O estUNIVERSIDADE FEDERAL DE PELOTAS
Faculdade de Odontologia
Programa de Pós-Graduação em Odontologia

Tese de Doutorado

Mapeamento da osseointegração a partir de respostas biológicas imuno-dirigidas: evidências a partir de uma revisão sistemática e de um estudo clínico randomizado com foco no carregamento oclusal de pacientes usuários de overdentures mandibulares implanto-retidas.

Amália Machado Bielemann

Pelotas, 2018

Amália Machado Bielemann

Mapeamento da osseointegração a partir de respostas biológicas imuno-dirigidas: evidências a partir de uma revisão sistemática e de um estudo clínico randomizado com foco no carregamento oclusal de pacientes usuários de overdentures mandibulares implanto-retidas.

Tese apresentada ao Programa de Pós-Graduação em Odontologia da Faculdade de Odontologia da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Doutor em Odontologia, área de concentração Prótese Dentária.

Orientadora: Profª. Drª. Fernanda Faot

Pelotas, 2018

Universidade Federal de Pelotas / Sistema de Bibliotecas
Catalogação na Publicação

B111m Bielemann, Amália Machado

Mapeamento da osseointegração a partir de respostas biológicas imuno-dirigidas : evidências a partir de uma revisão sistemática e um estudo clínico randomizado com foco no carregamento oclusal de pacientes usuários de overdentures mandibulares implanto-retidas / Amália Machado Bielemann ; Fernanda Faot, orientadora. — Pelotas, 2018.

144 f. : il.

Tese (Doutorado) — Programa de Pós-Graduação em Prótese Dentária, Faculdade de Odontologia, Universidade Federal de Pelotas, 2018.

Elaborada por Fabiano Domingues Malheiro CRB: 10/1955

Amália Machado Bielemann

Mapeamento da osseointegração a partir de respostas biológicas imuno-dirigidas: evidências a partir de uma revisão sistemática e de um estudo clínico randomizado com foco no carregamento oclusal de pacientes usuários de overdentures mandibulares implanto-retidas.

Tese aprovada, como requisito parcial, para obtenção do grau de Doutor em Odontologia, Programa de Pós-Graduação em Odontologia, Faculdade de Odontologia de Pelotas, Universidade Federal de Pelotas.

Data da defesa: 11 de maio de 2018.

Banca examinadora:

Profª. Drª. Fernanda Faot (Presidente)
Doutora em Clínica Odontológica (Área de Prótese Dentária) pela Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas.

Profª. Drª. Natália Marcumini Pola
Doutora em Odontologia (Área de Periodontia) pela Faculdade de Odontologia de Araçatuba da Universidade Estadual Paulista Júlio de Mesquita Filho.

Profª. Drª. Raissa Micaella Marcello Machado
Doutora em Clinica Odontológica (Área de Prótese Dentária) pela Faculdade de Odontologia de Piracicaba

Profª. Drª. Luciana de Rezende Pinto
Doutora em Clínica Odontológica (Área de Prótese Dentária) pela Faculdade de Bauru da Universidade de São Paulo

Prof. Dr. José Antônio Mesquita Damé (Suplente)
Doutor em Odontologia (Área de Concentração Periodontia) pela Faculdade de Odontologia de Bauru

Prof. Dr. Otacílio Luis Chagas Júnior (Suplente)
Doutor em Odontologia (Área de Concentração Cirurgia e Traumatologia Buco-Maxilo Facial) pela Pontifícia Universidade Católica do Rio Grande do Sul

AGRADECIMENTOS

À Universidade Federal de Pelotas, por meio do seu magnífico Reitor, **Prof. Dr. Pedro Rodrigues Curi Hallal**, à Faculdade de Odontologia, na pessoa de sua Diretora, **Profª. Drª. Adriana Etges**, e ao Programa de Pós-Graduação em Odontologia/UFPel, por meio da coordenadora **Profª. Drª. Tatiana Pereira Cenci**.

Ao **CNPq**, pelo financiamento deste trabalho (Edital Universal CNPq nº 476170/2013-3), e a **CAPES**, pela minha bolsa de Doutorado, possibilitando minha dedicação exclusiva ao programa de pós-graduação e ao desenvolvimento deste estudo.

À empresa **Neodent** pela aprovação do nosso trabalho no Programa de Auxílio a Pesquisa. Ao Dr. Sérgio Bernardes pela disponibilidade e colaboração na execução deste projeto.

A **Profª Drª Fernanda Faot**, minha orientadora, por me guiar, incentivar e acreditar na minha capacidade. Já são sete anos de convivência diária, obrigada pela amizade durante toda essa trajetória, pela confiança e encorajamento. Me fazes querer aprender mais e te tenho como um exemplo a ser seguido, espero poder retribuir a dedicação e a amizade. Obrigada!

Aos membros da banca, **Prof. Dr. José Antônio Damé, Profª Drª Luciana Rezende; Profª Drª Natália Marcumini Pola e Prof. Dr. Otacílio Luiz Chagas Jr.** Obrigada pelos momentos de discussão, parcerias, aprendizado e amizade.

As amigas e colaboradores desta tese, **Alessandra Julie Schuster, Raissa Micaella Marcello Machado**, obrigada pela amizade e apoio. Esta tese também é de vocês, sem a parceria, a colaboração e a ajuda não teria conseguido executar todas as etapas.

A minha família por serem a base e o encorajamento. A minha mãe, **Valquiria Bielemann**, sem teu apoio e incentivo não teria chegado aqui. Obrigada por me guiar a seguir para pós-graduação. Ao meu Pai, **José Antônio**, e a minha Avó, **Emília Bielemann**, hoje quem sou é reflexo da dedicação e carinho de vocês, obrigada pela compreensão. Ao meu irmão, **Vinicius Bielemann**, pela atenção e cuidado.

Obrigada as amigas **Ana Paula Perroni, Camilla, Laura, Luisa Backes, Luiza Monteiro, Marcella, Marina, Nathalia, Paula,** agradeço a amizade, apoio, incentivo e torcida. Obrigada, por todos os momentos compartilhados, nossas diferenças nos unem e nos completam. Vocês são a minha sanidade, parte do que sou é devido nossa amizade.

Aos amigos que acompanharam mais de perto minha trajetória acadêmica: **Prof. Dr. David Debruyne, Dr. Gustavo G. Nascimento; Ms. Anna Paula da Rosa Possebon, Ms. Karine Duarte da Silva, Ms. Marina Madruga, Ms. Lucas Brondani,** e **Ms. Wellington da Rosa**.

Ao **Ms. André Schinestsck** pela disponibilidade de sua clínica sempre que necessário à execução de exames radiológicos.

Por fim, aos funcionários da Faculdade de Odontologia da UFPel. Agradeço a todos que trabalharam de forma direta e indireta para que este trabalho pudesse ser realizado. Agradeço aos **técnicos da radiologia** (Jefersson, Josinei e Jonas) pela disposição e por atenderem nossos pedidos de urgência. Aos **enfermeiros** do bloco cirúrgico, gurias da **esterilização** e aos funcionários do segundo andar, sempre solícitos quando era necessário.

Muito obrigada a Todos!

Qualquer trabalho seria terrivelmente aborrecido se não jogássemos o jogo apaixonadamente.

(Simone de Beauvoir)

Notas Preliminares

A presente tese foi redigida segundo o Manual de Normas para trabalhos acadêmicos da UFPel, adotando o nível de descrição em Artigos capítulos não convencionais. Disponível no endereço eletrônico: http://sisbi.ufpel.edu.br/arquivos/PDF/Manual_Normas_UFPel_trabalhos_acad%C3%AAmicos.pdf.

O projeto de pesquisa referente a esta Tese, foi aprovado em 23 de fevereiro de 2017, pela Banca Examinadora composta pelos Professores Doutores Fernanda Faot, Gustavo Giacomelli Nascimento, Luciana de Rezende Pinto e Natália Marcumini Pola.

Resumo

BIELEMANN, Amália Machado. **Mapeamento da osseointegração a partir de respostas biológicas imuno-dirigidas: evidências a partir de uma revisão sistemática e de um estudo clínico randomizado com foco no carregamento oclusal de pacientes usuários de overdentures mandibulares implanto-retidas.** 2018. 144f Tese (Doutorado em Odontologia) – Programa de Pós-Graduação em Odontologia. Universidade Federal de Pelotas, Pelotas, 2018.

A osseointegração de implantes dentários ocorre devido ao processo desencadeado pela resposta imune do hospedeiro, entretanto os biomarcadores que modulam esse processo ainda não foram determinados. Com o intuito de investigar e compreender a ossointegração por meio dos biomarcadores foram delineados dois estudos: I) Revisão sistemática (RS) com foco na avaliação de coleta de fluido crevicular peri-implantar (FCPI) durante a cicatrização de óssea após a inserção de implantes dentários; II) Estudo clínico longitudinal randomizado de acordo com o carregamento oclusal, carga imediata (CI) ou convencional (CC), de overdentures mandibulares. O estudo II avaliou o comportamento clínico e biológico da osseointegração de implantes de diâmetro reduzido (IDR) submetidos a CI ou CC, inseridos em 20 pacientes desdentados totais, com elevado tempo de edentulismo e limitada disponibilidade óssea mandibular. Na RS foram selecionados 30 estudos clínicos e identificados 52 biomarcadores durante o período de osseointegração. Os biomarcadores mais estudados foram interleucina (IL) -1β, fator de necrose tumoral alfa (TNF-α) e óxido nítrico (NO). As coletas de FCPI foram realizadas imediatamente após inserção do implante até 16 semanas antes do carregamento oclusal. Através dos dados coletados nesta RS, não foi possível identificar qual o mecanismo pelos quais os biomarcadores inflamatórios e ósseos são liberados durante a osseointegração. No entanto, eventos já conhecidos da osseointegração foram associados com os resultados dos estudos clínicos disponíveis, sendo este, um guia aos futuros pesquisadores, devido o mapeamento de todos os biomarcadores já avaliados durante esse processo. No estudo “II”, o grupo CI apresentou estabilidade 8,95% menor que o CC, até a semana 12 ($p<0,05$). O cálculo no CI foi 50% maior que no CC na semana 1 ($p=0.006$), e 30% menor na semana 8 ($p=0.017$). A profundidade a sondagem do CI foi em média 21,49% inferior ao grupo CC em todos os períodos avaliados ($p=0.05$). O sangramento a sondagem foi 28,9% maior para o CI na semana 12 ($p=0,044$). Implantes que receberam CI apresentaram a concentração de TNF-α 40,75% maior até a semana 4 ($p<0.05$) e 57,78% mais IL-1β, após a semana 4 até a 12 que o grupo CC. A concentração de IL-6 foi 53,94% menor para o CI, até a semana 8. A concentração de IL-10 teve aumento progressivo significativo e similar para ambos os grupos até a semana 8, na semana 12 o grupo CI teve 45,74% maior concentração do que o grupo CC ($p=0.003$). A taxa de sobrevivência foi de 90% para ambos os grupos. Os implantes que receberam CI apresentaram resultados clínicos mais estáveis, mas resultados biológicos mais instáveis durante a cicatrização óssea. Diante disso, a reabilitação da população com baixa disponibilidade óssea em região anterior de mandíbula com overdentures mandibulares é mais segura quando feita com CC, pois a resposta inflamatória foi mais controlada neste grupo.

Palavras-chave: Implantes dentários; osseointegração; biomarcadores; edentulismo; atrofia óssea; interleucinas, implantes de diâmetro reduzido.

Abstract

BIELEMANN, Amália Machado. **Mapping the osseointegration using immuno-driven biological processes: evidences from a systematic review and a randomized clinical trial with focus on occlusal loading of implant-retained mandibular overdentures wearers.** 2018. 144f. Thesis (PhD in Dentistry). Graduate Program in Dentistry. Federal University of Pelotas, Pelotas, 2018.

The dental implant osseointegration occurs due to the process triggered by the host immune response, however the biomarkers that modulate this process have not yet been determined. In order to investigate and understand ossointegration through the biomarkers, two studies were designed: I) Systematic Review (SR) focusing on the evaluation of peri-implant crevicular fluid collection (FCPI) during bone healing after dental implants insertion; II) Longitudinal Randomized Clinical Trial according to the occlusal loading, immediate (IML) or conventional (CL), of implant retained mandibular overdentures. The study II evaluated the clinical and biological behavior of narrow diameter implants (NDR) osseointegration submitted to IML or CC, inserted in 20 totally edentulous patients, with a high edentulism time and limited mandibular bone availability. The SR selected 30 clinical studies and identify 52 biomarkers reported during osseointegration. The most studied biomarkers were interleukin (IL) -1β, tumor necrosis factor alpha (TNF-α) and nitric oxide (NO). PICF collections were performed immediately after implant insertion up to 16 weeks, prior to occlusal loading. Through the data collected, it was not possible to identify the mechanism by which inflammatory and bone biomarkers are released during osseointegration. However, it was possible to associate cellular and molecular events triggered with osseointegration with the results of available clinical studies. The data summarized can guide researchres to design future clinical studies and can help selecting target biomarkers already quantified. The study "II" showed that IML group presented implant stability 8.95% lower than the CL, until week 12 ($p < 0.05$). The calculus presence in IML group was 50% higher than the CL at week 1 ($p = 0.006$), and 30% lower at week 8 ($p = 0.017$). The IML probing in depth was about 21.49% lower than in the CL group for all evaluated periods ($p = 0.05$). The bleeding on probing was 28.9% higher for IML at week 12 ($p = 0.044$). IML group presented the highest TNF-α concentration by week 4 ($p < 0.05$) and 57.78% more IL-1β after week 4 to 12 than the CL group. The IL-6 concentration was 53.94% lower for IML group until week 8. The IL-10 concentration had a significant progressive and similar increase for both groups until week 8, and at week 12 the IML group had 45.74% higher concentration than the CL group ($p = 0.003$). The implant survival rate was 90% for IML and CL. The implants immediately loaded showed more stable clinical results, however presented unstable biological resposnes during bone healing. Therefore, the rehabilitation of the population with low bone availability in the anterior region of the mandible using implant retained mandibular overdentures seems to be safer when CL is adopted based on the inflammatory biomarker release.

Keywords: Dental implants; osseointegration; biomarkers; edentulism; bone atrophy; interleukins, narrow diameter implants.

SUMÁRIO

INTRODUÇÃO

A população brasileira com 60 anos ou mais passou de 12,8% para 14,4%, entre 2012 e 2016 (IBGE, 2017), há a expectativa de que esta população em 2050 suba para 22,7% (IBGE, 2008). Segundo o SB Brasil 2010, na população entre 65 e 74 anos 63,1% fazem uso de prótese total, 37,5% usam prótese total inferior, mas o mais alarmante é que 17,9% necessitam de prótese total em um dos maxilares e 15,4% em ambos os maxilares (BRASIL, 2012). Esses dados vão ao encontro do relatório sobre envelhecimento e saúde da Organização Mundial da Saúde (2015), no qual relata o aumento da expectativa de vida e consequente declínio na mortalidade de idosos. Entretanto, o mesmo, preconiza que o envelhecimento saudável deve preconizar a manutenção da habilidade funcional dos idosos, além da ausência de doença, assim, o processo de desenvolvimento e manutenção da capacidade funcional permite o bem-estar em idade avançada (WORLD HEALTH ORGANIZATION, 2015). Portanto, a conservação da saúde oral é fundamental ao bem-estar e saúde dessa população, e envolve a manutenção da habilidade e capacidade de se alimentar e se comunicar.

Em vista disso, o uso de implantes dentários tornou-se rotina na reabilitação oral de pacientes desdentados totais, principalmente pela garantia de sucesso, previsibilidade do tratamento (THOMASON et al., 2009). Bem como, seu impacto direto na força de mordida, habilidade e performance mastigatória, influenciando diretamente no controle da coordenação neuromuscular (FERRARIO et al., 2005). Entretanto, o sucesso da osseointegração de implantes é dependente da resposta do hospedeiro, ou seja, da capacidade de cicatrização óssea do paciente. Sendo, as contra-indicações para a reabilitação com implantes dentários limitadas às condições sistêmicas graves (como osteogênese imperfeita e artrite reumatóide), comprometimento do sistema imune ou relacionadas ao não acompanhamento adequado de doenças crônicas, como diabetes severa e desordens sanguíneas, além de história recente de radioterapia na região da cabeça ou pescoço (BORNSTEIN; CIONCA; MOMBELLI, 2009). Neste cenário, a maioria dos pacientes idosos apresenta pelo menos uma condição crônica, como hipertensão (50% da população) ou hiperglicemia (18% da população), além de portadores de múltiplas condições diversas decorrentes da idade, como visão reduzida e dificuldade de locomoção, as quais não os impede de serem reabilitados com implantes dentários (IBGE, 2014).

Outro fator que pode influenciar o sucesso da osseointegração nestes pacientes é o tempo de edentulismo prolongado, principalmente enquanto usuários

de próteses totais mandibulares. A perda dos elementos dentários ocasiona a reabsorção progressiva do osso alveolar, a qual é mais acentuada na mandíbula (NAERT et al., 2004). Desta forma, o prolongado tempo de edentulismo acarreta em modificações intra-orais, tais como, a perda da resiliência e sensibilidade da fibromucosa, redução da altura e espessura do osso alveolar e alteração do formato do rebordo alveolar (TALLGREN, 1972). Esses fatores influenciam todo o procedimento reabilitador, desde a escolha do tipo de prótese, dos implantes dentários até ao ato cirúrgico menos traumático. Diante disso, ainda é necessário investigar e compreender os processos de cicatrização óssea após instalação de implantes em populações desdentadas totais com idade avançada e com características anatômicas e fisiológicas específicas, frente a diferentes abordagens de tratamento baseadas na seleção do tipo de implante.

Os resultados encontrados no estudo clínico conduzido por Bielemann et al. (2018)(BIELEMANN et al., 2018), evidenciaram que populações com elevado tempo de edentulismo e pouco volume ósseo na região anterior de mandíbula, podem requerer um maior tempo de cicatrização óssea para que os implantes dentários alcancem uma adequada estabilidade secundária. Adicionalmente, relataram que leitos ósseos com osso tipo I e pacientes fumantes apresentam um metabolismo de remodelação óssea não linear (instável), enquanto pacientes com atrofia óssea mandibular apresentaram um metabolismo de remodelação óssea diferenciado notado pela liberação de fator de necrose tumoral alfa (TNF-α). A qual é, potencializadora da osteoclastogênese e responsável pelo recrutamento e ativação de células do sistema imune para o local da lesão (HALL et al., 2015; STOW et al., 2009). Os níveis de TNF-α foram elevados entre os 30 e 60 dias de cicatrização em pacientes atróficos ou seja, a atividade de remodelação por reabsorção óssea foi mais prolongada nessa população (BIELEMANN et al., 2018). Importante destacar que a liberação de TNF-α também têm sido positivamente correlacionada a perdas ósseas sistêmicas (osteoporose, menopausa e artrite) (LIM; PARK; KIM, 2016; WEI et al., 2016)**.** Em vista disso, ainda há a necessidade de investigação e compreensão da influência de biomarcadores da inflamação e do reparo ósseo durante a cicatrização de implantes nessas populações assim como, o estudo de protocolos de tratamentos com maior previsibilidade e sucesso.

Do ponto de vista biológico, o sucesso da reabilitação com implantes é totalmente dependente da resposta ao processo desencadeado no microambiente

ósseo ao receber e perceber o implante dentário, o qual resulta em uma resposta inflamatória de corpo estranho. O implante ao ser reconhecido como um corpo estranho, gera uma resposta imune-modulada de sinalização celular e ativação do sistema complemento resultando em uma reação inflamatória de cicatrização (ALBREKTSSON et al., 2001; TRINDADE et al., 2016). Esta reação é localmente modulada por biomarcadores inflamatórios como citocinas, proteínas ou peptídeos multifuncionais que exercem suas funções como fatores reguladores intercelulares em níveis locais e sistêmicos. Assim, a expressão de biomarcadores específicos regularizam a intensidade da resposta inflamatória tanto para os processos de iniciação da reação de corpo estranho, cicatrização e organização celular, quanto para os processos patológicos (ALBREKTSSON et al., 2001; CHANG; LANG; GIANNOBILE, 2010; TRINDADE; ALBREKTSSON; WENNERBERG, 2015). Diante deste contexto, a osseointegração tem sido descrita como um processo dinâmico dependente do equilíbrio da expressão de biomarcadores, em que a perturbação desse estado pode desencadear uma reação inflamatória exacerbada podendo levar a perda da osseointegração.

Um dos métodos que pode ser utilizado para investigar e mapear esse processo é a análise da liberação de citocinas pró- e anti-inflamatórias por meio da coleta de fluido crevicular peri-implantar (FCPI), o qual é um método não-invasivo de monitoramento do estado de saúde dos tecidos peri-implantares. Prati et al. (2013) (PRATI et al., 2013), salienta que apesar desse método prover informações sobre o microambiente ósseo, este ainda não é muito utilizado no monitoramento da cicatrização do implante, tendo em vista que inúmeros estudos utilizam o método para monitorar saúde peri-implantar somente após a osseointegração. Além disso, devido ao elevado custo laboratorial para este tipo de análise, informações sobre quais biomarcadores inflamatórios seriam mais adequados para o monitoramento de cada processo biológico ainda são escassas. Em contrapartida, revisões sistemáticas já abordaram o uso deste método afim de prever e contribuir ao diagnóstico precoce da saúde peri-implantar após a osseointegração (DUARTE et al., 2016; FAOT et al., 2015).

O alcance da osseointegração também pode estar ligado diretamente a estabilidade primária alcançada pelo torque de inserção (TI) dos implantes durante o ato cirúrgico. O TI é considerado uma propriedade preditiva da resistência biomecânica alcançada pela interface osso-implante imediatamente após sua

instalação (HOF et al., 2014). Embora o elevado torque, seja capaz de prenunciar uma osseointegração bem sucedida (JAVED et al., 2013), conforme a microarquitetura óssea e os protocolos de fresagem adotados, ele também pode gerar excessiva osseocompressão, ocasionando fraturas e induzindo a remodelação pronunciada do leito ósseo, favorecendo o insucesso do tratamento (DUYCK et al., 2015; ISIDOR, 2006). Diante do fato de que a baixa ou ausente estabilidade inicial poder estar relacionada ao aumento das falhas de osseointegração (ALBREKTSSON et al., 2017; MONJE et al., 2014), Duyck e colaboradores (2015) realizaram um estudo *in vivo* afim de, avaliar o efeito do torque de inserção na cicatrização óssea. Os autores, evidenciaram que, quando um baixo TI é utilizado, nota-se no leito ósseo uma menor área de contato entre o osso-implante gerando espaços que garantem o preenchimento entre as roscas do implante por coágulos sanguíneos, desencadeando rápida neoformação óssea (DUYCK et al., 2015). Desta forma, acredita-se que não é necessário que ocorra a reabsorção do osso velho para a formação do novo, e assim os implantes inseridos com baixo TI podem alcançar estabilidade biológica, enquanto implantes colocados com TI elevado desenvolvem uma estabilidade biológica logo após a estabilidade mecânica inicial (GREENSTEIN; CAVALLARO, 2017).

