível perda do implante.
- O conhecimento sobre a microbiota oral é de extrema importância para alertar sobre possíveis infecções durante procedimentos de implantodontia, não só durante a cirurgia de implantação de implantes dentários, mas também no momento da colocação dos cicatrizador, o que muitas vezes é considerado um procedimento simples.

Referências

1. Lemons J Natiella J. Biomaterials, biocompatibility, and peri-implant considerations. Dent Clin North America 30:3-23, 1986

2. Lee TC. History of dental implants. In Cranin AN (ed): Oral Implantology, Springfield, Charles C. Thomas, 1970 pp. 3-5.

3. Block, M. S. Dental Implants. The Last 100 Years. Journal of Oral and Maxillofacial Surgery, 2018. 76(1), 11–26.08.045.

4. Strock EA. Experimental work on a method for the replacement of missing teeth by direct manipulation of a metal support into the alveolus. Am J Orthodont Oral Surg 1939.25:467-472.

5. Shulman LB, Driskell TD. Dental Implants. An Historical Perspective. In Endosseous Implants for Maxillofacial Reconstruction, Eds Block MS, Kent JN, Saunders, Phila,1995. Chapter 1 pp 1-12.

6. Schnitman PA, Shulman LB. Dental Implants: Benefit and Risk. NIH Consens Statement Online 1978 Jun 13-14 [2021, dec 11];1(3):13-19.

7. Zarb G. Proceedings. Toronto Conference on Osseointegration in Clinical Dentistry, and George A. Zarb. 1983. Proceedings of the Toronto Conference on Osseointegration in Clinical Dentistry, May 1982. St. Louis. Mosby.

8. Branemark P-I, Zarb GA, Albrektsson T. Tissue-Integrated Prostheses: Osseointegration in Clinical Dentistry. Quintessence Publishing Co, Inc, Chicago, 1985.

9. Bastos Neto, FVR. Implantologia: ciência e prática / Fábio Valverde Rodrigues Bastos Neto. ~ 1ª ed. – Maringá – p. 58 – São Paulo: Dental Press,2011.

10. Niinomi M. Mechanical biocompatibilities of titanium alloys for biomedical applications. J Mech Behav Biomed Mater. 2008; 1:30-42.

11. Sabino, R. M., Rau, J. V., De Bonis, A., De Stefanis, A., Curcio, M., Teghil, R., & Popat, K. C. Manganese-containing bioactive glass enhances osteogenic activity of TiO2 nanotube arrays. Applied Surface Science, 2021. 570, 151163.

12. Mei S, Wang H, Wang W, et al. Antibacterial effects and biocompatibility of titanium surfaces with graded silver incorporation in titania nanotubes. Biomaterials. 2014; 35:4255-4265.

13. Nesabi M, Valanezhad A, Safaee S, Odatsu T, Abe S, Watanabe I. A novel multi-structural reinforced treatment on Ti implant utilizing a combination of alkali solution and bioactive glass sol. J Mech Behav Biomed Mater. 2021 Dec; 124:104837.

14. Borges, H., Correia, A., Castilho, R., & Fernandes, G. Zirconia Implants and Marginal Bone Loss*:* A Systematic Review and Meta-Analysis of Clinical Studies. The International Journal of Oral & Maxillofacial Implants, 2020. 35(4), 707–720.

15. Afrashtehfar, K. I., & Del Fabbro, M. Clinical performance of zirconia implants. A meta-review. The Journal of Prosthetic Dentistry. 2019.

16. Körtvélyessy, G., Tarjányi, T., Baráth, Z. L., Minarovits, J., & Tóth, Z. Bioactive coatings for dental implants: A review of alternative strategies to prevent peri-implantitis induced by anaerobic bacteria. 2021. Anaerobe 70, 102404.

17. Chai, M., An, M., & Zhang, X. Construction of a TiO2/MoSe2/CHI coating on dental implants for combating Streptococcus mutans infection. Materials Science and Engineering. 2021. C, 129, 112416.

18. Sedghi L, DiMassa V, Harrington A , Lynch SV , Kapila YL. The oral microbiome: Role of key organisms and complex networks in oral health and disease**.** Periodontol 2000. 2021 Oct;87(1):107-131.

19. Faveri M, Mayer MP, Feres M, de Figueiredo LC, Dewhirst FE, Paster BJ. Microbiological diversity of generalized aggressive periodontitis by 16S rRNA clonal analysis. Oral Microbiol Immunol. 2008. 23:112-118.

20. Kumar PS, Griffen AL, Moeschberger ML, Leys EJ. Identification of candidate periodontal pathogens and beneficial species by quantitative 16S clonal analysis. J Clin Microbiol. 2005. 43:3944-3955

21. Nobbs AH, Zhang Y, Khammanivong A, Herzberg MC. Streptococcus gordonii Hsa environmentally constrains competitive binding by Streptococcus sanguinis to saliva-coated hydroxyapatite. J Bacteriol. 2007. 189:3106-3114.

22. Baehni PC, Takeuchi Y. Anti-plaque agents in the prevention of biofilm-associated oral diseases. Oral Dis. 2003. 9(1 Suppl):23-29.

23. Brito LC, Teles FR, Teles RP, França EC, Ribeiro-Sobrinho AP, Haffajee AD, et al. Use of multiple-displacement amplification and checkerboard DNA-DNA hybridization to examine the microbiota of endodontic infections. J Clin Microbiol. 2007. 45:3039-3049

24. Hojo, K., Nagaoka, S., Ohshima, T., & Maeda, N. Bacterial Interactions in Dental Biofilm Development. Journal of Dental Research. 2009. 88(11), 982–990.