Os resultados clínicos de diferentes carregamentos oclusais adotados em reabilitações do tipo overdentures mandibulares (OM) têm sido descritos em revisões sistemáticas (SCHIMMEL et al., 2014; ZYGOGIANNIS et al., 2016), mostrando que o carregamento convencional (CC) apresenta tendências de sucesso e sobrevivência mais favoráveis que o carregamento imediato (CI). Entretanto, quando os estudos clínicos selecionados para análises comparativas nas referidas revisões, são analisados separadamente, diferenças significativas entre os tipos de carregamento não são evidenciadas. No que se refere a implementação de CI para overdentures, Zygogiannis et al. (2016) reforçam que a adoção de protocolos de carregamento precoce ou tardio ainda são preferíveis em virtude dos problemas apresentados pelo desenho dos estudos clínicos analisados, como tamanho amostral, diferentes desfechos e diversos tempos de acompanhamentos. Por fim, ainda recomenda que quando o CI for adotado, o estabelecimento da estabilidade primária e a otimização da distribuição biomecânica das cargas através de um desenho da prótese e de um ajuste oclusal apropriados devem ser criteriosamente observados (ZYGOGIANNIS et al., 2016). Ainda, de acordo com a revisão sistemática de Marcello-Machado et al.

(2018), pacientes com limitada espessura óssea podem ser reabilitados de forma previsível e satisfatória com OM retidas por IDRs. Este estudo demonstrou por meio de meta-análise que independente do carregamento oclusal, a perda óssea marginal em 1 ano pode ser de 0.18 mm (0.20–0.57mm), em 2 anos de 0.12 mm (0.36–0.60 mm) e em 3 anos -0.32 mm (- 1.29–0.64) com taxas de sobrevivência e sucesso de 98% e 96%, respectivamente em curto e longo prazo. Além disso, evidenciou que os IDRs apresentam melhor previsibilidade em longo prazo quando recebem CC. Entretanto, esta revisão apontou que o CI em IDRs ainda é inexplorado, uma vez que somente um estudo (CHO et al., 2007) foi encontrado descrevendo o desempenho clínico desta intervenção em apenas 10 pacientes.

Biologicamente, o estudo de Prati et al. (2013) ao acompanhar a reabilitação com próteses totais tipo protocolos Brånemark em pacientes com idade média de 55,5 anos, concluiu que o CI imediato induz a maior liberação de biomarcadores ósseos, gerando uma resposta mais rápida do organismo para a substituição do osso velho ao redor do implante e assim, ocasionando um maior contato entre osso-implante. Elsayd et al (2016), reabilitou pacientes com idade média de 62,6 anos com OM e CI retidas por componentes do tipo magnético ou do tipo botão. Após 1 ano de acompanhamento, implantes com componente do tipo botão apresentaram alta taxa de sobrevivência, 96.9%. Além disso, os referido implantes mostraram diminuição do ISQ e maior perda óssea, entretanto estes resultados podem ser atribuídos ao estresse funcional que implantes não-esplintados recebem (ELSYAD et al., 2016). Implantes com componente tipo botão também tiveram menores concentrações de IL-1β, imputada a menor presença de placa visível do que no componente magnético. Bielemann et al. (2018), em um estudo com OM acompanhou o período de cicatrização óssea de implantes com CC durante 3 meses, e evidenciou que que a IL-1β é um indicador de proteção tecidual, pelo fato de que seus resultados mostraram um pico de concentração desta citocina após a fase de trauma cirúrgico simultaneamente ao aumento do índice de placa visível. Frente a isto, ressalta-se a importância de compreender e identificar quais são as possíveis repostas biológicas frente a cada tipo de carregamento oclusal durante a fase de cicatrização óssea.

Por fim, ressalta-se a necessidade de mapear a reação de cicatrização óssea e identificar os biomarcadores inflamatórios presentes no fluído peri-implantar após a instalação de implantes dentários afim, de compreender o processo imune-modulado de osseointegração. Adicionalmente, a carência de estudos com pacientes com

elevado tempo de edentulismo mandibular, com deficiências de quantidade e qualidade óssea demostra ainda existir, a necessidade de investigações mais profundas nessas populações no que se refere a determinação do perfil de resposta de cicatrização óssea de implantes dentários. Devido a isso, esta Tese buscou por meio de 2 trabalhos científicos, relatar evidências de como o processo de osseointegração pode ser compreendido por meio de conceitos da osteoimunologia baseados no disparo da reação de corpo estranho e ainda, investigar como os diferentes carregamentos oclusais (imediato ou convencional) influenciam a cicatrização óssea e peri-implantar de IDR's instalados em uma população idosa de desdentados totais.

Dessa forma esta tese tem por objetivos específicos:

1. Revisar sistematicamente a literatura a fim de caracterizar a atuação dos biomarcadores no fluido crevicular peri-implantar durante os estágios da cicatrização óssea de implantes dentários;
2. Apresentar um ensaio clínico randomizado para monitorar clinicamente e fisiologicamente a cicatrização de implantes osseointegráveis instalados em pacientes desdentados mandibulares submetidos a 2 diferentes carregamentos oclusais.

Capítulo 1

Artigo Original publicado no periódico *Archives of Oral Biology*

Systematic review of wound healing biomarkers in peri-implant crevicular fluid during osseointegration

Amália Machado Bielemann[a], Raissa Micaella Marcello-Machado[b], Altair Antoninha Del Bel Cury[c], Fernanda Faot[d,*]

[a] Graduate Program in Dentistry, School of Dentistry, Federal University of Pelotas, RS, Brazil

[b] Faculty of Dentistry, Piracicaba Dental School, University of Campinas, Piracicaba, SP, Brazil

[c] Department of Prosthodontics and Periodontology, School of Dentistry, Piracicaba Dental School, University of Campinas, Piracicaba, Brazil

[d] Department of Restorative Dentistry, School of Dentistry, Federal University of Pelotas, Gonçalves Chaves Street 457, 96015-560 Pelotas, RS, Brazil

Running Title: Biomarkers in peri-implant crevicular fluid during osseointegration.

Corresponding author:

Prof. Fernanda Faot, PhD, Associate Professor, School of Dentistry, Federal University of Pelotas. Address: Gonçalves Chaves Street 457; 96015-560; Pelotas, RS, Brazil; e-mail: fernanda.faot@gmail.com

*Artigo publicado no periódico *Archives of Oral Biology*, sendo aqui apresentado com estrutura conforme normas do referido periódico.

https://doi.org/10.1016/j.archoralbio.2018.02.013

Abstract

Objective: To quantify and characterize the role of biomarkers in peri-implant crevicular fluid (PICF) at each stage of healing during osseointegration.

Design: This systematic review was performed in accordance with PRISMA guidelines using several databases: MedLine (PubMed), Embase, ISI Web of Science, Scopus, and Cochrane Library. Medical subject headings and their indexers were used with no other limitations until December 2017. The dataset was extended with relevant papers from the reference lists of selected papers and from the gray literature. Data was summarized for study objectives, patient demographics, methods used to analyze PICF, biomarker concentrations, results and main findings. Methodologic quality of each included study was assessed using the checklist created by Downs and Black.

Results: Electronic search resulted in 1698 articles. After excluding the duplicates, reading titles, abstracts and reference list reviews 30 prospective studies with longitudinal follow-up were selected. In total, 52 different biomarkers were identified. The most studied cytokines were interleukin (IL) IL-1β, tumor necrosis factor alpha (TNF-α), and nitric oxide (NO). The earliest PICF specimens were collected immediately after implanta- tion, and the latest at 16 weeks prior to occlusal loading. 36 biomarkers were quantified during week 1, 49 between day 10 and week 6, and 49 between weeks 8 and 12. Only 5 articles received good quality ratings. **Conclusion:** The mechanism by which inflammatory and bone biomarkers are released during osseointegration has not yet been identified. However, some hypotheses based on immune-modulated reactions are being explored to investigate early and asymptomatic implant failures. Given the available clinical studies, it was not possible to further explore the performance of all biomarkers already analyzed and to extrapolate their results to propose a consultable data system based on release volume or concentration because of clinical study and data heterogeneity.

Keywords: Biological markers; Cytokines; Dental implant; Osseointegration; Wound healing

1- Introduction

Successful rehabilitation after dental implants installation requires maximal bone-implant interaction after osseointegration, wherein osteoinductive and osteoconductive processes (Albrektsson & Johansson, 2001) generate the molecular and cellular events of neoformation and bone remodeling (Trindade, Albrektsson, & Wennerberg, 2015a; Trindade, Albrektsson, & Wennerberg, 2015b). The osseointegration process involves homeostasis, formation of granulation tissue, bone formation, and remodeling (Bosshardt, Chappuis, & Buser, 2017). Bone homeostasis is mainly driven by the periosteum and osteocytes activity at the tissue and cellular level. The periosteum plays an important role for implants with subcrestal positioning, such as those with the recently developed morse taper connections. In cases of guided bone regeneration therapy, the periosteal cells can also differentiate into osteoblasts contributing to the radial bone growth by continuously producing mature osteoblasts from periosteal progenitor cells, as observed during healing of long bone fractures (Roberts et al., 2015). Osteocytes cells are terminally differentiated osteoblasts that regulate the mineralization and form the connective dendritic processes (Bonewald, 2011; Insua, Monje, Wang, & Miron, 2017). The osteocytes are key regulators of bone homeostasis because they release signaling factors to recruit osteoclasts in bone remodeling sites, and can inhibit osteoblast activity (Bonewald, 2011; Insua et al., 2017). Thus, successful implant osseointegration requires de novo bone formation on the surface of the implant through a continued recruitment and migration of differ- entiating osteogenic cell to the implant site during the contact osteogenesis. This dynamic process operates in an identical fashion to re-modeling bone surfaces occupied by a cement line matrix. Simultaneously, the distance osteogenesis occurs together with contact osteogenesis in every endosseous healing site (Davies, 2003).

Even though well-established surgical implant protocols can yield ≥95% success rates (Buser, Sennerby, & De Bruyn, 2017), early failures are still a great concern among clinicians and researchers (Albrektsson, Chrcanovic, Östman, & Sennerby, 2017; Alsaadi, Quirynen, Komárek, & Van Steenberghe, 2007; Chrcanovic, Kisch, Albrektsson, & Wennerberg, 2016; Koka & Zarb, 2012; Manzano et al., 2016). These early failures occur without a known biological mechanism and there appears to be no evidence that primary infection is the major causative factor for marginal bone resorption (Albrektsson, Buser, & Sennerby, 2012; Qian, Wennerberg, & Albrektsson, 2012). One possible reason for early failures might be that the bone healing after implant insertion is impaired by local and systemic factors,

resulting in failure to establish an intimate bone-to-implant contact (Alsaadi et al., 2007; Chrcanovic et al., 2016). Another hypothesis states that peri-implant tissue around failing implants may contain cytokines with the potential to regulate the activity of osteoclasts, which lead to speculations about clinical interventions based on accessible targets for local therapies with cytokine modulators (Konttinen et al., 2006). Some studies hypothesized that early dental failures could be related to inflammatory reactions in the peri-implant tissues caused by particles derived from the dental implants surface (Franchi et al., 2004; Goodman, 2007; Kumazawa et al., 2002). These particles can be released by implant exposure to therapeutic corrosive substances and/or mechanical procedures (e.g. surgical insertion; micro-movements between contacting surfaces at implant connections), that result in a host-immune response (Noronha Oliveira et al., 2018). Finally, Albrektsson et al. (2014) proposed a model in which implant osseointegration is a long-term equilibrium between host immune cells and bone biomaterials, and the failures are related to healing dis-balance (Albrektsson et al., 2014). This model was later relabeled as the Foreign Body Reaction hypothesis, which states that the bone microenvironment responds to dental implants as foreign bodies. The latter initiates an immune-modulated reaction, cell signaling and complement system activation triggering an inflammatory healing reaction (Trindade et al., 2015a, 2015b; Trindade, Albrektsson, Tengvall, & Wennerberg, 2016).

Trindade et al. (2018) recently found evidence for the involvement of the immune system during the process of osseointegration around titanium implants (TI) in an animal pilot study. In this study, histological gene expression analyses indicated that the immune system activated displays type 2 inflammation that likely guides the host-biomaterial relationship. They found that the TI suppress bone resorption, favoring the bone formation and generating an immunological host reaction. The bone deposition on the implant surface is then initiated to isolate the implant from the bone marrow space, resulting in an accidental clinical osseointegration. After 10 days, the sites with TI had an initial bone formation and presented an increase in arginase-1, indicating a greater activation of type 2 macrophages (M2-macrophages) and cells of the innate responses suggesting an activation of the immune system. After the inflammatory period, at 28 days, TI showed a more active and organized bone remodeling and formation. In addition, the expression of factors related to M1- and M2-macrophages, leucocytes, type 2 innate lymphoid cells, neutrophils, and complement system components indicated a prolonged activation of the innate immune system

(Trindade et al., 2018). The role of immuno-biological responses during osseointegration was also highlighted in a recent in vitro study by Ma et al. (2018) describing the effects of the implant surface on immune cells and bone mesenchymal stem cells (bMSCs). These authors suggested that the alteration of the surface nanostructure can control the inflammatory response of the macrophages. The macrophages tend to facilitate the osteogenic behavior of bMSCs and attract fewer inflammatory cells, improving the clinical performance of the implants by manipulating the balance of bone regeneration/absorption. Their main results included increases in secretion of receptor activator of nuclear factor-B ligand (sRANKL) and macrophage colony-stimulating factor (M-CSF) that may result from increased concentrations of IL-1β and IL- 6, since increased osteoprotegerin (OPG) and OPG/sRANKL ratios are induced by transforming growth factor beta (TGF-β) alone or in com- bination with bone morphogenetic protein (BMP)-2. These findings indicate that the formation of osteoclasts can be induced by immunological factors secreted by bMSCs. Hence, Ma et al. stated that understanding and monitoring the profiles of cytokines secreted by macrophages and the retroregulative cytokines released by bMSCs is important, because they can provide a framework for systematically analyzing and predicting the performance of an implant (Ma et al., 2018).

In recent years, the correlation between clinical indicators of peri- implant health monitoring and marginal bone loss was questioned (Albrektsson et al., 2012; Lin, Kapila, & Wang, 2017; Qian et al., 2012; Sanz & Chapple, 2012). Common periodontal indices such as bleeding on probing and probing depth are not always a reliable tool for assessing peri-implant marginal soft- and hard-tissue conditions (Albrektsson et al., 2012; Coli, Christiaens, Sennerby, & Bruyn, 2017). Healthy peri-implant mucosa can show an increase of probing pocket depth over time (≥4 mm) and is not necessarily associated with bone loss or disease. Likewise, bleeding on probing does not always indicate the presence of acute inflammation in the peri-implant mucosa, but may reflect the nature of the scar tissue-implant contact, as the absence of bleeding on probing does not always appear to be a predictor of future stability (Coli et al., 2017). In an attempt to improve the methodology to evaluate the inflammatory status of gingival tissues, biochemical analysis of gingival crevicular fluid (GFC) are being done in addition to the standard clinical tests. The collection of crevicular fluid enables the measurement of biomarkers for periodontal diseases. They are secreted products of immune cells and represent the innate immune response against bacterial pathogens and danger signals (Bostanci & Belibasakis,

2018). These evaluations may be performed in peri-implant tissue to analyze the biomarkers such as cytokines, proteins, and multifunctional peptides function as intercellular regulatory factors locally and systemically present in peri-implant crevicular fluid (PICF). These biomarkers modulate inflammation intensity, foreign body reaction, cellular organization, healing, and disease pathogenesis. During early bone healing, this immunologically-driven process is proposed to be related primarily to osteoconduction (Albrektsson & Johansson, 2001; Chang, Lang, & Giannobile, 2010; Trindade et al., 2015a, 2015b).

Biomarkers are fundamental to the intercellular interactions and cellular activation that are needed to re-establish tissue bioequivalence (Stow & Murray, 2013; Stow et al., 2009). They remain in the tissue microenvironment for various lengths of time and are present in PICF. Many researchers have studied PICF seeking to find specific markers related to pathologic inflammation, failed bone repair, and failed implantation. Biomarkers from the peri-implant microenviroment have been quantified to develop early diagnostic techniques for peri-implant disease. Previous systematic reviews (Duarte et al., 2016; Faot et al., 2015; Kaklamanos & Tsalikis, 2002) have identified possible biomarker uses and relationships to pathologic processes. These reviews aggregated data from patients with osseointegrated implants, patients with systemic or local disease, and healthy controls. In 2002, Kaklamanos and Tsalikis (2002) called for a consensus to define and describe clinical conditions and tissue status based on PICF biomarkers to monitor and predict peri-implant tissue response. A subsequent study by Faot et al. (2015), identified IL-1β and TNF-α as pro-inflammatory biomarkers that can be used for early differentiation of peri-implantitis and mucositis. Furthermore, the results of Duarte et al.'s systematic review provided moderate evidence indicating that implants with peri-implantitis are associated with higher levels of proinflammatory cytokines in PICF than healthy implants (Duarte et al., 2016). They further showed that data that may be used to predict peri-implantitis based on PICF levels of anti-inflammatory cytokines, osteoclastogenesis-related cytokines, and chemokines are limited. Thus far, these reviews describe primarily biomarker roles in bioequilibrium maintenance after osseointegration and pathologic processes. Evidence is scarce and inconsistent regarding which biomarkers are present in PICF and which activities during bone healing are responsible for successful osseointegration.

Understanding the cytokine mechanisms and the retroregulative cytokines released by bMSC that operate during osseointegration will improve the researchers'

and clinician's abilities to determine the prognosis of failing implants and allow early diagnoses of peri-implant diseases based on patient susceptibility. In addition, mapping the clinical studies that investigated the biomarkers in the PICF before implant loading can allow to identify potential confounding factors that can impair implant healing. Potential confounding factors include patient's age and smoking habit, host bone immuno-response capacity, systemic diseases, bone quality, surgical steps, antibiotics prescription, and implant surface and design. Although cytokines are essential for activation, differentiation, and control of osseointegration, they are not routinely measured to monitor peri-implant health because their baseline levels are unknown. Therefore, the objective of this systematic review was to synthesize the results of previous studies of bone healing stages and known biologic events to show which markers have already been quantified and associated with the osseointegration phases.

2. Materials and methods

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher, Liberati, Tetzlaff, & Altman, 2009). The clinical questions were organized according to the PICOS (participant, intervention, comparison, outcome, and study design) strategy.

2.1. Focus question

"What is the evolution of inflammatory and bone biomarker levels in PICF from early healing until osseointegration? Which biomarkers are present and acts in each stage of the bone healing process after implant installation?"

2.2. Search strategy

Electronic searches were conducted by two examiners (AMB and RMMM). PubMed, Embase, Scopus, ISI Web of Science, and Cochrane Library databases were searched for articles published until December 2017. The search strings used combinations of medical subject heading terms: ('dental implants' or "dental implantation") and ("osseointegration" or "osteogenesis" or "bone resorption") and ("biological markers" or "macrophage inflammatory proteins" or "anti-inflammatory agents" or "cytokines" or "interleukins"). No date limit was applied. Terms were combined to fit each database search. The reference lists from retrieved papers were

hand-searched to identify additional eligible studies. The search strategy is summarized in Table S1 (Supplementary material).

2.3. Selection criteria

Two examiners (AMB and RMMM) selected suitable studies independently. Selections were then compared, and the differences were resolved through discussion. The same two reviewers also hand- searched reference lists of studies selected in the previous step independently to find additional pertinent articles.

Inclusion criteria

i) Original, prospective, longitudinal clinical trial involving participants who were partially or totally edentulous and received dental implants.

ii) At least one PICF collection performed during osseointegration (up to 12 weeks post-surgery or up to 16 weeks post-surgery after dental implant installation without occlusal loading).

iii) Inflammatory or bone biomarker expression (level or concentration) analyzed during healing by any technique.

iv) Report written in English.

Exclusion criteria

i) Literature reviews, case study, in vitro or in vivo animal model studies;

ii) Biomarkers quantified from blood, saliva, or mucosa (punches or biopsy specimens);

iii) PICF collected after implant osseointegration (≥4 months post-surgery or –loading);

iv) Focus on biological parameters, such as presence of keratinized mucosa, implant-prosthetic microgap size or implant stability;

v) Use of mini-implants for orthodontic anchorage;

vi) Studies that performed bone-augmentation procedures or guided bone-regeneration techniques.

2.4. Data synthesis

From each included paper, data were extracted and expressed in chronological order according to publication date. Data synthesis was based on evidence tables and a descriptive summary was produced to explain study variations, including patient characteristics and results. Several data elements were extracted: author names, publication year, study type, objectives, number of patients, number of implants, implant system, implant type, follow-up, outcome variables, bone site, prosthetic treatment, implant loading, inflammatory and bone markers, PICF analyses, biomarker concentration, significant biomarker results, and main findings. For selected studies in which experimental group members had a systemic disorder or received drug treatment, data was extracted only for patients in the control group.

2.5. Quality assessment

Two reviewers (AMB and RMMM) assessed the risk of bias of included trials independently in duplicate. The methodological quality of each included study was assessed based on Downs and Black's checklist (Downs & Black, 1998), which consists of 27 items encompassing five domains: reporting, external validity, bias, confounding, and power. Answers were scored as 0 or 1, except for one reporting subscale item (scored 0–2) and statistical power (scored 0–5). In accordance with previous systematic reviews (Chudyk, Jutai, Petrella, & Speechley, 2009; Jácome & Marques, 2014), statistical power was scored as 1 (power test reported) or 0 (power test absent). Finally, the Downs and Black's checklist scores were grouped into the following four-level quality index (Chudyk et al., 2009): ≤14, poor; 15–19, fair; 20–25, good; and 26–28, excellent.

3. Results

3.1. Study selection

The study selection process is summarized in Fig. 1. The electronic search resulted in 1698 articles. After removing duplicates, 1117 articles remained for title screening and abstract reading. Of these, 115 publications were read in full, 88 studies were excluded, and two papers could not be accessed. The remaining 25 studies were selected for inclusion. An additional five articles were included from the reference lists of the articles included from the electronic searches.

In total, 30 studies met the selection criteria for this review (Basegmez, Yalcin, Yalcin, Ersanli, & Mijiritsky, 2012; Bielemann et al., 2018; Boynueğri, Yalim, Nemli, Ergüder, & Gökalp, 2012; De Wilde et al., 2015; Dŏgan et al., 2015; Dolanmaz et al., 2015; Elsyad, Mahanna, Elshahat, & Elshoukouki, 2016; Emecen-Huja et al., 2013; Ghiraldini et al., 2016; Gokmenoglu, Ozmeric, Erguder, & Elgun, 2014; Gruber, Nadir, & Haas, 2010; Güncü, Tözüm, Güncü, & Yamalik, 2008; Güncü, Tözüm, Güncü, Yamalik, & Tümer, 2009; Khoury, Thomas, Walters, Sheridan, & Leblebicioglu, 2008; Mandić et al., 2015; Nogueira-Filho et al., 2014; Nomura et al., 2000; Nowzari, Yi, Chee, & Rich, 2008; Onuma et al., 2015; Peker Tekdal, Bostanci, Belibasakis, & Gürkan, 2016; Prati et al., 2013; Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd, & Thomsen, 2017; Slotte et al., 2012; Tirachaimongkol, Pothacharoen, Reichart, & Khongkhunthian, 2016; Tözüm, Türkyilmaz, & Yamalik, 2005; Tözüm, Turkyilmaz, Yamalik, Karabulut, & Eratalay, 2007; Tözüm, Türkyilmaz, Yamalik, Tümer et al., 2007; Tsoukaki et al., 2013; Verrastro Neto et al., 2017). The main study characteristics and results are summarized in Table 1. The selected articles were published between 2000 and 2018 and described longitudinal and prospective studies. Of the 30 studies, 16 were randomized with respect to loading implant protocol (Güncü, Tözüm et al., 2008; Güncü et al., 2009; Prati et al., 2013; Tözüm et al., 2005; Tözüm, Turkyilmaz, Yamalik, Karabulut et al., 2007; Tözüm, Türkyilmaz, Yamalik, Tümer et al., 2007; Verrastro Neto et al., 2017), type of abutment (Slotte et al., 2012), surgical protocol (Tsoukaki et al., 2013; De Wilde et al., 2015; Peker Tekdal et al., 2016), medication protocol (Gokmenoglu et al., 2014), or treatment procedure (Boynueğri et al., 2012; Mandić et al., 2015; Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017).

3.2. Study characteristics

Table 1 summarizes the demographic characteristics of study participants and the PICF analysis methods used. Biomarker concentrations released during early and late healing phases are listed in Tables S2 and S3, respectively (Supplementary material). A total of 634 patients and 1378 implants were registered (average of 2.17 implants per patient). The number of implants per study ranged from 10 (Nomura et al., 2000; Tirachaimongkol et al., 2016) to 205 (Prati et al., 2013), and the sample size ranged from 6 patients (Nomura et al., 2000) to 51 patients (Ghiraldini et al., 2016). The mean age was 50.81 ± 5.85 years. Three studies did not report mean patient age (Gruber et al., 2010; Nowzari et al., 2008; Slotte et al., 2012). Implant diameter ranged from 1.5 mm (De Wilde et al., 2015) to 5 mm (Tirachaimongkol et al., 2016), and implant length ranged from 8.0

mm (De Wilde et al., 2015) to 15.0 mm (Onuma et al., 2015; Tözüm et al., 2005; Tözüm, Turkyilmaz, Yamalik, Karabulut et al., 2007; Tözüm, Türkyilmaz, Yamalik, Tümer et al., 2007). The follow-up period varied from a post-surgical baseline assessment (Güncü, Tözüm et al., 2008; Güncü, Aslan, Tümer, Güncü, & Uysal, 2008; Prati et al., 2013; Tirachaimongkol et al., 2016) to 18 months (Basegmez et al., 2012; Tözüm, Türkyilmaz, Yamalik, Tümer et al., 2007), but the present systematic review considers only the osseointegration period (up to 12 weeks or up to 16 weeks for patients without loading implants). Information about bone site and prosthetic rehabilitation type was not reported for nine studies (Basegmez et al., 2012; Dŏgan et al., 2015; Gokmenoglu et al., 2014; Gruber et al.,2010 Nogueira-Filho et al., 2014; Nomura et al., 2000; Nowzari et al., 2008; Slotte et al., 2012; Tsoukaki et al., 2013). Nineteen studies (Bielemann et al., 2018; Boynueğri et al., 2012; De Wilde et al., 2015; Dolanmaz et al., 2015; Elsyad et al., 2016; Emecen-Huja et al., 2013; Ghiraldini et al., 2016; Güncü, Tözüm et al., 2008; Güncü et al., 2009; Khoury et al., 2008; Onuma et al., 2015; Prati et al., 2013; Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017; Tözüm et al., 2005; Tözüm, Turkyilmaz, Yamalik, Karabulut et al., 2007; Tözüm, Türkyilmaz, Yamalik, Tümer et al., 2007; Tirachaimongkol et al., 2016; Verrastro Neto et al., 2017) focused on mandibular jaw rehabilitation, and seven studies (De Wilde et al., 2015; Khoury et al., 2008; Mandić et al., 2015; Peker Tekdal et al., 2016; Prati et al., 2013; Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017) focused on maxillary jaw rehabilitation. The posterior region was re-habilitated in 13 studies, nine mandibular (De Wilde et al., 2015; Dolanmaz et al., 2015; Emecen-Huja et al., 2013; Ghiraldini et al., 2016; Güncü, Tözüm et al., 2008; Güncü et al., 2009; Khoury et al., 2008; Onuma et al., 2015; Tirachaimongkol et al., 2016) and four maxillary (De Wilde et al., 2015; Khoury et al., 2008; Mandić et al., 2015; Peker Tekdal et al., 2016). Rehabilitation with prosthetic mandibular overdentures was performed in six studies (Bielemann et al., 2018; Boynueğri et al., 2012; Elsyad et al., 2016; Tözüm et al., 2005; Tözüm, Turkyilmaz, Yamalik, Karabulut et al., 2007; Tözüm, Türkyilmaz, Yamalik, Tümer et al., 2007). Two studies used a Brånemark full-arch prosthesis for both maxillary jaws (Prati et al., 2013) and mandibular arch (Verrastro Neto et al., 2017). Seven studies used both conventional loading (CL) and immediate loading (IML) (Güncü, Tözüm et al., 2008; Güncü et al., 2009; Prati et al., 2013; Slotte et al., 2012; Tözüm et al., 2005; Tözüm, Turkyilmaz, Yamalik, Karabulut et al., 2007; Tözüm, Türkyilmaz, Yamalik, Tümer et al., 2007), whereas 12 studies used only CL (Basegmez et al., 2012; Bielemann et al., 2018; Boynueğri et al., 2012; De Wilde et al., 2015; Dŏgan et al., 2015; Ghiraldini et al., 2016; Khoury

et al., 2008; Mandić et al., 2015; Nogueira-Filho et al., 2014; Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017; Tirachaimongkol et al., 2016) and four studies used IML (Elsyad et al., 2016; Gruber et al., 2010; Onuma et al., 2015; Verrastro Neto et al., 2017). Seven studies did not describe loading time (Dolanmaz et al., 2015; Emecen-Huja et al., 2013; Gokmenoglu et al., 2014; Nomura et al., 2000; Nowzari et al., 2008; Peker Tekdal et al., 2016; Tsoukaki et al., 2013).

A total of 52 biomarkers were investigated. The biomarkers studied included (number of studies for each biomarker) were: interleukin (IL)- 1β; (n = 11) (Bielemann et al., 2018; Boynueğri et al., 2012; De Wilde et al., 2015; Dŏgan et al., 2015; Elsyad et al., 2016; Emecen-Huja et al., 2013; Gokmenoglu et al., 2014; Gruber et al., 2010; Khoury et al., 2008; Nowzari et al., 2008; Slotte et al., 2012); tumor necrosis factor-alpha (TNF-α; n = 9) (Bielemann et al., 2018; Boynueğri et al., 2012; Dŏgan et al., 2015; Emecen-Huja et al., 2013; Nogueira-Filho et al., 2014; Nowzari et al., 2008; Slotte et al., 2012; Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017); osteoprotegerin (OPG; n = 7) (Dolanmaz et al., 2015; Ghiraldini et al., 2016; Onuma et al., 2015; Peker Tekdal et al., 2016; Prati et al., 2013; Sayardoust, Omar, Norderyd et al., 2017; Verrastro Neto et al., 2017); nitric oxide (NO; n = 6) (Gokmenoglu et al., 2014; Güncü, Tözüm et al., 2008; Güncü et al., 2009; Tözüm et al., 2005; Tözüm, Turkyilmaz, Yamalik, Karabulut et al., 2007; Tözüm, Türkyilmaz, Yamalik, Tümer et al., 2007); osteocalcin (OC; n = 6) (Ghiraldini et al., 2016; Prati et al., 2013; Slotte et al., 2012; Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017; Tirachaimongkol et al., 2016); IL-6 (n = 6) (Bielemann et al., 2018; De Wilde et al., 2015; Emecen-Huja et al., 2013; Nogueira-Filho et al., 2014; Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017); IL- 8 (n = 6) (De Wilde et al., 2015; Emecen-Huja et al., 2013; Khoury et al., 2008; Nogueira-Filho et al., 2014; Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017); matrix metalloproteinase (MMP)-8; (n = 5) (Basegmez et al., 2012; De Wilde et al., 2015; Emecen-Huja et al., 2013; Nomura et al., 2000; Tsoukaki et al., 2013), alkaline phosphatase (ALP; n = 5) (Mandić et al., 2015; Slotte et al., 2012; Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017; Tirachaimongkol et al., 2016), vascular endothelial growth factor (VEGF; n = 5) (Emecen-Huja et al., 2013; Nogueira-Filho et al., 2014; Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017; Verrastro Neto et al., 2017), the receptor activator of nuclear factor-B ligand (sRANKL; n = 5) (Dolanmaz et al., 2015; Onuma et al., 2015; Peker Tekdal et al., 2016; Sayardoust, Omar, Norderyd et al., 2017; Tsoukaki et al., 2013), IL-10 (n = 3) (Bielemann et al., 2018; Emecen-Huja et al., 2013; Nogueira- Filho et al., 2014); bone morphogenetic protein (BMP)-2 (n = 3) (Dolanmaz et al., 2015;

Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017); transforming growth factor beta (TGF-β; n= 3) (Ghiraldini et al., 2016; Gokmenoglu et al., 2014; Sayardoust, Omar, Norderyd et al., 2017); cathepsin k (CatK; n = 3) (Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017; Slotte et al., 2012); tartrate resistant acid-phosphatase (TRAP) (n = 2) (Slotte et al., 2012; Verrastro Neto et al., 2017); osteopotin (OPN; n = 2) (Ghiraldini et al., 2016; Prati et al., 2013); tissue inhibitor of metalloproteinases (TIMP)-1 (n = 2) (Emecen-Huja et al., 2013; Nomura et al., 2000); and prostaglandin-E2 (PGE2; n = 2) (Basegmez et al., 2012; Gokmenoglu et al., 2014). Additionally, investigated biomarkers that were represented by only a single clinical study included: BMP-7 (Dolanmaz et al., 2015), TGF-β1, IL-1-α, IL-4, IL-12 p70; interferon gamma (IFN-γ) (Nogueira-Filho et al., 2014), TGF-β2, chemokine ligand-3 (CCL-3) (De Wilde et al., 2015), TGF-α, parathyroid hormone (PTH) (Prati et al., 2013), fibroblast growth factor (FGF) (Ghiraldini et al., 2016), FGF-β, IL-7, IL-12, IL-1 receptor antagonist, eotaxin, MMP-9, TIMP-2 TIMP-3, TIMP-4, monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein (MIP-1β) (Emecen-Huja et al., 2013), MMP-1, collagenase (Nomura et al., 2000), neutrophil elastase (Gruber et al., 2010), active human cytomegalovirus (HCMV) (Nowzari et al., 2008); chemokine C-X-C receptor 4 (CXCR4), hypoxia inducible factor 1 alpha (HIF-1a) (Sayardoust, Omar, Norderyd et al., 2017); placental growth factor (PLGF) and periostin (Verrastro Neto et al., 2017).

In two studies, PICF was collected immediately after implant insertion for analysis of the biomarkers OPG, PTH, TGF-α (Prati et al., 2013), OC (Prati et al., 2013; Tirachaimongkol et al., 2016), and ALP (Tirachaimongkol et al., 2016). One study at the first day of healing ALP, BMP-2, CatK, IL-6, IL-8, OC, TNF-α and VEGF (Sayardoust, Omar, & Thomsen, 2017). In one study, PICF was collected on day 2 for quantification of ALP, CatK, IL-1β, OC, TRAP, and TNF-α (Slotte et al., 2012). In twelve studies, cytokines were collected and analyzed during the first week (Bielemann et al., 2018; De Wilde et al., 2015; Emecen- Huja et al., 2013; Khoury et al., 2008; Mandić et al., 2015; Nomura et al., 2000; Onuma et al., 2015; Prati et al., 2013; Sayardoust, Omar, & Thomsen, 2017; Tirachaimongkol et al., 2016; Tsoukaki et al., 2013; Verrastro Neto et al., 2017). In one study, neutrophil elastase and IL-1 were collected and analyzed during and after 10 days of the implant installation (Gruber et al., 2010). A total of 37 biomarkers were evaluated on day 14 (Bielemann et al., 2018; Emecen-Huja et al., 2013; Elsyad et al., 2016; Ghiraldini et al., 2016; Mandić et al., 2015; Nomura et

al., 2000; Peker Tekdal et al., 2016; Prati et al., 2013; Sayardoust, Omar, & Thomsen, 2017; Slotte et al., 2012; Tirachaimongkol et al., 2016; Tözüm, Turkyilmaz, Yamalik, Karabulut et al., 2007; Tsoukaki et al., 2013; Verrastro Neto et al., 2017) and 21 biomarkers were assessed on day 21 (Emecen-Huja et al., 2013; Mandić et al., 2015; Tirachaimongkol et al., 2016). After 30 days of healing, 34 biomarkers were quantified (Bielemann et al., 2018; Dŏgan et al., 2015; Dolanmaz et al., 2015; Gokmenoglu et al., 2014; Güncü, Tözüm et al., 2008; Güncü et al., 2009; Mandić et al., 2015; Nogueira-Filho et al., 2014; Nomura et al., 2000; Nowzari et al., 2008; Peker Tekdal et al., 2016; Prati et al., 2013; Sayardoust, Omar, & Thomsen, 2017; Slotte et al., 2012; Tirachaimongkol et al., 2016; Tözüm et al., 2005; Tözüm, Turkyilmaz, Yamalik, Karabulut et al., 2007; Tözüm, Türkyilmaz, Yamalik, Tümer et al., 2007; Verrastro Neto et al., 2017). Investigators assessed 27 biomarkers on day 44 (Emecen-Huja et al., 2013; Nogueira- Filho et al., 2014; Tirachaimongkol et al., 2016; Tsoukaki et al., 2013), 36 biomarkers on day 60 (Bielemann et al., 2018; Emecen-Huja et al., 2013; Nogueira-Filho et al., 2014; Peker Tekdal et al., 2016; Prati et al., 2013; Sayardoust, Omar, Norderyd et al., 2017; Tirachaimongkol et al., 2016), and 45 biomarkers on day 90. These late PICF samples were collected before occlusal loading (Basegmez et al., 2012; Bielemann et al., 2018; Boynueğri et al., 2012; Dolanmaz et al., 2015; Emecen-Huja et al., 2013; Ghiraldini et al., 2016; Gokmenoglu et al., 2014; Güncü, Tözüm et al., 2008; Güncü et al., 2009; Nomura et al., 2000; Nowzari et al., 2008; Peker Tekdal et al., 2016; Prati et al., 2013; Sayardoust, Omar, Norderyd et al., 2017; Slotte et al., 2012; Tirachaimongkol et al., 2016; Tözüm et al., 2005; Tözüm, Türkyilmaz, Yamalik, Tümer et al., 2007; Tsoukaki et al., 2013). Three studies considered the end of osseointegration to be by week 16, at which point seven biomarkers were analyzed (Nogueira-Filho et al., 2014; Prati et al., 2013; Verrastro Neto et al., 2017). Fig. 2 summarizes which biomarkers were analyzed relative to time in the presently reviewed articles.

Eight papers describe the use of postoperative antibiotics for different periods: 500 mg amoxicillin three times daily (Bielemann et al., 2018; Dolanmaz et al., 2015; Mandić et al., 2015; Nogueira-Filho et al., 2014; Prati et al., 2013), 1 g amoxicillin three times daily (Tsoukaki et al., 2013), 900 mg clindamycin two times daily (Onuma et al., 2015), and 300 mg clindamycin plus 2 g phenoxymethylpenicillin two times daily (Slotte et al., 2012). Seven studies describe using 2 g amoxicillin an hour prior to surgery for antimicrobial prophylaxis (Elsyad et al., 2016; Ghiraldini et al., 2016; Peker Tekdal et al., 2016; Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017; Tirachaimongkol et al., 2016; Verrastro Neto et al., 2017). Another study reports that amoxicillin and clavulanate potassium (1 g + 250 mg) dual therapy was used for 5 days prior to surgery without postoperative

chlorhexidine (Dŏgan et al., 2015). One study group compared preoperative and postoperative antimicrobial prophylaxis: 2 g amoxicillin one hour before surgery vs 500 mg amoxicillin three times daily for 7 days after surgery (Khoury et al., 2008). Chlorhexidine gluconate was used at concentrations of 0.1% (Khoury et al., 2008; Slotte et al., 2012), 0.12% (Elsyad et al., 2016; Emecen-Huja et al., 2013; Ghiraldini et al., 2016; Nogueira-Filho et al., 2014; Onuma et al., 2015; Prati et al., 2013; Tirachaimongkol et al., 2016; Tsoukaki et al., 2013; Verrastro Neto et al., 2017), and 0.2% (Dolanmaz et al., 2015; Peker Tekdal et al., 2016; Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017) for various lengths of time. One study protocol did not include prescription medication (Gruber et al., 2010), and eleven study reports did not include information about medication usage (Basegmez et al., 2012; Boynueğri et al., 2012; De Wilde et al., 2015; Gokmenoglu et al., 2014; Güncü, Tözüm et al., 2008; Güncü et al., 2009; Nomura et al., 2000; Nowzari et al., 2008; Tözüm et al., 2005; Tözüm, Turkyilmaz, Yamalik, Karabulut et al., 2007; Tözüm, Türkyilmaz, Yamalik, Tümer et al., 2007).

Several clinical questions were investigated in the selected studies: potential differences between implants subjected to immediate loading (IML) versus conventional loading (CL) (Güncü, Tözüm et al., 2008; Güncü et al., 2009; Prati et al., 2013; Tözüm et al., 2005; Tözüm, Turkyilmaz, Yamalik, Karabulut et al., 2007; Tözüm, Türkyilmaz, Yamalik, Tümer et al., 2007; Verrastro Neto et al., 2017); comparisons between macrogeometry and microgeometry of various implants (Boynueğri et al., 2012; De Wilde et al., 2015; Dolanmaz et al., 2015; Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017); differences between surgical protocols, such as “effect of piezoelectric surgery” and “osteotomy” (Peker Tekdal et al., 2016); flapped versus flapless surgery (Tsoukaki et al., 2013); differences in PICF marker levels after 3 months of osseointegration and occlusal loading (Basegmez et al., 2012); effects of systemic diseases such as diabetes (Dŏgan et al., 2015; Ghiraldini et al., 2016) and osteopenia (Onuma et al., 2015) on biomarker levels; application of various antibiotic therapy protocols (Khoury et al., 2008); treatment with light-emitting diode (LED) photobiomodulation (Gokmenoglu et al., 2014) or low-level laser treatment (Mandić et al., 2015); differences between PICF biomarker levels in GCF (Elsyad et al., 2016; Emecen-Huja et al., 2013; Gruber et al., 2010; Nogueira-Filho et al., 2014; Tirachaimongkol et al., 2016); biomarkers released during bone healing process after implant insertion (Bielemann et al., 2018), effect of insertion torque in IML protocols (Verrastro Neto et al., 2017); pathogenic

inflammation (Nomura et al., 2000; Nowzari et al., 2008; Slotte et al., 2012; Tözüm, Turkyilmaz, Yamalik, Karabulut et al., 2007) and acute inflammation (Elsyad et al., 2016; Tözüm et al., 2005) and differences in the early and late osseointegration between smokers and non-smokers (Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017).

The quality ratings of the studies included in this review are listed in Table S4 (Supplementary material) and presented in Fig. 3. Four studies were classified as poor (mean score = 12.25 ± 2.48) (De Wilde et al., 2015; Gruber et al., 2010; Nomura et al., 2000; Nowzari et al., 2008) 21 as fair (mean score = 17.25 ± 1.26) (Basegmez et al., 2012; Bielemann et al., 2018; Boynueğri et al., 2012; Dŏgan et al., 2015; Dolanmaz et al., 2015; Elsyad et al., 2016; Emecen-Huja et al., 2013; Gokmenoglu et al., 2014; Güncü, Tözüm et al., 2008; Güncü et al., 2009; Khoury et al., 2008; Mandić et al., 2015; Nogueira-Filho et al., 2014; Onuma et al., 2015; Prati et al., 2013; Sayardoust, Omar, & Thomsen, 2017; Slotte et al., 2012; Tirachaimongkol et al., 2016; Tözüm et al., 2005; Tözüm, Turkyilmaz, Yamalik, Karabulut et al., 2007; Tözüm, Türkyilmaz, Yamalik, Tümer et al., 2007), and five as good (mean score = 20.67 ± 1.11) (Ghiraldini et al., 2016; Peker Tekdal et al., 2016; Sayardoust, Omar, Norderyd et al., 2017; Tsoukaki et al., 2013; Verrastro Neto et al., 2017). Analysis of checklist questions showed that only eight studies (Bielemann et al., 2018; Elsyad et al., 2016; Ghiraldini et al., 2016; Peker Tekdal et al., 2016; Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017; Tirachaimongkol et al., 2016; Verrastro Neto et al., 2017) provided partial reporting of the distributions of principal confounders (Question 5). No study fulfilled the requirement that the sample be representative of the population (Question 12) and in only one study (Verrastro Neto et al., 2017), an attempt was made to blind study participants (Question 14). The confounding subscale (Questions 21–26), which addresses bias in participant selection, showed that 17 studies were randomized (Question 23) (Boynueğri et al., 2012; De Wilde et al., 2015; Elsyad et al., 2016; Gokmenoglu et al., 2014; Güncü, Tözüm et al., 2008; Güncü et al., 2009; Mandić et al., 2015; Peker Tekdal et al., 2016; Prati et al., 2013; Slotte et al., 2012; Tözüm et al., 2005; Tözüm, Turkyilmaz, Yamalik, Karabulut et al., 2007; Tözüm, Türkyilmaz, Yamalik, Tümer et al., 2007; Tsoukaki et al., 2013; Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017; Verrastro Neto et al., 2017), but only six (Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017; Tozum, Yamalik, Karabulut et al., 2007; Tözüm, Türkyilmaz, Yamalik, Tümer et al., 2007; Tsoukaki et al., 2013; Verrastro Neto et al., 2017) had blinded participants and health care staff until recruitment completion (Question 24). Eight studies (Bielemann et al., 2018; Dolanmaz et al., 2015;

Ghiraldini et al., 2016; Mandić et al., 2015; Onuma et al., 2015; Prati et al., 2013; Sayardoust, Omar, Norderyd et al., 2017; Tsoukaki et al., 2013) reported main conclusions based on intention to treat analyses and in consideration of possible confounders (Question 25), and eight studies reported power analyses (Bielemann et al., 2018; Elsyad et al., 2016; Emecen-Huja et al., 2013; Nogueira-Filho et al., 2014; Onuma et al., 2015; Peker Tekdal et al., 2016; Sayardoust, Omar, Norderyd et al., 2017; Tsoukaki et al., 2013).