25. Kolenbrander PE, Andersen RN, Blehert DS, Egland PG, Foster JS, Palmer RJ Jr. Communication among oral bacteria. Microbiol Mol Biol Rev. 2002. 66:486-505.

26. Ghannoum MA, Jurevic RJ, Mukherjee PK et al. Characterisation of the oral fungal microbiome (mycobiome) in healthy individuals. PLoS Pathog. 2010. 6: e1000713.

27. Yoda N. Zheng K. Chen J et al.: Bone morphological effects on post-implantation remodeling of maxillary anterior buccal bone: A clinical and biomechanical study. *J Prosthodont Res.* 2017; 61: 393.

28. Tolstunov, L. Peri-Implant Disease: Peri-Implantitis Versus “Peri-Implantosis. Journal of Oral and Maxillofacial Surgery, Volume 78, Issue 5, 680 – 681. May 2020.

29. Marsh PD, Devine DA. How is the development of dental biofilms influenced by the host? J Clin Periodontol. 2011;38(Suppl 11):28–35.

30. Lasserre JF, Brecx MC, Toma S. Oral Microbes, Biofilms and Their Role in Periodontal and Peri-Implant Diseases. Materials (Basel). 2018 Sep 22;11(10):1802.

31. Zhuang LF, Watt RM, Mattheos N, Si MS, Lai HC, Lang NP. Periodontal and peri-implant microbiota in patients with healthy and inflamed periodontal and peri-implant tissues. Clin Oral Implants Res 2016; 27:13-21.

32. Wang HL, Garaicoa-Pazmino C, Collins A, Ong HS, Chudri R, Giannobile WV. Protein biomarkers and microbial profiles in peri-implantitis. Clin Oral Implants Res2016; 7:1129-36.

33. Lafaurie, G. I., Sabogal, M. A., Castillo, D. M., Rincón, M. V., Gómez, L. A., Lesmes, Y. A., & Chambrone, L. (2017). Microbiome and Microbial Biofilm Profiles of Peri-Implantitis: A Systematic Review. Journal of Periodontology, 88(10), 1066–1089.

34. Persson GR, Renvert S. Cluster of bacteria associated with peri-implantitis. Clin Implant Dent Relat Res 2014;16 :783-93.

35. Albertini M, López-Cerero L, O'Sullivan MG, et al. Assessment of periodontal and opportunistic flora in patients with peri-implantitis. Clin Oral Implants Res 2015; 26:937-41.

36. Mulla M, Mulla M, Hegde S, Koshy AV. In vitro assessment of the effect of probiotic lactobacillus reuteri on peri-implantitis microflora. BMC Oral Health. 2021 Aug 19;21(1):408.

37. Flichy-Fernández AJ, Ata-Ali J, Alegre-Domingo T, Candel-Martí E, Ata-Ali F, Palacio JR, Peñarrocha-Diago M. The effect of orally administered probiotic Lactobacillus reuteri-containing tablets in peri-implant mucositis: a double-blind randomized controlled trial. J Periodontal Res. 2015 Dec;50(6):775-85.

38. Peña M, Barallat L, Vilarrasa J, Vicario M, Violant D, Nart J. Evaluation of the effect of probiotics in the treatment of peri-implant mucositis: a triple-blind randomized clinical trial. Clin Oral Investig. 2019 Apr;23(4):1673-1683.

39. Vacca C, Contu MP, Rossi C, Ferrando ML, Blus C, Szmukler-Moncler S, Scano A, Orrù G. In vitro Interactions between *Streptococcus intermedius* and *Streptococcus salivarius* K12 on a Titanium Cylindrical Surface. Pathogens. 2020 Dec 20;9(12):1069.

40. Renvert S, Polyzois I. Risk indicators for peri-implant mucositis: a systematic literature review. J Clin Periodontol 2015; 42(Suppl 16): S172–S186.

41. Velasco-Ortega E, Jiménez-Guerra A et al. Immediate Loading of Implants Placed by Guided Surgery in Geriatric Edentulous Mandible Patients. Int J Environ Res Public Health. 2021 Apr 13;18(8):4125.

42. Luo H, Pan W, Sloan F, et al. Forty-year trends in tooth loss among american adults with and without diabetes mellitus: an age-period-cohort analysis. Prev Chronic Dis. 2015;12: E211.

43. Shurtz-Swirski R, Sela S, Herskovits AT, et al. Involvement of peripheral polymorphonuclear leukocytes in oxidative stress and inflammation in type 2 diabetic patients. Diabetes Care. 2001;24: 104–110.

44. Liu YY, Lu RJ, Guo B, Gao LL, Zhang N, Chang WX. The effects of hyperglycemia on the biological behavior of human gingival fibroblasts on a titanium surface. Ann Palliat Med. 2021 Sep;10(9):9685-9691.

45. Wang IC, Ou A, Johnston J, Giannobile WV, Yang B, Fenno JC, Wang HL. Association between Peri-implantitis and Cardiovascular Diseases: A Case-control Study. J Periodontol. 2021 Nov 1. Epub ahead of print.

46. Bottino MA, Todescan FF. A importância da oclusão na Implantodontia. In: Dinato JC, Polido WD. Implantes osseointegraos: cirurgia e prótese. 1ª ed. São Paulo: Artes Médicas; 2001. P.103-10.

47. Wada S, Kojo T, Wang YH, Ando H, Nakanishi E, Zng M, et al. Effect of loading on the development of nerve fibers around oral implant in the dog mandible. Cin oral implants Res. 2001; 12:219-24.

48. Consolaro a, Carvalho RS, Francischone CE Jr, Francischone CE. Mecanismo da saucerização nos implantes osseointegrados. Rev. Dental Press Periodontia Implantol. 2010;4(1):37-54.