4.Discussion

Surgical rehabilitation for dental implantation is characterized by successive physiologic stages of bone trauma, bone debris formation, bone hemostasis, blood clot formation, and tissue hypoxia (Shanbhag, Shanbhag, & Stavropoulos, 2015). These stages can be influenced by the activity and involvement of the immune system activated during implant recognition (Trindade et al., 2018) and can be amplified due to chronic and/or systemic disorders of the host such as diabetes, osteopenia, smoking habits, drug therapy, and atrophy of bones. Local factors arising from iatrogenic surgery such as debris released from the implant surface, insertion torque, overheating during drilling, and micro-implant movements can also trigger inflammatory reactions. A review by Noronha Oliveira et al. (2018) showed that proinflammatory cytokines, infiltration of inflammatory cells and activation of osteoclasts in the peri-implant tissues are stimulated by the presence of metal particles and ions. Thus, each step stimulates the release of healing- related factors that have the potential to be employed as clinical biomarkers. The results of Ma et al. (2018) highlighted the confounding influence of the implant surface nanostructure, macrophage inflammatory response, and osteogenic differentiation of bMSCs. In addition, the retro-regulative effects of bMSCs on the osteoclastic differentiation of macrophages and the culture system as a function of implant surface and culture medium could provide a prospective approach for improving implant osseointegration via immune-regulation.

Therefore, we aimed to identify the biomarkers and their levels in PICF during osseointegration based on available data from clinical studies. In total, 52 different biomarkers were investigated during the 16 weeks of osseointegration following implant insertion. We included studies of patients receiving implants with and without occlusal loading, studies of patients with

systemic complications and healthy control groups. Some confounding factors can influence the host-bone response mediating the release of pro and anti-inflammatory cytokines, chemokines, growth factors, T-cells modulators and bone and angiogenesis markers. These factors include age, diabetes, smoking habit, oral hygiene, bone site characteristics, preoperative and postoperative antibiotics administration, along with surgical aspects such as flap technique, drilling protocols and insertion torque. Some studies (Bielemann et al., 2018; Prati et al., 2013; Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017; Tsoukaki et al., 2013; Verrastro Neto et al., 2017) tailored their experimental design and patient selection to investigate the influence of these factors on the cytokines release during the early and late stages of osseointegration.

4.1. Biomarker release during early healing

The initial inflammatory response is important for cellular recruitment and subsequent immunologically-regulated processes. The first stage of peri-implant bone healing, known as osteoinduction, is characterized by mesenchymal cell differentiation into pre-osteoblasts, which initiate osteogenesis (Albrektsson & Johansson, 2001) by synthesizing growth factors and cytokine-rich extracellular bone matrix (Raghavendra, Wood & Taylor, 2005). In the first 3–4 days, inflammation is characterized by high levels of pro-inflammatory cytokines (e.g., ILs, TNF, and IFN-γ) and recruitment of lymphocytes and macrophages (Shanbhag et al., 2015). Pro-inflammatory cytokine levels decline between days 7 and 14 (Shanbhag et al., 2015), and only soft tissue, a primitive matrix composed of varying amounts of old bone debris, remains at day 7 (Lang et al., 2011).

Immediately after implant insertion, Prati et al. (2013) found that the bone markers TGF-α, OC, OPG, OPN, and PTH levels were reduced to negligible levels. These data may suggest that immediate PICF collection is not ideal given that blood clot stabilization and neutrophil migration occur during the first 24 h after injury (Wang, Zhang, & Miron, 2016). In addition, blood contamination of PICF is likely to occur during this time. Sayardoust, Omar, and Thomsen (2017) registered a peak expression for IL-6, IL-8 and VEGF levels at the first day of healing, both in smokers and nonsmokers with implants submitted to CL (Sayardoust, Omar, & Thomsen, 2017). Interestingly, Verrastro Neto et al. (2017)

observed higher concentrations of VEGF and OPG at the 7th day in implants inserted with lower torque (< 30 N cm) using IML implants. These findings suggest that reduced implant insertion torque favors the angiogenesis and the microcirculation (Verrastro Neto et al., 2017).

The implant surface assists initial blood cell anchorage (Terheyden, Lang, Bierbaum, & Stadlinger, 2012), sustains the fibrin clot and ensures resistance, controls displacement forces generated by cell migration, maintains a migratory pathway of osteogenic cells, and stimulates angiogenesis (likely through extracellular matrix and growth factor mediation) (Raghavendra et al., 2005). Slotte et al. (2012) collected PICF on postoperative day 2 to analyze gene expression for IL-1β, TNF- α, OC, ALP, CatK, and TRAP. As expected, they found elevated expression of the pro-inflammatory marker IL-1β for the CL implants in the group with smooth abutment and a positive correlation with the implant stability quotient (ISQ). They further noted that increased TNF-α expression on days 2 and 14 predicted implant complications, including bone dehiscence at the implant surface, implant rotation instability, implant looseness, and implant removal (Slotte et al., 2012). TIMP-1 levels increase during week 1 and decrease after week 2 (Nomura et al., 2000), suggesting that release occurs gradually over 24 h in inflamed sites where it functions as a biomarker and primary main inhibitor of MMPs (Nomura, Ishii, Oishi, Kohma, & Hara, 1998). This role may also explain why MMP-1, MMP-8, and collagenase are significantly increased during this period but gradually decrease over time, the results showed that, collagenase activity was significantly increased only during the first week (Nomura et al., 2000). In 2015, Mandić et al. (2015) observed increased ALP activity during week 1 compared to week 4, indicating the intense osteoblastic activity (Bonewald, 2011). The ALP is considered a marker of osteoblast differentiation and activity, it can also be produced by polymorphonuclear cells during inflammation (Plagnat et al., 2002). Researchers believe that increased ALP during the first week reflects early inflammatory healing following surgical trauma (Mandić et al., 2015).

Tsoukaki et al. (2013), found high MMP-8 levels on day 7 that were more pronounced in bone sites receiving more intense surgical trauma and believe that this early healing period response is primarily due to extracellular matrix degradation (Nomura et al., 2000). Prati et al. (2013) reported that bone markers,

TGF-α, OPN, OC, and PTH, levels on day 7 were higher in patients with IML implants compared to the CL (Prati et al., 2013). In contrast, Onuma et al. (2015) reported low OPG levels in patients with osteopenia who received IML implants. However, a marked increase in sRANKL was observed in both osteopenic and healthy patients (Onuma et al., 2015), suggesting a relationship between surgical trauma and inflammation caused by the surgery. Significant increases in IL-6, IL-8, MIP-1β, and TIMP-1 release have been associated with trauma (Emecen-Huja et al., 2013). More specifically, significant IL-6 increases have been observed in response to acute surgical trauma in both soft and hard tissue (Bielemann et al., 2018; Jawa, Anillo, Huntoon, Baumann, & Kulaylat, 2011a; Jawa, Anillo, Huntoon, Baumann, & Kulaylat, 2011b) and IL-8 increases have been observed up to 2 weeks after trauma (Khoury et al., 2008). The pro- inflammatory characteristics of IL-8 (Schierano et al., 2003) indicate its role as a potent neutrophil chemotactic and selective recruitment (Petković et al., 2010). Because IL-8 concentrations are significantly reduced one week after healing (Emecen-Huja et al., 2013), IL-8 may be involved only in the initial control of exacerbated inflammation.

Interestingly, out of twelve studies investigating early implant healing (Bielemann et al., 2018; De Wilde et al., 2015; Emecen-Huja et al., 2013; Khoury et al., 2008; Mandić et al., 2015; Nomura et al., 2000; Onuma et al., 2015; Prati et al., 2013; Sayardoust et al., 2017a; Tirachaimongkol et al., 2016; Tsoukaki et al., 2013; Verrastro Neto et al., 2017), only one study (Khoury et al., 2008) investigated the effect of antimicrobial prophylaxis or post-surgical medication on implant healing. Khoury et al. (2008) reported that prophylactic amoxicillin affected clinical parameters but not IL-1β or IL-8 pro-inflammatory biomarkers, suggesting that treatment did not suppress inflammation during early osseointegration. There appear to be no changes in implant success rate based on prophylactic or post-operative timing of anti-microbial therapy (Chrcanovic, Albrektsson, & Wennerberg, 2014). However, if the aseptic chain is followed during surgery, it is unclear what benefit post-surgical medication would provide and whether this therapy would affect PICF quantity or quality, or influence biomarker release. There is evidence that prophylactic antimicrobials reduce implant failure rates, although the mechanism for this reduction is un- known, it is assumed that the more aseptic surgical bed provides an improved environment for healing (Chrcanovic et al., 2014). In contrast, chlorhexidine for hygiene maintenance may affect periodontal clinical parameters and PICF content (Khoury et al., 2008), mainly due to its residual effects. Considering these biologic effects, research

studies addressing pre-surgical and post-surgical disinfection should include data analysis during the 7–14 day period and should be carefully analyzed.

4.2. Biomarker release during intermediate healing

After 14 days of healing, bone implant contact (BIC) is increased 6% to 14.8%, and bone formation partially extends along the old bone trabeculae to the implant surface in transitional regions from compact bone to soft tissue interfaces. At this stage, the osteoid matrix is surrounded by osteoblasts that assist in trabecular formation (Lang et al., 2011). While OC is known to be released by mature osteoblasts and is responsible for the connection of calcium ions to extracellular bone matrix (Sato, Matsuzaka, Kokubu, & Inoue, 2011), only one study found increased OC expression in implants with CL implants with rough surface abutments compared to IML implants (Slotte et al., 2012). Although not statistically significant, this scenario was maintained until week 12, with increased OC levels for implants with CL compared to IML (Slotte et al., 2012). At week 2, there was a positive correlation with OC and ISQ (Slotte et al., 2012). Prati et al. (2013) have identified similarly increased OC levels in IML compared to CL during weeks 2 to 4 that remained stable until week 16.

OPN and OPG are bone maturation and resorption modulators (Belibasakis & Bostanci, 2012). Previous studies have shown that pa- tients who received IML implants had higher OPN concentrations compared to those who received CL implants (Ghiraldini et al., 2016; Prati et al., 2013). Increased OPG levels have been described among patients receiving IML implants at 15 days, which suggests that there is already ongoing osteoblastic activity during this period (Prati et al., 2013). However, other studies showed a progressive increase in OPG from week 2 onward (Ghiraldini et al., 2016; Peker Tekdal et al., 2016), indicating an augmentation of OPG levels with the advancement of bone healing.

TGF family presence during the second week of healing has been associated with collagen synthesis and osteoblast proliferation, differentiation, and activity (Zhang, Ahmad, & Gronowicz, 2003). TGF-α plays an important role during early healing and vascularization when the fibrin-rich and fibroblast-rich matrix provides a scaffold for future bone growth (Zhang, Ahmad, & Gronowicz, 2003), and also controls the conversion of this initial matrix into new bone through the release of MMPs (MMP-9, MMP-13, and MMP-14), formation of blood vessels, and matrix remodeling (Stein & Lian, 1993). Prati et al. (2013) observed that, in patients with IML implants, TGF-α levels peak at day 15

and then decrease, after the concentration decreased but remain elevated compared to levels in patients with CL. Thus, IML protocols may stimulate the first phase of osseointegration and, consequently, hasten the subsequent healing phases. In contrast, TGF-α concentrations are significantly higher in patients with CL implants compared to those with IML implants between 60 and 120 days (Prati et al., 2013).

Emecen-Huja et al. (2013) showed that IL-1β levels in PICF de- creased significantly from the initial healing period (weeks 1–3) to week 12, with similar decreasing trends for IL-6 and IL-1β levels. Slotte et al. (2012) and Bielemann et al. (2018) reported that IL-1β and IL-6 data at 15 days in patients with CL implants were consistent with Emecen-Huja et al.'s (2013) findings. In addition, patients with rough-surface IML implants had a 67.33% increase in IL-1β expression com- pared to patients with CL implants (Slotte et al., 2012). Elsyad et al. (2016) also showed that IL-1β concentrations were low 15 days before occlusal loading of implants and high 12 months later. These previous studies suggest that bone remodeling and osseointegration occur simultaneously with functional loading of implants and that the inter- action of biological and mechanical forces are fundamental to implant success (Raghavendra et al., 2005). Accordingly, we can infer that ocClusal loading stimulates the release of pro-inflammatory factors. Conversely, the study by Bielemann et al. (2018) observed a higher IL-1β concentration in first week, which was mainly attributed to the surgical bone trauma. After the first week, no significant changes during the healing process were noted, showing that this cytokine is unable to discriminate physiologic bone-change in totally edentulous patients over longer time periods (Bielemann et al., 2018).

4.3. Biomarker release during late healing

At 30 days, BIC has reached nearly 30% of the implant surface, with large surface areas covered by newly formed bone; the process of bone apposition and deposition is directed towards greater activity (Lang et al., 2011). IL-1β and TNF-α activity potentiate osteoclastogenesis and alveolar bone resorption (Hall, Pehrson, Ekestubbe, Jemt, & Friberg, 2015; Nowzari, Phamduong, Botero, Villacres, & Rich, 2012). During late healing, IL-1β, which was expressed at higher levels in IML smooth abutment implants than with CL rough abutment implants, has been correlated with complications such as bone dehiscence and implant rotation or instability (Slotte et al., 2012). Deregulated IL-1β release may induce pathologic bone resorption and intensely inhibits bone formation

(Mundy, 1991; Panagakos, Aboyoussef, Dondero, & Jandinski, 1996), mainly because it is directly involved in inflammation and immune processes during infection (Duitman, Orinska, & Bulfone-Paus, 2011). IL-1 has been detected in endolysosomes after its early release from monocytes triggered by adenosine triphosphate stimulation (ATP) (Carta et al., 2006). Thus, this IL may act during early bone healing, then decrease 3–6 weeks into the osseointegration process (Emecen-Huja et al., 2013).

Unlike other researchers, Dŏgan et al. (2015) did not find differences in IL-1β or TNF-α levels between healthy patients and those with diabetes. However, their data suggest that overproduction of these pro-inflammatory cytokines may modulate periodontal destruction induced by dental biofilm in patients with diabetes. Slotte et al. (2012) also did not find differences in TNF-α expression 30 days after the operation, despite its reduction compared to baseline at day 2. They found a non- significant increase in TNF-α expression in patients with IML implants with smooth and rough abutments that correlated with peri-implant surgical flap healing. Because TNF-α is produced quickly by macrophages (Hehlgans & Pfeffer, 2005) to recruit and activate immune cells to the site of injury or infection (Kugimiya et al., 2005; Stow et al., 2009), it serves as a marker of peri-implant status (Emecen-Huja et al., 2013). In contrast, Bielemann et al. (2018) observed significantly higher concentrations at week 2 and 4. At week 4, the TNF-α release was significantly higher in patients with atrophic mandibular ridges and non-smokers, suggesting that the intensity of the cellular events required for bone remodeling process is different and more intense in these patients (Bielemann et al., 2018). It is logical, therefore, that TNF-α levels will be reduced at day 30 of osseointegration.

The TRAP role in the molecular events that occur during late peri-implant healing was described in two studies (Slotte et al., 2012; Verrastro Neto et al., 2017). Primary stability and TRAP expression correlated positively over the first 30 days (Slotte et al., 2012), in-dicating that TRAP is a bone resorption marker involved in osteoclast recruitment and function (Hall et al., 2015). Verrastro Neto et al. (2017) reported reduced levels of TRAP in implants with low insertion torque, suggesting a positive impact on the local host response around these implants when implants are inserted under high torque.

Primary stability and secondary stability at days 2 and 30 correlated with ALP expression (Slotte et al., 2012; Tirachaimongkol et al., 2016), an index of bone formation-related metabolic activity (Paknejad, Emtiaz, Khoobyari, Gharb, & Yazdi, 2006). However,

Mandić et al. (2015) found a similarly high ALP activity only during the first post-operative week. Furthermore, Sayardoust, Omar, and Thomsen (2017) reported a peak in ALP expression after 28 days for non-smokers that had surface-modified implants installed.

A previous finding related to CatK, a bone resorption marker, showed that its releasing at 28 days was negativelly correlated with peri-implant surgical flap healing at day 2, supporting the notion that it has an active role in early healing at the peri-implant bone site (Slotte et al., 2012). This biomarker was recently investigated in a population consisting of non-smokers and smokers. In implants with modified- surface in non-smokers, a gradual increase over time with a peak expression after 28 days was observed, while a low expression was observed in smokers at week 8 (Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017). OC correlated positively with good bone quality at 28 days, as evidenced by its decreasing expression with IML implants from day 14 to day 28 and its increasing expression with CL implants from day 14 to 28 (Slotte et al., 2012). Ghiraldini et al. (2016) reported a 46% increase in levels in CL implants in week 12 that did not persist 12 months later.

After 6 weeks, the implant surface reaches 60% of BIC, and advanced bone maturation is evident, as primary osteocyte formation is visible further from the implant surface and old bone remodeling occasionally leads to secondary osteocyte formation (Lang et al., 2011). The two studies with 6-week follow-up examinations showed that IL-8 did not change (Emecen-Huja et al., 2013) and that high and stable MMP-8 levels highlight the intense bone remodeling that occurs during this period (Tsoukaki et al., 2013).

TGF-α increased 62% and OC increased 23% from baseline to 8 weeks in patients with CL implants compared to patients with IML implants and remained stable up to 120 days (Prati et al., 2013). These findings may be explained by the OC increase in response to mechanical stimuli (Pavlin, Zadro, & Gluhak-Heinrich, 2001), possibly because immediate loading activates pre-osteoblasts to release OC (Sato et al., 2011). During this same period, RANKL concentrations were higher in patients with implants installed using conventional osteotomy with drilling compared to implants inserted using piezoelectric surgery (Peker Tekdal et al., 2016).

At 3 months, it is believed that the phase of osseointegration with continuous bone matrix deposition ends, and a new phase of bone remodeling begins (Raghavendra et al., 2005). At week 12, IL-1β increases have been reported in patients receiving Standard Straumann CL implants versus esthetic plus Straumann SLA® implants.

Standard implants were associated with the highest visible plaque scores, PICF volume, and IL-1β levels, all of which may have been caused by plaque accumulation (Boynueğri et al., 2012). Similar peri-implant biological behavior has been shown with IL-1β in patients with CL implants with similar designs and smooth surface abutments from other manufacturers (Slotte et al., 2012). However, a study that followed patients with CL Straumann SLActive® implants did not show IL-1β concentration changes during 7 months of follow-up despite occlusal loading (Dŏgan et al., 2015), the surface treatment did not affect IL release. As with IL-1β, TNF-α expression data have been inconsistent. One study showed that TNF-α decreased 60% on average at 90 days compared to the postoperative period (Slotte et al., 2012). Other studies reported significant increases in 12- and 18-week concentrations (Boynueğri et al., 2012; Dŏgan et al., 2015). Interestingly, inflammatory marker quantification in late implant healing was reported to correlate negatively with secondary stability, as measured by ISQ and PGE2 quantification in patients receiving weekly LED treatments at week 12 (Gokmenoglu et al., 2014). This finding supports the idea that LED therapy may produce anti-inflammatory activity through PGE2 inhibition, even after bone remodeling.

At 90 days, patients with CL implants and rough abutments had increased ALP release compared to patients with IML implants (Slotte et al., 2012). There was a negative correlation between ALP and peri-implant surgical flap healing during the first 2 days (Slotte et al., 2012), as evidenced by ALP expression and subsequent bone neogenesis originating from and linked to pre-existing bone structures (Stucki et al., 2001). This finding indicates that bone maturation proceeds prior to increases in ALP, which serves as a general marker of bone metabolism and plays a major role in progression of bone mineralization (Plagnat et al., 2002). Consistent with this finding, Tirachaimongkol et al. (2016) found a positive correlation between ALP and ISQ in patients with CL implants. During the same period, TRAP was positively correlated with bone quality and complications but negatively correlated with bleeding on probe (Slotte et al., 2012).

4.4. Other biomarkers released during osseointegration

IL-10, an anti-inflammatory cytokine, produced by T-helper 2 cells (Th2), macrophages, and B cells (Ata-Ali et al., 2015), seems to be a promising biomarker due to its importance as an endogenous suppressor of infection and bone resorption by suppressing osteoclastic differentiation (Zhang et al., 2014). Bielemann et al. (2018) reported a progressive increase release of IL-10 during weeks 1-12 of bone healing.

This finding can be interpreted as an attempt of the host bone to resolve the inflammation process, suggesting that IL10 can play an important role in the regulation of bone homeostasis, and cellular and humoral immune responses (Zhang et al., 2014).

MMP-8 and TIMP-1 decreased during weeks 1–12 of bone healing following implant placement (Nomura et al., 2000; Tsoukaki et al., 2013). Thus, MMP-8 and TIMP-1 action during initial healing appears to be related to the response to surgical trauma, MMP-8 also rather than to bone remodeling (end of osseointegration). TGF-β1 was not detected during the first 2 months but was detected at 4 months (point of osseointegration completion) and increased until 12 months (Nogueira- Filho et al., 2014). The authors believe that this finding illustrates the course of inflammation in response to biofilm and delayed fibrotic tissue formation. However, one study showed that TGF-β levels were stable for up to 2 weeks of osseointegration, increased 24.7% between 2 weeks and 12 weeks, trended toward a decrease (non-significant, 42.81%) at 3–6 months, increased (54%) between 6 months and 12 months, and finally decreased after 12 months (Ghiraldini et al., 2016). Gokmenoglu et al. (2014) demonstrated that a similar reduction in TGF- β release over time was associated with increased accumulation of bacterial biofilm in peri-implant sites (Gokmenoglu et al., 2014).

BMPs, which are signaling proteins of the TGF-β superfamily, also play essential roles in osteogenesis (Kugimiya et al., 2005). They induce bone formation as nearby mesenchymal cells differentiate into chondroblasts and osteoblasts (Yilgor, Tuzlakoglu, Reis, Hasirci, & Hasirci, 2009). At 30 days, IML implants installed with higher insertion torque showed high levels of BMP-9 release, indicating high osteoblastic activity during the late phase of osseointegration (Verrastro Neto et al., 2017). At 90 days, BMP-2 and BMP-7 release were more pronounced compared to 30 days, but not significantly so (Dolanmaz et al., 2015). However, their high concentrations indicate their role in inducing complete bone morphogenesis (Kugimiya et al., 2005). BMP-2 plays an important role in bone and cartilage development, and BMP-7 plays a key role in mesenchymal cells transformation into bone and cartilage (Chen, Zhao, & Mundy, 2004).

OPG increased progressively up to the twelfth week, with significant increases from 2 weeks to 4 weeks (42.6% increase for patients undergoing piezoelectric surgery and 107.6% increase for patients undergoing osteotomy with drill). An OPG decrease was observed from 12 to 24 weeks that was 44% lower for patients receiving implants in association with less invasive surgical protocols using piezoelectric surgery (Peker Tekdal et al., 2016). Dolanmaz et al. (2015) also observed this OPG increase between 4

weeks and 12 weeks. OPG and OPN increases also appear related to maintenance of implant stability during (15 days) and the post-osseointegration period (12 months) (Ghiraldini et al., 2016). It is known that OPG binds RANKL to prevent RANK membrane receptor binding in pre-osteoclasts to modulate bone maturation and resorption (Belibasakis & Bostanci, 2012). The latter enables to identify the cooperation of angiogenic and osteogenic signaling that benefits peri-implant bone formation (Verrastro Neto et al., 2017). OPN is associated with binding of calcium-based biominerals to extra-cellular bone matrix and bone mineralization (McKee, Pedraza, & Kaartinen, 2011). The finding by Prati et al. (2013) that patients with IML implants had higher OPN levels compared to patients with CL implants suggests that IML implants stimulate greater metabolic activity than do CL implants during bone mineralization.

Ghiraldini et al. (2016) did not find significant differences between healthy patients, patients with well-controlled type 2 diabetes, and patients with poorly-controlled type 2 diabetes with respect to FGF levels. However, our analysis of their results shows that FGF concentrations were highest for healthy patients, lower for patients with poorly-controlled diabetes, and lowest for patients with well-controlled patients. In addition, this clinical study also reports that patients with well-controlled diabetes had higher levels of OPG compared to patients with poorly-controlled diabetes. OPG reduces FGF release (Hadjidakis & Androulakis, 2006), likely because higher OPG concentrations are re- flected in FGF results (Ghiraldini et al., 2016).

RANKL also plays an important role in osteoclastogenesis of bone metabolism. Despite its importance, sRANKL levels did not change significantly from 2 weeks to 12 weeks (Dolanmaz et al., 2015; Tsoukaki et al., 2013). RANKL release was low at weeks 2, 4, 8, and 12 (Peker Tekdal et al., 2016), and from 1 week to 18 weeks with the highest concentrations occurring soon after inflammation caused by surgical trauma (Onuma et al., 2015). It has been speculated that low RANKL concentrations in PICF indicate that ELISA is not sensitive enough to detect this biomarker (Belibasakis & Bostanci, 2012; Tsoukaki et al., 2013).

Some biomarkers, such as NO and PTH hormone, had long-term effects on osseointegration. NO is involved only in bone remodeling and peri-inflammatory processes of periodontal soft tissues. Studies showing that NO concentrations do not increase until there has been 3 months of osseointegration, regardless of type of implant loading, support the notion that NO is not important for early healing (Güncü, Tözüm, et al., 2008; Güncü et al., 2009; Tözüm, Turkyilmaz, Yamalik, Karabulut et al., 2007; Tözüm,

Türkyilmaz, Yamalik, Tümer et al., 2007). Additionally, only one study found higher NO levels in patients receiving LED light therapy compared to those not receiving LED therapy (Gokmenoglu et al., 2014). LEDs may activate cell respiration by pumping NO from the intracellular space into the extracellular matrix (Gokmenoglu et al., 2014). PTH hormone increases between 15 days and 90 days (Prati et al., 2013), consistent with a gradual action on bone mass in association with high OPG levels; the OPG-PTH complex inhibits exacerbated osteoclast activity (Pierroz et al., 2010). PTH release is also augmented in patients with IML implants, favoring more pronounced osteoblastic activity, as supported by the finding of PTH receptors on osteoblast membranes (Pierroz et al., 2010).

4.5. Confounding factors and biomarker release

Two studies reported the clinical and physiological healing in patients with glycemic disorders (Dŏgan et al., 2015; Ghiraldini et al., 2016) and one related to bone disorders (Onuma et al., 2015). Dŏgan et al. (2015) monitored the IL-1B and TNF-α release in the GCF and PICF in patients with glycemic-controlled type 2 diabetes (T2DM) until 7 months after installation. They found no differences in implant stability, bone levels around the implants and in the levels of the 2 pro-inflammatory cytokines in the GCF and PICF. Ghiraldini et al. (2016) also investigated the effect of glycemic control T2DM patients on local levels of PICF during the healing process analyzing the following key bone markers in PICF: TGF-β, FGF, OPN, OC, and OPG. Three types of patients were monitored until 12 months post-therapy: i) healthy patients, ii) better-controlled patients (diabetics with glycated hemoglobin (HbA1c) levels ≤8%) and iii) poorly-controlled patients (diabetics with HbA1c levels > 8%). The results revealed that diabetic patients with compromised glycemic control exhibit a distinct profile of bone-related factors that could impair the bone repair. Osteogenic and/ or bone mineralization markers were downregulated in poorly-controlled diabetics, as the lowest OPN concentrations were found at 12 months. The OC and TGF-β levels also decreased after 12 months compared to 15-day and 3 months follow-up periods, respectively. However, no differences in implant failure or clinical complications were observed between the groups. Onuma et al. (2015) did not find significant differences in the levels of osteoclastogenesis-related factors (sRANKL and OPG) in the PICF of immediately loaded implants in patients with and without osteopenia. This finding suggests that bone turnover in the peri-implant environment was not affected by

osteopenia. However, the levels of the biomarkers in the PICF differed between baseline and 4 months after surgery, showing that the surgical trauma associated with implant loading generates increased RANKL levels and decreased OPG levels in the PICF for both groups.

It is known that the influence of antibiotics on the PICF volume and marker release is dose and type dependent (Escalante et al., 2015; Khoury et al., 2008). Khoury et al. (2008), investigated the clinical and biologic markers of early soft tissue healing around dental implants in the presence and absence of antibiotic prophylaxis. Their main finding was that systemic amoxicillin may have a modest effect on clinical parameters during the first postoperative week and may have a limited effect, if any, on biomarkers. Escalante et al. (2015), performed an RCT comparing the administration of a single dose of two different antibiotics (azithromycin e amoxicillin) prior to surgical placement and analyzed the presence of these drugs in the PICF. They observed that azithromycin was available at the surgical site for a long period of time (until the 13th day) unlike amoxicillin, which presented concentrations below the detection limit. In addition, a single prophylactic dose of azithromycin appeared to alter several potentially important aspects of inflammation and early healing after surgery. Azithromycin in PICF seems to inhibit the cytokine release by balancing the release of pro- and anti-inflammatory cytokines suggesting that the administration of azithromycin speeds up the post-surgical healing progress. However, the inhibition mechanism of cytokine production by different anti- biotics and doses has not yet been established (Desaki et al., 2004; Kikuchi, 2002; Matsumura et al., 2011). Therefore, it is important to highlight in our results the studies that administered antibiotics, so that the clinician can interpret the results of each study according to the different methodologies used.

Two recent studies (Sayardoust, Omar, & Thomsen, 2017; Sayardoust, Omar, Norderyd et al., 2017) investigated the clinical and molecular behavior of osseointegration through eight biomarkers (IL-8, IL-6, TNF-α; ALP, OC, CatK; BMP-2 and VEGF) in smokers and non-smokers, rehabilitated with fixed implant-retained prostheses. In the first study, smoking did not influence IL-8, IL-6 and TNF-a expression during the initial osseointegration phase 1, 7, 14, and 28 days after implant placement. In non-smoking patients, the CatK, bone remodeling gene showed a gradual increase with peak expression after 28 days. In smokers, the maximum level of CatK expression occurred after 14 days, in addition they usually presented an expression of the OC marker. Non-smokers also showed a peak of BMP-2 after 7 days, followed by constant expression,

whereas smokers presented only a mean peak, always lower than nonsmokers, which remained un- changed. VEGF in both groups had maximum expression levels on day 1, followed by a gradual reduction until day 28. There are thus a few differences in gene expression between smokers and nonsmokers, which include a positive expression of OC and a late peak of BMP-2 in smokers. In nonsmokers, the involvement of a strong inflammatory response and low regenerative capacity is implicated in the failure process. In smokers, failure can be attributed to an altered structure and composition of the host bone. The second study by Sayardoust, Omar, Norderyd et al. (2017), focused on the late stages of osseointegration (60- and 90-day after implant installation). They observed that smoking did not significantly influence the release of TNF-α and IL-8. However, IL-6 increased from 60 to 90 days in the smoker group. This study asserted that smoking did not influence the gene expression of the ALP, OC, CatK, BMP-2, and VEGF factors at 60 and 90 days. From these two studies, it was concluded that smoking has an early effect on osseointegration, which depends on the surface properties of the implant and the local response of the host.

Bielemann et al. (2018) analyzed the concentration of cytokines during the osseointegration period in edentulous patients with high edentulism time rehabilitated with mandibular overdentures retained by two narrow diameter implants. The effect of potential confounding factors such as mandibular bone atrophy, smoking habit, bone type, and insertion torque on the cytokine expression in the PICF were also investigated. Smoking habit influenced the release of TNF-α, IL-1β, IL-6, and IL-10. IL-10 was the most affected cytokine, as non-smokers presented a higher release of these cytokines at 2, 4, 8, and 12 weeks. Bone atrophy influenced the release of TNF-α, IL-1β, and IL-6. Atrophic patients had greater pro-inflammatory cytokines release (IL-1β and IL-6) during the first month after installation. TNF-α show the highest release from the first month of installation, and at 3 months the release was reversed being higher in non-atrophic patients. Bone type I only influenced the IL-1β and IL-6 release. Bone type I was associated with a greater IL-1β release at week 2, while bone type II was associated with a greater release of IL-6 after weeks 2 and 4. The insertion torque only influenced the IL-6 release at week 8, being greater for implants with torque > 32 N cm. The authors concluded that smoking, bone atrophy, and bone type can greatly influence in the cytokine release during osseointegration. In addition, this study showed that cytokines are likely to interact synergistically with each other and show an association with clinical parameters, population-specific characteristics, and that

variations in cytokine concentrations observed throughout the study can be attributed to the healing balance.

Regarding the surgical approach and its relationship with the bone characteristics, a recent study by Verrastro Neto et al. (2017), was the first to investigate the influence of insertion torque on the bone and vascular biomarker release during the healing process of implants with immediate loading quantified 7, 14, 30, and 120 days after implant placement. The hypothesis proposed by the authors was confirmed and different levels of insertion torque for the immediately loaded dental implants was able to modulate the local pattern of bone and vascular mediators during early bone healing around implants. Benefits in terms of the release of at least some bone and vascular mediators were expected using reduced torque. For instance, the upregulation of angio- genic factors such as the VEGF and the placental growth factor (PLGF), and osteoblastogenic factors such as BMP-9 and OPG, and the down-regulation of TRAP, a marker of osteoclastogenesis. The levels of bone- and angiogenesis-related markers during early peri-implant repair was significantly influenced by the different levels of torque. The main findings were: i) inter-group comparisons showed that VEGF and OPG levels were higher in the low torque group than in the conventional torque group on days 7 and 30, respectively; ii) BMP-9 and periostin levels were higher in the conventional group than in the low torque group on day 120, and iii) TRAP was up-regulated around implants inserted with conventional torque at all time points.

4.6. Influence of mucositis and peri-implantitis

Biofilms play a significant role in determining the outcome and success of an implant. Local PICF biomarker events may very well represent an inflammatory reaction in response to plaque accumulation and microbial challenge, not just osseointegration. The latter is especially likely during the initial stages of wound healing, when patients are less likely to perform optimal oral hygiene. The effect of plaque on implants on peri-implant mucosa was reported in most studies selected in this systematic review. However, no study investigated the potential relationship between clinical parameters, microbiological profiles of the healthy and failing implants, and the immunological response of the host. It is well known that plaque accumulation can trigger an inflammatory process that affect both the soft and the hard tissue around a functional implant (Ata-Ali, Flichy-Fernandez, Ata-Ali, Penarrocha- Diago, & Penarrocha-Diago,

2013). Mucositis is the first signal of the peri-implant tissue response to accumulation of bacterial deposits and it is defined by its restriction to the inflammation of the soft tissues with bleeding on probing. In the presence of overload of microbial plaque, when a consortium of bacteria called “red complex” (Socransky, Haffajee, Cugini, Smith, & Kent, 1998) is present, they can alter the host response to inflammation increasing mainly the IL-1β and IL-6 release (Petković et al., 2010).

This relationship was investigated by Ata-Ali et al. (2013) in patients (age range 60–63 years) with healthy dental implants and with peri-implant mucositis. Similar studies focused on the healing process of the implants. When plaque accumulation and gingival inflammation was detected in those patients, transitory mucositis was diagnosed accompanied by significant increase in the IL-6 expression. Although IL-1β expression also increased in the mucositis group, the levels were similar to those observed in the healthy implants. The analysis of the periodontal pathogens of the red complex showed no differences between healthy peri-implant sites and sites with mucositis. Furthermore, no specific association with the studied bacterial species was established. In this context, the main difference between mucositis and peri-implant health may not be the prevalence, but rather the amount of putative pathogens present. In addition, the level of hard tissue de- struction is believed to be the result of the activation of the host immunoinflammatory response to bacterial challenge.

In a population of partially edentulous patients aged ≥70, the effects of the experimental mucositis were also clinically and biologically evaluated to determine a cause and effect relationship (Meyer et al., 2017). Twelve inflammatory markers were measured in a non-current smoker population in which the implants had been placed at various time points at least 1 year before the study. The results showed no statistical difference between teeth and implants for any inflammatory marker. During the plaque accumulation phase of over 3 weeks, a tendency of more severe inflammation around implants was described and the different biomarkers reacted variably. In the implant sites, only IL-1β was released in significantly higher concentrations. Small differences were observed for IFN-y, IL-8, granulocyte–macrophage colony-stimulating factor (GM-CSF), MIP-1β and TNF-α, generally with lower concentrations compared to the baseline. After re-establishing oral hygiene, the level of the different biomarkers tended to return to the median concentrations found at baseline within 1 week.

Early and late dental implant failures can be associated with peri-implantitis. A clinical study by Ata-Ali et al. (2015) reported some interesting findings that can help

diagnosing peri-implantitis. In terms of clinical parameters, there was a significant relationship between smoking and the presence of peri-implantitis, and patients with peri-implantitis were also significantly younger than patients with healthy peri-implant tissues. Microbiologically, there was a significant relation between peri-implantitis and *P. gingivalis; P gengibvalis and P denticola*, were in turn associated with the total bacterial load. In terms of PICF analyses, the fluid volume was similar between healthy implants and implants with peri-implantitis, but greater levels of IL-1β, IL-6, IL-10 e TNF-α were found in the peri-implantitis group. Furthermore, a significant relationship between the concentration of these four biomarkers and the inflammatory response in peri-implantitis tissue was described, and the IL-1β/IL-10 ratio was 3.75 times higher in the peri-implantitis group. However, the authors also concluded that in terms of bone loss in cases with peri-implantitis, the contribution factors such as peri-implant microbiota and the dental arch involved could be equally important.

The local peri-implant health status is easily monitored by routine clinical exams assessing peri-implant probing depths and presence of inflammation and suppuration. In conjunction with the bone loss detected by radiographic evaluation to complement clinical exams, an adequate monitoring of peri-implant health is allowed. However, since peri-implantitis might be latent in its early stages, biomarker analysis in PICF might serve as tool for an early diagnosis and/or determination of patient susceptibility. The identification of specific biomarkers involved in the onset and progression of peri-implant disease may also contribute to the determination of the prognosis of affected implants (Zani et al., 2016). In addition, PICF biomarkers might help guide distinct treatment approaches for target individuals (Petković et al., 2010). In attempt to explore the use of multi-biomarker models to examine the diagnostic properties of the PICF inflammatory mediators, a panel of 20 analytes potentially involved in different stages of peri-implant disease pathogenesis was selected by Zani et al. (2016) to investigate if combinations of PICF biomarkers could be used to distinguish healthy implants from implants affected by peri-implantitis. Implants with peri-implantitis had significantly higher levels of 12/20 biomarkers compared to healthy implants: GM-CSF, macrophage-derived chemoattractant (MDC), IL- 12p70, IL-13, platelet-derived growth factor BB (PDGF-BB), IL-15, soluble human CD40 ligand (sCD40L), IL-17, Il-1β, IL-2, IL-6, and TNF-α. An interesting approach of this study was the proposal of multi-analyte models to discriminate implants with peri-implantitis from healthy implants. First, the logistic models indicated that the combination of six biomarkers, Fms-like

tyrosine kinase-3 ligand (Flt-3L), GM-CSF, IL-10, sCD40L, IL-17, and TNF-α increased the diagnostic properties of the model compared to the isolated biomarkers considerably. Second, a multi-biomarker approach involving IL-17, IL-1-ra, Flt-3L, IL-10, sCD40L, GM-CSF, TNFα, PDGF-BB, and IL-15 could be of diagnostic value for the detection of diseased implants. Moreover, these pre- liminary results indicated that even PICF collected from a clinically healthy site of the peri-implantitis affected fixture might contain bio- markers of peri-implant disease.

5. Conclusions

This systematic review identified the biomarkers levels in PICF during osseointegration based on data from 30 clinical studies that quantified 52 different biomarkers during the 16 weeks following implant insertion. No study selected for this review achieves the maximum score for bias risk analysis, due mainly to the absence of information about study sample recruitment, representativeness of the study samples to the population of interest, and the potential influence of researcher bias on results. The absence of reports accounting for potentially confounding factors (e.g. age, sex, bone atrophy, smoking habits, or systemic diseases) may lead to erroneous results. In addition, there is a lack of reporting of adverse treatment effects, and caution is required in extrapolating the reported findings to other populations. The included studies did not examine potential associations with bone healing and repair, metabolic bone activity, bone neoformation, or osseointegration of dental implants.

Finally, it was impossible to conduct a meta-analysis of extracted data because the biomarker concentrations in PICF reported in the selected studies could not be grouped by biological time or biomarker type. These limitations are related to the methodological heterogeneity of the analyses; different commercial biomarker analysis kits produce diferent models for processing and reporting of results. Moreover, non-standardized measurement units cannot be directly converted to consistent units across studies, primarily because of the proprietary nature of the kits. Polymerase chain reaction (PCR) methods are not appropriate for biomarker quantification because they evaluate biomarker expression only.

Due to the data heterogeneity in the literature, it was not possible to quantify the biomarkers present during osseointegration. Consequently, we also could not define which markers are most important at each phase of osseointegration. Therefore, this systematic review reports results according to the biological events of osseointegration

to serve as a catalog and reference for new studies. At this stage, the established clinical parameters for monitoring peri-implant health should still be considered the gold standard for clinical practice. When reference values of biomarkers in the peri-implant fluid are known in the future, PICF collection can become a useful additional tool in clinical practice for acute monitoring of peri-implant health.

Sources of funding

There was no grant support or funding for this study.

Conflicts of interest

The authors declare they have no conflicts of interest for this pub- lication.

Acknowledgements

The authors thank Prof. J.A. Shibli and Prof. R.C.V. Casarin for providing the original data of their trials.

Appendix A

Supplementary data

References

Albrektsson, T., & Johansson, C. (2001). Osteoinduction, osteoconduction and osseoin- tegration. European Spine Journal, 10(2), 96–101.

Albrektsson, T., Buser, D., & Sennerby, L. (2012). Crestal bone loss and oral implants. Clinical Implant Dentistry and Related Research, 14(6), 783–791.

Albrektsson, T., Dahlin, C., Jemt, T., Sennerby, L., Turri, A., & Wennerberg, A. (2014). Is marginal bone loss around oral implants the result of a provoked foreign body re- action? Clinical Implant Dentistry and Related Research, 16(2), 155–165.

Albrektsson, T., Chrcanovic, B., Östman, P.-O., & Sennerby, L. (2017). Initial and long- term crestal bone responses to modern dental implants. Periodontology 2000, 73(1), 41–50.

Alsaadi, G., Quirynen, M., Komárek, A., & Van Steenberghe, D. (2007). Impact of local and systemic factors on the incidence of oral implant failures, up to abutment con- nection. Journal of Clinical Periodontology, 34(7), 610–617.

Ata-Ali, J., Flichy-Fernandez, A. J., Ata-Ali, F., Penarrocha-Diago, M., & Penarrocha- Diago, M. (2013). Clinical, microbiologic, and host response characteristics in pa- tients with peri-implant mucositis. The International Journal of Oral & Maxillofacial Implants, 28(3), 883–890.

Ata-Ali, J., Flichy-Fernández, A. J., Alegre-Domingo, T., Ata-Ali, F., Palacio, J., & Peñarrocha-Diago, M. (2015). Clinical, microbiological, and immunological aspects of healthy versus peri-implantitis tissue in full arch reconstruction patients: A pro- spective cross-sectional study. BMC Oral Health, 15(1), 1–11.

Basegmez, C., Yalcin, S., Yalcin, F., Ersanli, S., & Mijiritsky, E. (2012). Evaluation of periimplant crevicular fluid prostaglandin e2 and matrix metalloproteinase-8 levels from health to periimplant disease status: A prospective study. Implant Dentistry, 21(4), 306–310.

Belibasakis, G. N., & Bostanci, N. (2012). The RANKL-OPG system in clinical period- ontology. Journal of Clinical Periodontology, 39(3), 239–248.

Bielemann, A. M., Marcello-Machado, R. M., Leite, F. R. M., Martinho, F. C., Chagas- Júnior, O. L., Del Bel Cury, A. A., et al. (2018). Comparison between inflammation- related markers in peri-implant crevicular fluid and clinical parameters during osseointegration in edentulous jaws. Clinical Oral Investigations, 22(1), 531–543.

Bonewald, L. F. (2011). The amazing osteocyte. Journal of Bone and Mineral Research, 26(2), 229–238.

Bosshardt, D. D., Chappuis, V., & Buser, D. (2017). Osseointegration of titanium, titanium alloy and zirconia dental implants: Current knowledge and open questions. Periodontology 2000, 73(1), 22–40.

Bostanci, N., & Belibasakis, G. N. (2018). Gingival crevicular fluid and its immune mediators in the proteomic era. Periodontol 2000, 76(1), 68–84.

Boynueğri, A. D., Yalim, M., Nemli, S. K., Ergüder, B. I., & Gökalp, P. (2012). Effect of different localizations of microgap on clinical parameters and inflammatory cytokines in peri-implant crevicular fluid: A prospective comparative study. Clinical Oral Investigations, 16(2), 353–361.

Buser, D., Sennerby, L., & De Bruyn, H. (2017). Modern implant dentistry based on osseointegration: 50 years of progress, current trends and open questions. Periodontology 2000, 73(1), 7–21.

Carta, S., Tassi, S., Semino, C., Fossati, G., Mascagni, P., Dinarello, C. A., et al. (2006). Histone deacetylase inhibitors prevent exocytosis of interleukin-1beta containing secretory lysosomes: Role of microtubules. Blood, 108(5), 1618–1626.

Chang, P.-C. C., Lang, N. P., & Giannobile, W. V. (2010). Evaluation of functional dynamics during osseointegration and regeneration associated with oral implants. Clinical Oral Implants Research, 21(1), 1–12.

Chen, D., Zhao, M., & Mundy, G. R. (2004). Bone morphogenetic proteins. Growth Factors, 22(4), 233–241.

Chrcanovic, B. R., Albrektsson, T., & Wennerberg, A. (2014). Reasons for failures of oral implants. Journal of Oral Rehabilitation, 41(6), 443–476.

Chrcanovic, B. R., Kisch, J., Albrektsson, T., & Wennerberg, A. (2016). Factors influencing early dental implant failures. Journal of Dental Research, 95(9), 995–1002.

Chudyk, A. M., Jutai, J. W., Petrella, R. J., & Speechley, M. (2009). Systematic review of hip fracture rehabilitation practices in the elderly. Archives of Physical Medicine and Rehabilitation, 90(2), 2460262.

Coli, P., Christiaens, V., Sennerby, L., & Bruyn, H. D. (2017). Reliability of periodontal diagnostic tools for monitoring peri-implant health and disease. Periodontology 2000, 73(1), 203–217.

Dŏgan, Ş. B., Kurtiş, M. B., Tüter, G., Serdar, M., Watanabe, K., & Karakış, S. (2015). Evaluation of clinical parameters and levels of proinflammatory cytokines in the crevicular fluid around dental implants in patients with type 2 diabetes mellitus. The International Journal of Oral & Maxillofacial Implants, 30(5), 1119–1127.

Davies, J. E. S. (2003). Understanding peri-implant endosseous healing. Journal of Dental Education, 67(8), 932–949.

De Wilde, E. A. W. J., Jimbo, R., Wennerberg, A., Naito, Y., Coucke, P., Bryington, M. S., et al. (2015). The soft tissue immunologic response to hydroxyapatite-coated trans-mucosal implant surfaces: A study in humans. Clinical Implant Dentistry and Related Research, 17(Suppl. 1 (1)), e65–e74.

Desaki, M., Okazaki, H., Sunazuka, T., Omura, S., Yamamoto, K., & Takizawa, H. (2004). Molecular mechanisms of anti-inflammatory action of erythromycin in human bronchial epithelial cells: Possible role in the signaling pathway that regulates nu- clear factor-kappaB activation. Antimicrobial Agents and Chemotherapy, 48(5), 1581–1585.

Dolanmaz, D., Saglam, M., Inan, O., Dundar, N., Alniacık, G., Gursoy Trak, B., et al. (2015). Monitoring bone morphogenetic protein-2 and -7, soluble receptor activator of nuclear factor-κB ligand and osteoprotegerin levels in the peri-implant sulcular fluid during the osseointegration of hydrophilic-modified sandblasted acid-etched and sandblaste. Journal of Periodontal Research, 50(1), 62–73.

Downs, S. H., & Black, N. (1998). The feasibility of creating a checklist for the assessment of the methodological quality both of randomized and non-randomized studies of health care interventions. Journal of Epidemiology and Community Health, 52, 377–384.

Duarte, P. M., Serrão, C. R., Miranda, T. S., Zanatta, L. C. S. S., Bastos, M. F., Faveri, M., et al. (2016). Could cytokine levels in the peri-implant crevicular fluid be used to distinguish between healthy implants and implants with peri-implantitis? A systematic review. Journal of Periodontal Research, 51(6), 689–698.

Duitman, E. H., Orinska, Z., & Bulfone-Paus, S. (2011). Mechanisms of cytokine secretion: A portfolio of distinct pathways allows flexibility in cytokine activity. European Journal of Cell Biology, 90(6–7), 476–483.

Elsyad, M. A., Mahanna, F. F., Elshahat, M. A., & Elshoukouki, A. H. (2016). Locators versus magnetic attachment effect on peri-implant tissue health of immediate loaded two implants retaining a mandibular overdenture: A 1-year randomized trial. Journal of Oral Rehabilitation, 43(4), 297–305.

Emecen-Huja, P., Eubank, T. D., Shapiro, V., Yildiz, V., Tatakis, D. N., & Leblebicioglu, B. (2013). Peri-implant versus periodontal wound healing. Journal of Clinical Periodontology, 40(8), 816–824.

Escalante, M. G., Eubank, T. D., Leblebicioglu, B., Walters, J. D., Gülses, A., & Ayna, M. (2015). Comparison of azithromycin and amoxicillin before dental implant placement: An exploratory study of bioavailability and resolution of postoperative inflammation. Journal of Periodontology, 86(11), 1190–1200.

Faot, F., Nascimento, G. G., Bielemann, A. M., Campão, T. D., Leite, F. R. M., & Quirynen, M. (2015). Can peri-implant crevicular fluid assist in the diagnosis of peri-im- plantitis? A systematic review and meta-analysis. Journal of Periodontology, 86(5),631–645.

Franchi, M., Bacchelli, B., Martini, D., De Pasquale, V., Orsini, E., Ottani, V., et al. (2004). Early detachment of titanium particles from various different surfaces of endosseous dental implants. Biomaterials, 25(12), 2239–2246.

Güncü, G. N., Tözüm, T. F., Güncü, M. B., & Yamalik, N. (2008). Relationships between implant stability, image-based measures and nitric oxide levels. Journal of Oral Rehabilitation, 35(10), 745–753.

Güncü, G. N., Tözüm, T. F., Güncü, M. B., Yamalik, N., & Tümer, C. (2009). A 12-month evaluation of nitrite oxide metabolism around immediate and conventionally loaded dental implants. Implant Dentistry, 18(1), 27–37.

Ghiraldini, B., Conte, A., Casarin, R. C., Casati, M. Z., Pimentel, S. P., Cirano, F. R., et al. (2016). Influence of glycemic control on peri-Implant bone healing: 12-month out-comes of local release of bone-related factors and implant stabilization in type 2 diabetics. Clinical Implant Dentistry and Related Research, 18(4), 801–809.

Gokmenoglu, C., Ozmeric, N., Erguder, I., & Elgun, S. (2014). The effect of light-emitting diode photobiomodulation on implant stability and biochemical markers in peri-implant crevicular fluid. Photomedicine and Laser Surgery, 32(3), 138–145.

Goodman, S. B. (2007). Wear particles, periprosthetic osteolysis and the immune system.Biomaterials, 28(34), 5044–5048.

Gruber, R., Nadir, J., & Haas, R. (2010). Neutrophil elastase activity and concentrations of interleukin1-β in crevicular fluid after immediate replacement and immediate loading of implants. British Journal of Oral and Maxillofacial Surgery, 48(3), 228–231.

Hadjidakis, D. J., & Androulakis, I. I. (2006). Bone remodeling. Annals of the New York Academy of Sciences, 1092, 385–396.

Hall, J., Pehrson, N.-G., Ekestubbe, A., Jemt, T., & Friberg, B. (2015). A controlled, cross- sectional exploratory study on markers for the plasminogen system and

inflammation in crevicular fluid samples from healthy, mucositis and peri-implantitis sites.European Journal of Oral Implantology, 8(2), 153–166.

Hehlgans, T., & Pfeffer, K. (2005). The intriguing biology of the tumour necrosis factor/ tumour necrosis factor receptor superfamily: Players, rules and the games. Immunology, 115(1), 1–20.

Insua, A., Monje, A., Wang, H., & Miron, R. J. (2017). Basis of bone metabolism around dental implants during osseointegration and peri-implant bone loss. Journal of Biomedical Materials Research Part A, 105(7), 2075–2089.

Jácome, C., & Marques, A. (2014). Pulmonary rehabilitation for mild COPD: A sistematic review. Respiratory Care, 59(4), 588–594.

Jawa, R. S., Anillo, S., Huntoon, K., Baumann, H., & Kulaylat, M. (2011a). Analytic re-view: Interleukin-6 in surgery, trauma, and critical care: Part I: Basic science. Journal of Intensive Care Medicine, 26(1), 3–12.

Jawa, R. S., Anillo, S., Huntoon, K., Baumann, H., & Kulaylat, M. (2011b). Interleukin-6 in surgery, trauma, and critical care part II: Clinical implications. Journal of Intensive Care Medicine, 26(2), 73–87.

Kaklamanos, E. G., & Tsalikis, L. (2002). A review on peri-implant crevicular fluid assays potential in monitoring and predicting peri-implant tissue responses. Journal of the International Academy of Periodontology, 4(2), 49–59.

Khoury, S. B., Thomas, L., Walters, J. D., Sheridan, J. F., & Leblebicioglu, B. (2008). Early wound healing following one-stage dental implant placement with and without antibiotic prophylaxis: A pilot study. Journal of Periodontology, 79(10), 1904–1912.

Kikuchi, T. (2002). Clarithromycin suppresses lipopolysaccharide-induced interleukin-8 production by human monocytes through AP-1 and NF-kappaB transcription factors. Journal of Antimicrobial Chemotherapy, 49(5), 745–755.

Koka, S., & Zarb, G. (2012). On osseointegration: The healing adaptation principle in the context of osseosufficiency, osseoseparation, and dental implant failure. The International Journal of Prosthodontics, 25(1), 48–52.

Konttinen, Y. T., Lappalainen, R., Laine, P., Kitti, U., Santavirta, S., & Teronen, O. (2006). Immunohistochemical evaluation of inflammatory mediators in failing implants. The International Journal of Periodontics & Restorative Dentistry, 26(2), 135–141.

Kugimiya, F., Kawaguchi, H., Kamekura, S., Chikuda, H., Ohba, S., Yano, F., et al. (2005). Involvement of endogenous bone morphogenetic protein (BMP) 2 and BMP6 in bone formation. Journal of Biological Chemistry, 280(42), 35704–35712.

Kumazawa, R., Watari, F., Takashi, N., Tanimura, Y., Uo, M., & Totsuka, Y. (2002). Effects of Ti ions and particles on neutrophil function and morphology. Biomaterials, 23(17), 3757–3764.

Lang, N. P., Salvi, G. E., Huynh-Ba, G., Ivanovski, S., Donos, N., & Bosshardt, D. D. (2011). Early osseointegration to hydrophilic and hydrophobic implant surfaces in humans. Clinical Oral Implants Research, 22(4), 349–356.

Lin, G.-H., Kapila, Y., & Wang, H.-L. (2017). Parameters to define peri-Implantitis: A review and a proposed multi-domain scale. Journal of Oral Implantology, 43(6), 491–496.

Ma, Q. L., Fang, L., Jiang, N., Zhang, L., Wang, Y., Zhang, Y. M., et al. (2018). Bone mesenchymal stem cell secretion of sRANKL/OPG/M-CSF in response to macrophage-mediated inflammatory response influences osteogenesis on nanostructured Ti surfaces. Biomaterials, 154, 234–247.

Mandić, B., Lazić, Z., Marković, A., Mandić, B., Mandić, M., Djinić, A., et al. (2015). Influence of postoperative low-level laser therapy on the osseointegration of self-tapping implants in the posterior maxilla: A 6-week split-mouth clinical study. Vojnosanitetski Pregled, 72(3), 233–240.

Manzano, G., Montero, J., Martín-Vallejo, J., Fabbro, M., Del Bravo, M., & Testori, T. (2016). Risk factors in early implant failure: A meta-analysis. Implant Dentistry, 25(2), 272–280.

Matsumura, Y., Mitani, A., Suga, T., Kamiya, Y., Kikuchi, T., Tanaka, S., et al. (2011). Azithromycin may inhibit interleukin-8 through suppression of rac1 and a nuclear factor-Kappa B pathway in KB cells stimulated with lipopolysaccharide. Journal of Periodontology, 82(11), 1623–1631.

McKee, M. D., Pedraza, C. E., & Kaartinen, M. T. (2011). Osteopontin and wound healing in bone. Cells Tissues Organs, 194(2–4), 313–319.

Meyer, S., Giannopoulou, C., Courvoisier, D., Schimmel, M., Müller, F., & Mombelli, A. (2017). Experimental mucositis and experimental gingivitis in persons aged 70 or over. Clinical and biological responses. Clinical Oral Implants Research, 28(8), 1005–1012.

Moher, D., Liberati, A., Tetzlaff, J., & Altman, D. G. (2009). Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Medicine, 6(7), e1000097.

Mundy, G. R. (1991). Cytokines and bone remodeling. Journal of Bone and Mineral Metabolism, 9(2), 34–38.

Nogueira-Filho, G., Pesun, I., Isaak-Ploegman, C., Wijegunasinghe, M., Wierzbicki, T., & McCulloch, C. A. (2014). Longitudinal comparison of cytokines in peri-implant fluid and gingival crevicular fluid in healthy mouths. Journal of Periodontology, 85(11), 1582–1588.

Nomura, T., Ishii, A., Oishi, Y., Kohma, H., & Hara, K. (1998). Tissue inhibitors of metalloproteinases level and collagenase activity in gingival crevicular fluid: The relevance to periodontal diseases. Oral Disease, 4(4), 231–240.

Nomura, T., Ishii, A., Shimizu, H., Taguchi, N., Yoshie, H., Kusakari, H., et al. (2000). Tissue inhibitor of metalloproteinases-1, matrix metalloproteinases-1 and -8, and collagenase activity levels in peri-implant crevicular fluid after implantation. Clinical Oral Implants Research, 11(5), 430–440.

Noronha Oliveira, M., Schunemann, W. V. H., Mathew, M. T., Henriques, B., Magini, R. S., Teughels, W., et al. (2018). Can degradation products released from dental implants affect peri-implant tissues? Journal of Periodontal Research, 53(1), 1–11.

Nowzari, H., Yi, K., Chee, W., & Rich, S. K. (2008). Immunology, microbiology, and virology following placement of NobelPerfect™ scalloped dental implants: Analysis of a case series. Clinical Implant Dentistry and Related Research, 10(3), 157–165.

Nowzari, H., Phamduong, S., Botero, J. E., Villacres, M. C., & Rich, S. K. (2012). The profile of inflammatory cytokines in gingival crevicular fluid around healthy osseointegrated implants. Clinical Implant Dentistry and Related Research, 14(4), 546–552.

Onuma, T., Aquiar, K., Duarte, P., Feres, M., Giro, G., Coelho, P., et al. (2015). Levels of osteoclastogenesis-Related factors in the peri-implant crevicular fluid and clinical parameters of immediately loaded implants in patients with osteopenia: A short-term report. The International Journal of Oral & Maxillofacial Implants, 30(6), 1431–1436.

Paknejad, M., Emtiaz, S., Khoobyari, M. M., Gharb, M. T., & Yazdi, M. T. (2006). Analysis of aspartate aminotransferase and alkaline phosphatase in crevicular fluid from im- plants with and without peri-implantitis. Implant Dentistry, 15(1), 62–69.

Panagakos, F. S., Aboyoussef, H., Dondero, R., & Jandinski, J. J. (1996). Detection and measurement of inflammatory cytokines in implant crevicular fluid: A pilot study. The International Journal of Oral & Maxillofacial Implants, 11(6), 794–799.

Pavlin, D., Zadro, R., & Gluhak-Heinrich, J. (2001). Temporal pattern of stimulation of osteoblast-associated genes during mechanically-induced osteogenesis in vivo: Early

responses of osteocalcin and type I collagen. Connective Tissue Ressearch, 42(2), 135–148.

Peker Tekdal, G., Bostanci, N., Belibasakis, G. N., & Gürkan, A. (2016). The effect of piezoelectric surgery implant osteotomy on radiological and molecular parameters of peri-implant crestal bone loss: A randomized, controlled, split-mouth trial. Clinical Oral Implants Research, 27(5), 535–544.

Petković, A. B., Matić, S. M., Stamatović, N. V., Vojvodić, D. V., Todorović, T. M., Lazić, Z. R., et al. (2010). Proinflammatory cytokines (IL-1beta and TNF-alpha) and chemokines (IL-8 and MIP-1alpha) as markers of peri-implant tissue condition. International Journal of Oral and Maxillofacial Surgery, 39(5), 478–485.

Pierroz, D. D., Bonnet, N., Baldock, P. A., Ominsky, M. S., Stolina, M., Kostenuik, P. J., et al. (2010). Are osteoclasts needed for the bone anabolic response to parathyroid hormone? A study of intermittent parathyroid hormone with denosumab or alendronate in knock-in mice expressing humanized RANKL. Journal of Biological Chemistry, 285(36), 28164–28173.

Plagnat, D., Giannopoulou, C., Carrel, A., Bernard, J. P., Mombelli, A., & Belser, U. C. (2002). Elastase, alpha2-macroglobulin and alkaline phosphatase in crevicular fluid from implants with and without periimplantitis. Clinal Oral Implants Research, 13(3), 227–233.

Prati, A. J., Casati, M. Z., Ribeiro, F. V., Cirano, F. R., Pastore, G. P., Pimentel, S. P., et al. (2013). Release of bone markers in immediately loaded and nonloaded dental im- plants. Journal of Dental Research, 92(12_Suppl), 161S–167S.

Qian, J., Wennerberg, A., & Albrektsson, T. (2012). Reasons for marginal bone loss around oral implants. Clinical Implant Dentistry and Related Research, 14(6), 792–807.

Raghavendra, S., Wood, M. C., & Taylor, T. D. (2005). Early wound healing around en- dosseous implants: A review of the literature. The International Journal of Oral & Maxillofacial Implants, 20(3), 425–431.

Roberts, S. J., van Gastel, N., Carmeliet, G., Luyten, F. P., Gastel, N., Van Carmeliet, G., et al. (2015). Uncovering the periosteum for skeletal regeneration: The stem cell that lies beneath. Bone, 70, 10–18.

Sanz, M., & Chapple, I. L. (2012). Clinical research on peri-implant diseases: Consensus report of Working Group 4. Journal of Clinical Periodontology, 39(Suppl. 12), 202–206.

Sato, R., Matsuzaka, K., Kokubu, E., & Inoue, T. (2011). Immediate loading after implant placement following tooth extraction up-regulates cellular activity in the dog mandible. Clinical Oral Implants Research, 22(12), 1372–1378.

Sayardoust, S., Omar, O., & Thomsen, P. (2017a). Gene expression in peri-implant cre- vicular fluid of smokers and nonsmokers. 1. The early phase of osseointegration. Clinical Implant Dentistry and Related Research, 19(4), 681–693.

Sayardoust, S., Omar, O., Norderyd, O., & Thomsen, P. (2017b). Clinical, radiological, and gene expression analyses in smokers and non-smokers, Part 2: RCT on the late healing phase of osseointegration. Clinical Implant Dentistry and Related Research, 19(5), 901–915.

Schierano, G., Bellone, G., Cassarino, E., Pagano, M., Preti, G., & Emanuelli, G. (2003). Transforming growth factor-beta and interleukin 10 in oral implant sites in humans. Journal of Dental Research, 82(6), 428–432.

Shanbhag, S., Shanbhag, V., & Stavropoulos, A. (2015). Genomic analyses of early peri- implant bone healing in humans: A systematic review. International Journal of Implant Dentistry, 1(5), 1–12.

Slotte, C., Lennerås, M., Göthberg, C., Suska, F., Zoric, N., Thomsen, P., et al. (2012). Gene expression of inflammation and bone healing in peri-implant crevicular fluid after placement and loading of dental implants. A kinetic clinical pilot study using quan- titative real-time PCR. Clinical Implant Dentistry and Related Research, 14(5), 723–736.

Socransky, S. S., Haffajee, A. D., Cugini, M. A., Smith, C., & Kent, R. L. (1998). Microbial complexes in subgingival plaque. Journal of Clinical Periodontology, 25(2), 134–144. Stein, G. S., & Lian, J. B. (1993). Molecular mechanisms mediating proliferation/differentiation interrelationships during progressive development of the osteoblast phenotype. Endocrine Reviews, 14(4), 424–442.

Stow, J. L., & Murray, R. Z. (2013). Intracellular trafficking and secretion of inflammatory cytokines. Cytokine & Growth Factor Reviews, 24(3), 227–239.

Stow, J. L., Ching Low, P., Offenhäuser, C., Sangermani, D., Low, P. C., Offenhauser, C., et al. (2009). Cytokine secretion in macrophages and other cells: Pathways and mediators. Immunobiology, 214(7), 601–612.

Stucki, U., Schmid, J., Hammerle, C. F., Lang, N. P., Hämmerle, C. F., & Lang, N. P. (2001). Temporal and local appearance of alkaline phosphatase activity in early stages

of guided bone regeneration. A descriptive histochemical study in humans. Clinical Oral Implants Research, 12(2), 121–127.

Tözüm, T. F., Türkyilmaz, I., & Yamalik, N. (2005). Analysis of the possible impact of inflammation severity and early and delayed loading on. International Journal of Oral and Maxillofacial Implants, 20, 547–556.

Tözüm, T. F., Turkyilmaz, I., Yamalik, N., Karabulut, E., & Eratalay, K. (2007a). Analysis of the potential association of implant stability, laboratory, and image-based mea- sures used to assess osteotomy sites: Early versus delayed loading. Journal of Periodontology, 78(9), 1675–1682.

Tözüm, T. F., Türkyilmaz, I., Yamalik, N., Tümer, C., Kilinç, A., Kilinç, K., et al. (2007b). The effect of delayed versus early loading on nitric oxide metabolism around dental implants: An 18-month comparative follow-up study. The International Journal of Oral & Maxillofacial Implants, 22(1), 53–62.

Terheyden, H., Lang, N. P., Bierbaum, S., & Stadlinger, B. (2012). Osseointegration—Communication of cells. Clinical Oral Implants Research, 23(10), 1127–1135.

Tirachaimongkol, C., Pothacharoen, P., Reichart, P. A., & Khongkhunthian, P. (2016). Relation between the stability of dental implants and two biological markers during the healing period: A prospective clinical study. International Journal of Implant Dentistry, 2(1), 27.

Trindade, R., Albrektsson, T., & Wennerberg, A. (2015a). Current concepts for the biological basis of dental implants. Oral and Maxillofacial Surgery Clinics of North America, 27(2), 175–183.

Trindade, R., Albrektsson, T., & Wennerberg, A. (2015b). Current concepts for the biological basis of dental implants: Foreign body equilibrium and osseointegration dynamics. Oral and Maxillofacial Surgery Clinics of North America, 27(2), 175–183.

Trindade, R., Albrektsson, T., Tengvall, P., & Wennerberg, A. (2016). Foreign body reaction to biomaterials: On mechanisms for buildup and breakdown of osseointegration. Clinical Implant Dentistry and Related Research, 18(1), 192–203.

Trindade, R., Albrektsson, T., Galli, S., Prgomet, Z., Tengvall, P., & Wennerberg, A. (2018). Osseointegration and foreign body reaction: Titanium implants activate the immune system and suppress bone resorption during the first 4 weeks after implantation. Clinical Implant Dentistry and Related Research, 20(1), 82–91.

Tsoukaki, M., Kalpidis, C. D. R., Sakellari, D., Tsalikis, L., Mikrogiorgis, G., & Konstantinidis, A. (2013). Clinical, radiographic, microbiological, and immunological

outcomes of flapped vs. flapless dental implants: A prospective randomized controlled clinical trial. Clinical Oral Implants Research, 24(9), 969–976.

Verrastro Neto, A., Andrade, R., Corrêa, M. G., Casarin, R. C. V., Casati, M. Z., Pimentel, S. P., et al. (2017). The impact of different torques for the insertion of immediately loaded implants on the peri-implant levels of angiogenesis- and bone-related markers. International Journal of Oral and Maxillofacial Surgery, 1–7.

Wang, Y., Zhang, Y., & Miron, R. J. (2016). Health, maintenance, and recovery of soft tissues around implants. Clinical Implant Dentistry and Related Research, 18(3), 618–634.

Yilgor, P., Tuzlakoglu, K., Reis, R. L., Hasirci, N., & Hasirci, V. (2009). Incorporation of a sequential BMP-2/BMP-7 delivery system into chitosan-based scaffolds for bone tissue engineering. Biomaterials, 30(21), 3551–3559.

Zani, S. R., Moss, K., Shibli, J. A., Teixeira, E. R., de Oliveira Mairink, R., Onuma, T., et al. (2016). Peri-implant crevicular fluid biomarkers as discriminants of peri-implant health and disease. Journal of Clinical Periodontology, 43(10), 825–832.

Zhang, H., Ahmad, M., & Gronowicz, G. (2003). Effects of transforming growth factor-beta 1 (TGF-β1) on in vitro mineralization of human osteoblasts on implant materials. Biomaterials, 24(12), 2013–2020.

Zhang, Q., Chen, B., Yan, F., Guo, J., Zhu, X., Ma, S., et al. (2014). Interleukin-10 inhibits bone resorption: A potential therapeutic strategy in periodontitis and other bone loss diseases. BioMed Research International, 2014.

Table 1: Demographic characteristics of the studies and methodology used to analyze the PICF and the main results (n=30).

Authors, year/ Study design/ Country	Population (n, W/M) Age Mean(SD) [range]	Implants (n) Brand	Follow-up	Outcome Variables	Bone Site/ Prosthetic treatment	Implant Loading	Biomarkers	Antibiotic Yes/No	Methodology (PICF analyses)	Main results
Nomura et al. 2000, LONG Japan	6 53.5 (12.4)	10 (Straumann, Waldenburg, Switzerland; implant type: 9 solid screws and 1 hollow screw)	1, 2, 4, 12 w	PICF; CAL; GI; PD; PI; MBL	NO INF	NO INF	Collagenase Activity; MMP-1; MMP-8; TIMP-1	N	- hTIMP-1 assay kit (Fuji Chemical Industries, Toyama city, Japan); - MMP-1, -8: ELISA systems (Amersham, Japan); - Collagenase Activity: Analysed on SDS-PAGE	Over production of TIMP-1 in the wound area after implantation could inhibit excessive tissue destruction and degradation of the neo-matrix in wound repair due to MMPs.
Tözüm et al. 2005, RCT Turkey	17 (9/8) 53 [42-65]	34 Ø3.75 x 15 mm (Brånemark System, Nobel Biocare, Göteborg, Sweden).	2,4, 24 w	PICF; GI; PD; PI; GBTI	Overdenture Mandibular 2 ball attachments	IML= 18 CL= 16	Nitric Oxide	N	Extraction of nitrite into water (139 ml distilled water/sample); Staining with Griess reagent	Presence and severity of inflammation, and loading seem to have an impact on NO metabolism around dental implants.
Tözüm et al. 2007a, RCT Turkey	17 (9/8) 53 [42-65]	34 Ø3.75 x 15mm (Brånemark System; Nobel Biocare, Göteborg, Sweden).	1, 3, 6, 9, 12,18 m	PICF; GI; GBTI; PD; PI;	Overdenture Mandibular 2 ball attachments	IML= 18 CL= 16	Nitric Oxide	N	Extraction of nitrite into water (139 ml distilled water/sample); Staning with Griess reagent	NO is involved in bone repair and remodeling around dental implants, that mechanical loading influences NO metabolism, and NO production at dental implant sites is closely regulated.
Tozum et al. 2007b, RCT Turkey	17 (9/8) 53 [42-65]	34 Ø3.75 x 15mm (Brånemark System; Nobel Biocare, Göteborg, Sweden).	2, 4, 24 w	PICF; ISQ; MBL	Overdenture Mandibular 2 ball attachments	IML= 18 CL= 16	Nitric Oxide	N	Extraction of nitrite into water (139 ml distilled water/sample); Staning with Griess reagent	Loading seemed to have an impact on the relatedness of PICF volume and NO levels. Early NO release may be associated with an ‘‘early’’ stage of the sulcus ‘‘developing’’ around the surgically placed implants.

Khoury et al. 2008, OBS USA	20(9/11) 52.6 CG: 54.2(13.0) AG:51.0(17.0)	20 13: Type 1: Astra Tech Dental Implant, Astra Tech, Mölndal, Sweden 7 Type 2: Zimmer Dental Implant, Zimmer Dental, Carlsbad, CA	7 d	PICF; GI; PD; PI	Maxillary and Mandibular Anterior and Posterior Sextants	CL	IL-1β; IL-8	Y	ELISA (Quantikine, R&D Systems, Minneapolis, MN)	PICF can be studied as early as 1 week following one-stage implant placement. Amoxicillin prophylaxis seemed to have a modest effect on the examined clinical parameters, although it did not seem to affect the observed changes in the investigated biomarkers
Güncü et al. 2008, RCT Turkey	10(8/2) 41.3(8.5) [27–56]	20 Ø4mm x 11.5mm (Brånemark System, TiUnite,MkIII)	0, 1, 3, 6, 9, 12m	PICF; ISQ; MBL	Bilateral mandibular first molar	IML= 10 CL= 10	Nitric Oxide	N	Extraction of nitrite into water (139 ml distilled water/sample); Staning with Griess reagent	NO metabolism around IML and CL implants may demonstrate almost a similar pattern that resulted with a decrease at the end of 12-month follow-up period.
Nowzari et al. 2008, LONG USA	7(5/2) [26-71]	19	3,6, 12 m	PICF; MBL; Microbial examination	NO INF	NO INF	HCMV; IL-1β; INF-γ. TNF-α;	N	RT-PCR	Pro-inflammatory cytokine production was unrelated to heavy bacterial challenge. When periodonto pathogenic bacteria were detected by culture, cytokine levels were increased.
Güncü et al. 2009, RCT Turkey	12(8/4) 40(8.47) [27-56]	24 Ø4 x 11.5mm (Branemark System, TiUnite, Mk III Nobel Biocare AB, Goteborg, Sweden).	1, 3, 6, 9, 12 m	PICF; GBTI; GI; PD; PI	Bilateral mandibular first molar	CL= 11 IML= 11	Nitric Oxide	N	Extraction of nitrite into water (100µl distilled water/sample) Staning with Griess reagent	Association of NO in the bone metabolism around dental implants and further suggest the impact of different loading regimens on NO metabolism.
Gruber et al. 2010, LONG Sweden	11(6/5)	11 implants	10 d: Baseline, d1-d10, >d10	PICF	NO INF	IML	IL-1β; Neutrophil elastase	N	IL-1: immunoassay (#DLB50; R&D Systems, Inc.,Minneapolis, MN); SyntheticsubstrateSuc-Ala-Ala-Pro-Val p-nitroaniline (Sigma, St. Louis, MO);	Activity of neutrophil elastase and concentration of IL-1β in the PICF did not change significantly between teeth before extraction, after replacement, and after immediate loading of the implants. Placement of implants according to the immediate loading protocol does not provoke an inflammatory reaction.
Boynuegri et al. 2012, RCT Turkey	10(4/6) 57.0(10.0)	40 Ø4.1 x 10 mm (SLA coating, Straumann, Waldenburg, Switzerland) GA: Standard Straumann®	Baseline, 3, 6, 9, 15 m	PICF; BOP; GI; PD; PI	Mandibular Overdendure Dolder bars and clip	CL	IL-1β; TNF-α	N	ELISA kit (Biosource, Invitrogen Corporation, Carlsbad, CA, USA)	After 12 months of evaluation, the results showed that moving microgap coronally from the alveolar crest would be related with less inflammatory markers to maintain the peri-implant health status, mainly in implant sites without esthetic priority.

		GB: 1 mm subcrestal of the polished surface of group A GC: esthetic plus Straumann® GD: subcrestal of the polished surface of group C							
Basegmez et al. 2012, LONG Turkey	28 42.24(8.79)	72 implants (Brånemark; Nobel Biocare, Westmont, IL)	**3,** 6, 12,18 m	PICF; GI; PD; PI	NO INF	CL	MMP-8; PGE2	N	PGE2 (Assay Designs, Inc, Ann Arbor, MI); MMP-8 (Quantikine; R&D Systems, Minneapolis, MN);	The detection of PGE2 and MMP-8 in PICF is useful for monitoring the course of peri-implant disease. MMP-8 promises to be an early signal of peri-implant inflammation.
Slotte et al. 2012, RCT Sweden	18 IML: 9 CL:9 Age NO INF	50 (Brånemark System MkIIITiUnite; Nobel Biocare, Gothenburg, Sweden)	2, 14, 28, 90 d	PICF; BOP; BQ; COMPL; ISQ; WHI	NO INF	IML CL	ALP; CK; IL-1β; OC; TNF-α; TRAP	Y	PCR	The genes ALP, TNF-α, and lL-1β, showed correlation with clinical findings, WHI, ISQ, and COMPL. Some gene expressions even predicted complications (TNF-a at 2 and 14d, ALP and CK at 14d).
Emecen-Huja et al. 2013, LONG USA	40 (22/18) 54.4(2) [21-74]	40 (Astra Tech, Moindal, Sweden; Straumann USA, LLC, Andover, MA, USA; Zimmer Dental, Carsbal, CA, USA)	1,2,3,6,8,12 w	PICF; GI; ISQ; PI;	Mandibular Posterior Sextant 26: molars 14: premolar	NO INF	Eotaxin; FGF-β; IL-10; IL-12; IL-1ra; IL-1β; IL-6; IL-7; IL-8; TNF-α; VEG; MCP-1; MIP-1β; MMP-8; MMP-9; TIMP-1; TIMP-2; TIMP-3; TIMP-4	N	Multiplex bead-based assay kits (human cytokine group I kit, BioplexTM Cytokine Assay, Bio-Rad Laboratories, Inc., and MMP/TIMP kit, R&D Laboratories LTD., Antrim, Northern Ireland)	The results suggested that peri-implant tissues, compared to periodontal tissues, represent a higher pro-inflammatory state.
Prati et al. 2013, RCT Brazil	40(26/14) 55.45 IML: 11/9 58.9 (4.7) CL:15/5 52.0(9.5)	205 (SIN, São Paulo ,Brazil)	Baseline, 7, 15, 30, 60, 90, 120 d	PICF; BOP; PD; MBL	Maxillary and Mandibular edentulous arch Brånemark full-arch prosthesis	IML: 105 30 maxilla 75 Mandible CL: 100 50 maxilla 60 Mandible	OC; OPG; OPN; PTH; TGFα	Y	LUMINEX/Magpix system (HBN1A-51K and HCCBP1MAG-58K, Millipore Corporation, Billerica, Massachusetts).	Immediate loading promotes a higher and accelerated release of bone mediators around implants when compared with non-loaded implants.

Tsoukaki et al. 2013, RCT Grece	20(11/9) 46.94[30-62] FS:5/5 46.40 (9.52) FG:6/4 47.47(9.72)	30 (Osseospeed; Astra Tech Dental, MoIndal, Sweden)	1, 2, 6, 12 w	PICF; GI; MBL; Pain scale–VAS; PD; PI	NO INF	NO INF	MMP-8; sRANKL,	Y	ELISA (Quantikine-Human Total MMP-8 Immunoassay; R&D Systems, Inc., Minneapolis, MN, USA)	Flapless implant placement yielded improved clinical, radiographic, and immunological outcomes compared with flapped implantation.
Nogueira-Filho et al. 2014, LONG Canada	21(12/9) 49.0(13.4) [29-79]	27	1, 2, 4, 6, 12 m	PICF; BOP; CAL; PD; PI; MBL	NO INF	CL	IFN-γ; IL-10; IL-1α; IL-4; IL-6; IL-8; IL-12p70; TGF-β1; TNF-α; VEGF	Y	Quantibody Custom Array, RayBiotech, Norcross, GA.	Peri-implant tissues exhibit a similar cytokine profile to healthy tissues around teeth. Levels of TGF-β1 were undetectable at 2m, reflecting ongoing inflammatory processes. Low levels of IL-8 and IL-1α at the 1y follow-up. Low levels of VEGF and increased levels of the TGF-β1 at the end of the follow-up may indicate clinical stability in peri-implant tissues.
Gokmenoglu et al. 2014, RCT Turkey	15(6/9) 48.15 CG: 2/6 45.87 (13.46) LED: 4/3 50.43 (9.25)	22 (Xive, Dentsply - Friadent, Mannheim, Germany) CG:12 // Ø3.8[3.5–4.5] x 11.0mm LED:10 // Ø4.5[3.8–4.5] x 11.0mm [10.6–11.0]	4 ,12 w	PICF; BOP; GI; ISQ; PD; PI;	NO INF	NO INF	IL-1β; Nitric Oxide; PGE2; TGF-β;	N	IL-1β: ELISA kit (Bendermed Systems, Diagnostics, Vienna, Austria); TGF-β1: Instant ELISA kit (Bendermed Systems, Diagnostics,Vienna, Austria); PGE2: EIA Kit Monoclonal (Cayman Chemical Company, Ann Arbor, MI); Colorimetric NO Assay Kit (Oxford Biomedical Research Inc. Oxford, MI)	LED application to surgical area has a positive effect on the osseointegration process, and implant stability were maintained. The changes in the biochemical parameters suggested that LED therapy has an effect on the tissues surrounding the implant positively affecting bone healing.
Dolanmaz et al. 2015, LONG Turkey	47(22/25) 47.34(10.11)	47 Group A1: 16 (Standard plus SLA) Group A2: 16 (Standard plus SLActive) Group B: 15 (SLA; Nucleoss, Izmir,Turkey)	1, 3 m	PICF; GI; PD; PI	Mandible 30 molar 17 premolar	NO INF	BMP-2; BMP-7; OPG; sRANKL;	Y	ELISA kit (HumanOsteoprotegerin ELISA; BioVendor, Brno, CzechRepublic)	Changes in OPG/sRANKL and BMP-2 and -7 during early osseointegration are short term and transient. Relationships between clinical and biochemical parameters showed that the levels of sRANKL, OPG, BMP-2 and BMP-7 reflects the degree of peri-implant inflammation, rather than differences in the implant surfaces.
Dogan et al. 2015, LONG Turkey	20(10/10) 52.84 CG=5/2	39 (SLActive Standard Plus, InstitutStraumann) CG=12	1, 4, 7 m	PICF; BOP; CAL;	NO INF	CL	IL-1β; TNF-α	Y	Orgenium Laboratories Oy/Ani Biotech Oy	Dental implant therapy can be offered to patients with well-controlled T2DM, as there were no significant differences between control

	52.14(3.93) T2DM= 5/8 53.54(4.01)	T2DM= 27		GI; ISQ; KGW; PD; PI; VBL						and diabetic patients in terms of clinical parameters or GCF and PICF cytokine levels.
Onuma et al. 2015, LONG Brazil	22(22/0) CG=11 61.81(5.26) OP=13 61.5(6.6)	88 Ø3.5 x 10 -11.5 -13 -15mm (Morse taper connection-Titanium Fix)	7, 120 d	PICF; BOP; CAL; GI; MBL; PD; PI; Success; SUP	Mandible 37-47	IML	sRANKL; OPG	Y	ELISA kit (BiomedicaMedizinprodukte)	Osteopenia does not influence the PICF levels of osteoclastogenesis-related factors in immediately loaded implants after 4 months of loading.
Mandić et al. 2015, RCT-Split-mouth Russia	12(6/5) 61.28[55-75]	40 Ø4mm x 10mm Self-tapping BlueSky® (Bredent, Germany) CG=20 TG (LLLT) = 20	7, 14, 21, 28 d	PICF; ISQ	Maxilla Posterior Bilaterally premolar and molar	CL	ALP	Y	Spectrophotometer at 405 nm (Secomam Basic, France)	Low-level laser therapy expressed no significant influence on the osseointegration of self-tapping implants placed into low density bone of the posterior maxilla. Self-tapping macro-designed implants into low density bone could be a predictable therapeutic procedure with a high early success rate.
De Wild et al. 2015, RCT Belgium	13(6/7) 58.8[45–72]	25 Ø 1.5 mm x 8 mm	1 w	PICF; Number of inflammatory cells; ROI	Maxillary and Mandibular Posterior sextants 5 maxilla premolar 16 maxilla molar 1 Mandible premolar 3 Mandible molar	CL	CCL-3; IL-8; IL-1β; IL-6; TGF-β2; MMP-8	N	PCR Measurement	HA-coated nano-surface do not provoke greater inflammation as compared to the turned cpTi surface. Surface modification in the nano-level has not changed the biocompatibility of the abutment.

Ghiraldini et al. 2016, CC Brazil	51(23/28) 54.29[37-70] CG= 9/10 51.58(7.74) BC= 7/9 54.91(13.95) PC= 7/9 56.38(13.69)	51 Ø3.75mm x 8.5-11.5mm (external-hexagon connections _ SIN, São Paulo, Brazil)	15 d, 3,6,12 m	PICF ISQ; FPG; HbA1c	Single Posterior Mandibular	CL	FGF; OC; OPG OPN; TGF-β	Y	LUMINEX Human plex (HBNMAG-51K and TGFBMAG-64K, Millipore Corporation, Billerica, MA, USA); Multiplexing instrument (MAGpix™, MiraiBio, Alameda, CA, USA).	The pattern of release of bone markers in peri-implant fluid is altered by glycemic control. Poor glycemic control negatively modulated the bone factors during healing, although T2DM, regardless of glycemic status, had no effect on implant stabilization.
PekerTekdal et al. 2016, RCT Split-mouth Turkey	14(10/4) 50(8.4) [31–64]	38 Ø4.1mm x 8-10-12mm (Biodenta, Bone Level Implant; Biodenta Swiss AG, Berneck, Switzerland)	2,4,8,12,24 w	PICF; EHI; GI; MBL; Pain scale - VAS; PD; PI;	Maxillary Posterior Sextant 22 premolar 16 molar	NO INF	sRANKL; OPG	Y	ELISA kit (Biovender R&D, Brno, CzechRepublic)	Piezoelectric surgery may modify and reduce bone-destructive inflammatory response during implant osseointegration. Detectible limits of RANKL and OPG at all time points indicated that RANKL-OPG system is one of the key bone remodeling mechanisms involved in the establishment of a biological connection between implant and bone.
Tirachaimongkol et al. 2016, LONG Thailand	10(7/3) 42.4(11.99) [28-64]	10 Ø5 x 10 mm (One PW Plus® implant (PW Plus, Nakhon Pathom, Thailand)	0 (post-surgical), 1, 2, 3, 4, 6, 8, 10, 12 w	PICF; ISQ	Mandibular Posterior Sextant 10: molars	CL	ALP OC	Y	ALP activity = colorimetric analysis; OC= ELISA kit (Human Osteocalcin Quantikine ELISA Kit, R&D Systems, Inc., Minneapolis, MN, USA)	ISQ values were weakly correlated with both ALP and OC. OC may be used as a biological marker for monitoring implant healing at 6, 8, 10, and 12 weeks after implant placement.
Elsyad et al. 2016, RCT Egypt	30(11/19) 62.6	60 Ø3.7 x 13 mm (tioLogic, Dentaurum, Germany)	2 w, 6 w, 12 m	PICF; BOP; HBL; GI; PD; PI; VBL	Mandibular Overdenture GI: Magnetic attachments GII: Locator attachments	IML	IL-1β	Y	ELISA (Oraflow Inc., New York, USA)	Locator attachments for IML implants retaining mandibular overdentures are associated with decreased PI, ISQ, IL-1β concentration and increased the VBL compared to magnetic attachments after 1 year. IL-1b increase after 1 year in both groups, could be attributed to the increased PI. IL-1b may be present in PICF even though BOP did not significantly increase over time.

Sayardoust et al., 2017 a RCT Sweden	32(15/17) 61.8(2.3)	96 implants (Smokers(48); Non-Smokers(NSm) 1-Machined (smooth) (Brånemark Integra- tion, Gothenburg, Sweden) 2- Oxidized (moderately rough) (Nobel Biocare, Gothenburg, Sweden) 3- Laser-modified (combination of smooth and moderately rough) (BioHelix; Brånemark Integration, Gothenburg, Sweden).	1, 7, 14, 28 d	PICF; GI; ISQ; Pain scale-VAS; PI	Maxilla and Mandible	CL	ALP; BMP-2; CatK; IL-6; IL-8; OC; TNF-α; VEGF	Y	QPCR (using TATAA SYBR GrandMaster Mix (TATAA Biocenter)	Sm showed an upregulated expression of OC and a later and lower peak of BMP-2 (at 7 days) compared to NSm. Surface-modified implants were associated with higher expression of ALP and CatK at 28 days in Nsm.
Sayardoust et al., 2017 b RCT Sweden	32(15/17) 61.8(2.3)	Same sample from Sayardoust et al., 2017a	Baseline, 60, 90 d	PICF; Bone biopsy of implant site; BOP; GI; ISQ; MBL; Pain scale - VAS PI	Maxilla and Mandible	CL	ALP; BMP-2; CatK; CXCR4; HIF-1α; IL-6; IL-8; OC; RANK; RANK-L; OPG; TGF-β; TNF-α; VEGF	Y	QPCR (using TATAA SYBR GrandMaster Mix (TATAA Biocenter)	The HIF-1a at baseline expression in the bone site and IL-6 expression in PICF are important molecular determinants for MBL after 90 days. Smoking had an early effect on osseointegration, which was dependent on the implant surface properties and the local host response.
Verrastro et al., 2017 RCT Brazil	18 (11/7) 58.06(7.42) [39–65]	36 cylindrical dental implants with external hexagon connections (diameter 4.1 mm) (Implacil de Bortoli, São Paulo, Brazil)	7,14,30,120 d	PICF; IT	Mandible Branemark full-arch prostheses	IL	BMP-9; Periostin; OPG; PLGF; TRAP; VEGF	Y	Multiplex immunoassay instrument (MAGpix; MiraiBio, Alameda, CA, USA) Kits: HBNMAG–51 K, HRNKLMAG-31K-01, and HAGP1MAG–12 K; Millipore Corporation, Billerica, MA, USA)	Different levels of torque for implants insertion of may modulate the release of angiogenesis- and bone-related markers. Low IT in IML protocols increased VEGF and OPG levels during the first month. High levels of BMP-9 and Periostin were higher on day 120 in the conventional IT group followed by TRAP up-regulation.

Bielemann et al., 2018 LONG Brazil	30 (20/10) 67.3 [49 - 89]	60 implants Narrow Diameter Implants (Facility Neodent - 2.9 X10 mm)	1, 2, 4, 8, 12 w	PICF; BOP; Calculus; GI; ISQ; PD; PI	Mandible Overdenture	CL	IL-1β; IL-6; IL-10; TNF-α	Y	micro-ELISA reader (Ultramark, Bio-Rad, CA, USA)	Not all evaluated cytokines showed potential to be markers for peri-implant monitoring health. IL-10 seemed to be the most promising to diagnose imbalances or failures. Bone type, smoking habit, and atrophy all affected the cytokines release in different stages of mandible healing.

Legend: (C: Case-Controlled; LONG: Longitudinal; OBS: Observational; RCT: Randomized Controlled Trial; d: Days; y: Years; m: Months; CG: Control Group/Health Group; FS: Flapped Surgery; FG: Flapless Group; AG: Antibiotic Group; LED: Light-Emitting Diode Photomodulation; T2DM: Glycemic-Controlled Type 2 Diabetes Mellitus; BC:T2DM Better Control; PC: T2DM Poor Control; OP: Osteopenia; BOP: Bleeding On Probing; BQ: Bone Quality; CAL: Clinical Attachment Level; COMPL: Implant Complications; EHI: Early Healing Index; FPG: Fasting Plasma Glucose; GI: Gingival Index; GTBI: Gingival Bleeding Time Index; Hba1c: Glycated Hemoglobin Levels; HBL: Horizontal Bone Loss; ISQ: Implant Quotient Stability; IT: Insertion Torque; KGW: Keratinized Gingival Width; LLLT: Low-Level Laser Therapy; MBL: Marginal Bone Level; PD: Probing On Depth; PI: Plaque Index; PICF: Peri-Implant Crevicular Fluid; SUP: Suppuration; VBL: Vertical Bone Loss; WHI: Wound Healing Index; ROI : region of interest (zone of the connective tissue lateral to the neck at the implant-soft tissue interface and zone located away from the implant interface); CL: Conventional Loading; IML: Immediate Loading; ELISA: Enzyme-Linked Immunosorbent Assay; PCR, Polymerase Chain Reaction)

Figure. 1. Flowchart of screened process during literature search.

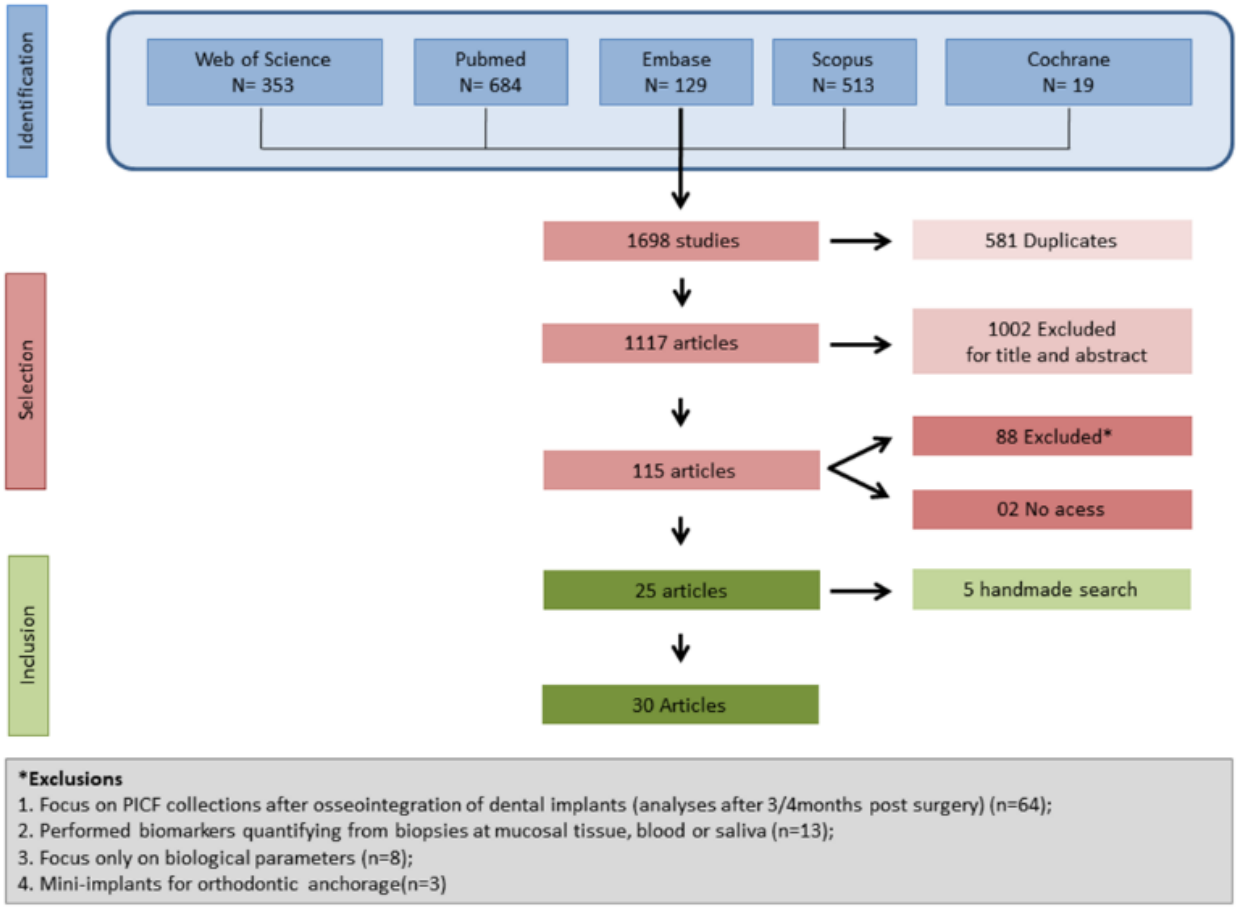
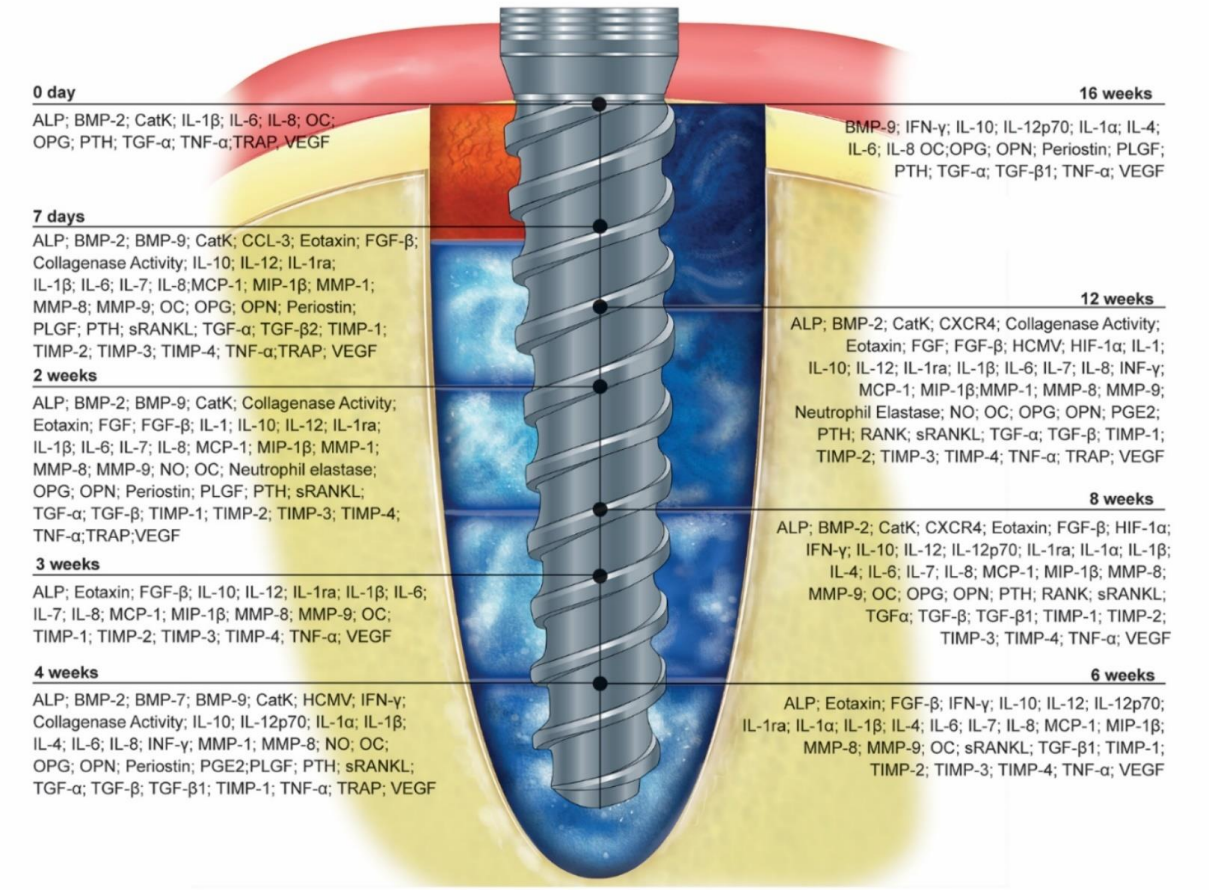
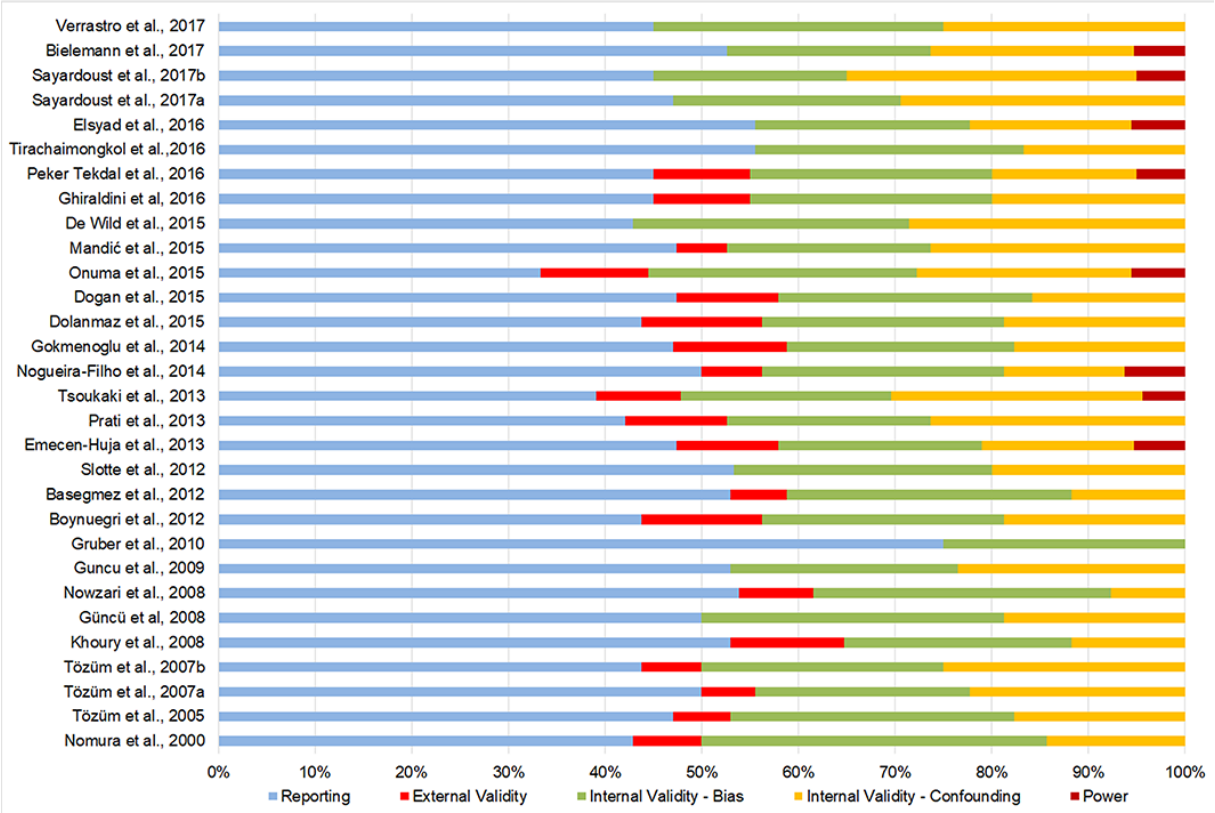


Figure. 2. Time line illustration of the cytokines releasing already described according to selected studies.



During the first 24 hours before implant insertion, the threads of the implant were filled with blood clot, containing neutrophil, macrophages, cytokines and growth factors. This first stage of bone healing, known as osteoinduction, is characterized by mesenchymal cell differentiation into pre-osteoblasts, which initiate osteogenesis (Albrektsson & Johansson, 2001) by synthesizing extracellular bone matrix (Raghavendra, Wood, & Taylor, 2005). Until the 7th day, only soft tissue and a primitive matrix composed of varying amounts of old bone debris remains (Lang et al., 2011). At 14 days, bone implant contact (BIC) is increased 6% to 14.8%, and bone formation partially extends along the old bone trabeculae to the implant surface in transitional regions from compact bone to soft tissue interfaces. At this stage, the osteoid matrix is surrounded by osteoblasts that assist in trabecular formation (Lang et al., 2011). At 30 days, BIC has reached nearly 30% of the implant surface, with large surface areas covered by newly formed bone; the process of bone apposition and deposition is directed towards greater activity (Lang et al., 2011). After 6 weeks, the implant surface reaches 60% of BIC, and advanced bone maturation is evident, as primary osteocyte formation is visible further from the implant surface and old bone remodeling occasionally leads to secondary osteocyte formation (Lang et al., 2011). At 12 -16 weeks, is the phase of osseointegration with continuous bone matrix deposition ends, and a new phase of bone remodeling begins (Raghavendra et al., 2005).

Figure. 3. Histograms presenting the frequency distribution (%) of the scores assessed for five domains (reporting, external validity, bias, confounding, and power) by the quality bias measurement of the studies according to Downs and Black.



Appendix A. Supplementary data

Table S1. Search strategy in PubMed (MedLine).

Pub Med: (#1 AND #2 AND #3)
#1: Search (("Dental Implants"[Mesh] OR "Dental Implants" OR "Implants, Dental" OR "Dental Implant" OR "Implant, Dental" OR "Dental Prostheses, Surgical" OR "Dental Prosthesis, Surgical" OR "Surgical Dental Prostheses" OR "Surgical Dental Prosthesis" OR "Prostheses, Surgical Dental" OR "Prosthesis, Surgical Dental" OR "Dental Implantation, Endosseous"[Mesh] OR "Dental Implantation, Endosseous" OR "Endosseous Dental Implantation" OR "Implantation, Endosseous Dental" OR "Osseointegrated Dental Implantation" OR "Implantation, Osseointegrated Dental" OR "Dental Implantation, Osseointegrated" OR "Implantation, Endosseous" OR "Endosseous Implantation" OR "Dental Implantation"[Mesh] OR "Dental Implantation" OR "Dental Prosthesis Implantation" OR "Prosthesis Implantation, Dental" OR "Implantation, Dental" OR "Implantation, Dental Prosthesis" OR "Dental Prosthesis Implantations" OR "Implantations, Dental Prosthesis" OR "Prosthesis Implantations, Dental" OR "Dental Implantation, Subperiosteal"[Mesh] OR "Dental Implantation, Subperiosteal" OR "Subperiosteal Dental Implantation" OR "Subperiosteal Implantation" OR "Implantation, Subperiosteal" OR "Implantation, Subperiosteal Dental")
#2: ("Osseointegration"[Mesh] OR "Osseointegration" OR "Osteogenesis"[Mesh] OR "Osteogenesis" OR "Bone Formation" OR "Physiologic Ossification" OR "Ossification, Physiologic" OR "Ossification, Physiological" OR "Physiological Ossification" OR "Bone Resorption"[Mesh] OR "Bone Resorption" OR "Bone Resorptions" OR "Resorption, Bone" OR "Resorptions, Bone" OR "Osteoclastic Bone Loss" OR "Bone Loss, Osteoclastic" OR "Bone Losses, Osteoclastic" OR "Loss, Osteoclastic Bone" OR "Losses, Osteoclastic Bone" OR "Osteoclastic Bone Losses"))
#3: (("Macrophage Inflammatory Proteins"[Mesh] OR "Macrophage Inflammatory Proteins" OR "Inflammatory Proteins, Macrophage" OR "Macrophage Inflammatory Protein-1" OR "Anti-Inflammatory Agents"[Mesh] OR "Anti-Inflammatory Agents" OR "Anti Inflammatory Agents" OR "Agents, Antiinflammatory" OR "Antiinflammatories" OR "Antiinflammatory Agents" OR "Agents, Anti-Inflammatory" OR "Agents, Anti Inflammatory" OR "Anti-Inflammatories" OR "Anti Inflammatories" OR "Biological Markers"[Mesh] OR "Biological Markers" OR "Markers, Biological" OR "Biomarkers" OR "Marker, Biological" OR "Biological Marker" OR "Biologic Marker" OR "Marker, Biologic" OR "Biologic Markers" OR "Markers, Biologic" OR "Markers, Clinical" OR "Clinical Markers" OR "Marker, Clinical" OR "Clinical Marker" OR "Markers, Immunologic" OR "Marker, Immunologic" OR "Immune Markers" OR "Markers, Immune" OR "Immunologic Markers" OR "Immunologic Marker" OR "Immune Marker" OR "Marker, Immune" OR "Serum Markers" OR "Markers, Serum" OR "Serum Marker" OR "Marker, Serum" OR "Surrogate Markers" OR "Markers, Surrogate" OR "Surrogate Marker" OR "Marker, Surrogate" OR "Biochemical Marker" OR "Marker, Biochemical" OR "Markers, Biochemical" OR "Biochemical Markers" OR "Cytokines"[Mesh] OR "Cytokines" OR "Interleukins"[Mesh] OR "Interleukins" OR "Metalloproteases"[Mesh] OR "Metalloproteases" OR "Metalloproteinases" OR "Metallopeptidases" AND "Alkaline Phosphatase"[Mesh] OR "Alkaline Phosphatase" OR "Prostaglandins"[Mesh] OR "Prostaglandins" OR "Prostanoids" OR "Cathepsins"[Mesh] OR "Cathepsins" OR "Cathepsin" OR "Cathepsin K"[Mesh] OR "Cathepsin K" OR "Osteoprotegerin"[Mesh] OR "Osteoprotegerin" OR "Osteoclastogenesis Inhibitory Factor" OR "Receptors, Tumor Necrosis Factor, Member 11b" OR "Tumor Necrosis Factor Receptor Superfamily, Member 11b" OR "FDCR-1 Protein" OR "FDCR 1 Protein" OR "OCIF Protein" OR "Tumor Necrosis Factor Receptor 11b" OR "Tumor Necrosis Factor"[Mesh] OR "Tumor Necrosis Factor" OR "Necrosis Factors, Tumor" OR "TNF Receptor Ligands" OR "Receptor Ligands, TNF" OR "Tumor Necrosis Factor Superfamily Ligands" OR "Tumor Necrosis Factor-alpha"[Mesh] OR "Tumor Necrosis Factor-alpha" OR "Transforming Growth Factor beta"[Mesh] OR "Transforming Growth Factor beta" OR "Milk Growth Factor" OR "Factor, Milk Growth" OR "Growth Factor, Milk" OR "TGF-beta" OR "TGFbeta" OR "Platelet Transforming Growth Factor" OR "Bone-Derived Transforming Growth Factor" OR "Bone Derived Transforming Growth Factor" OR "Receptor Activator of Nuclear Factor-kappa B"[Mesh] OR "Receptor Activator of Nuclear Factor-kappa B" OR "Receptor Activator of Nuclear Factor kappa B" OR "NF-KappaB Receptor Activator" OR "Activator, NF-KappaB Receptor" OR "NF KappaB Receptor Activator" OR "Receptor Activator, NF-KappaB" OR "RANK Protein" OR "Receptor Activator of NF-kappa B" OR "Receptor Activator of NF kappa B" OR "Tumor Necrosis Factor Receptor Superfamily, Member 11a" OR "Receptor Activator of Nuclear Factor-kappaB" OR "Receptor Activator of Nuclear Factor kappaB" OR "Receptors, Tumor Necrosis Factor, Member 11a" OR "TNFRSF11A Protein" OR "TRANCE Receptor" OR "TRANCE-R" OR "TRANCE R" OR "NF-Kappa B Receptor Activator" OR "NF Kappa B Receptor Activator" OR "Receptor Activator of NF-kappaB" OR "Receptor Activator of NF kappaB" OR "Chemokines"[Mesh] OR "Chemokines" OR "Cytokines, Chemotactic" OR "Intercrines" OR "Chemotactic Cytokines" OR "Macrophage-Activating Factors"[Mesh] OR "Macrophage-Activating Factors" OR "Factors, Macrophage-Activating" OR "Macrophage Activating Factors" OR "Matrix Metalloproteinases"[Mesh] OR "Matrix Metalloproteinases" OR "Metalloproteinases, Matrix" OR "MMPs") OR ("Interleukin-1"[Mesh] OR "Interleukin-1" OR "Interleukin 1" OR "IL-1" OR "T Helper Factor" OR "Lymphocyte-Activating Factor" OR "Lymphocyte Activating Factor" OR "Macrophage Cell Factor" OR "Interleukin I" OR "Interleukin 1 Receptor Antagonist Protein"[Mesh] OR "Interleukin 1 Receptor Antagonist Protein" OR "Interleukin-1beta"[Mesh] OR "Interleukin-1beta" OR "Interleukin 1beta" OR "Interleukin-1 beta" OR "Interleukin 1 beta" OR "Catabolin" OR "IL-1 beta" OR "Interleukin-10"[Mesh] OR "Interleukin-10" OR "Interleukin 10" OR "IL10" OR "IL-10" OR "CSIF-10" OR "Cytokine Synthesis Inhibitory

Factor" OR "Interleukin-12"[Mesh] OR "Interleukin-12" OR "Edodekin Alfa" OR "IL 12" OR "Natural Killer Cell Stimulatory Factor" OR "IL-12 p70" OR "IL12" OR "Interleukin 12" OR "Interleukin-12 p70" OR "Interleukin 12 p70" OR "p70, Interleukin-12" OR "Cytotoxic Lymphocyte Maturation Factor" OR "IL-12" OR "Interleukin-6"[Mesh] OR "Interleukin-6" OR "Interleukin 6" OR "Plasmacytoma Growth Factor" OR "Growth Factor, Plasmacytoma" OR "B-Cell Differentiation Factor-2" OR "B-Cell Differentiation Factor 2" OR "B-Cell Stimulatory Factor 2" OR "B-Cell Stimulatory Factor-2" OR "BSF-2" OR "Differentiation Factor, B-Cell" OR "Differentiation Factor, B Cell" OR "Differentiation Factor-2, B-Cell" OR "Differentiation Factor 2, B Cell" OR "Hybridoma Growth Factor" OR "Growth Factor, Hybridoma" OR "IFN-beta 2" OR "IL-6" OR "IL6" OR "B Cell Stimulatory Factor-2" OR "B Cell Stimulatory Factor 2" OR "B-Cell Differentiation Factor" OR "B Cell Differentiation Factor" OR "Interleukin-8"[Mesh] OR "Interleukin-8" OR "Interleukin 8" OR "IL8" OR "Monocyte-Derived Neutrophil Chemotactic Factor" OR "Neutrophil Activation Factor" OR "Neutrophil-Activating Peptide, Lymphocyte-Derived" OR "Lymphocyte-Derived Neutrophil-Activating Peptide" OR "Neutrophil Activating Peptide, Lymphocyte Derived" OR "Neutrophil-Activating Peptide, Monocyte-Derived" OR "Monocyte-Derived Neutrophil-Activating Peptide" OR "Neutrophil Activating Peptide, Monocyte Derived" OR "Alveolar Macrophage Chemotactic Factor-I" OR "Alveolar Macrophage Chemotactic Factor I" OR "Granulocyte Chemotactic Peptide-Interleukin-8" OR "Chemotactic Peptide-Interleukin-8, Granulocyte" OR "Granulocyte Chemotactic Peptide Interleukin 8" OR "Anionic Neutrophil-Activating Peptide" OR "Anionic Neutrophil Activating Peptide" OR "Neutrophil-Activating Peptide, Anionic" OR "Peptide, Anionic Neutrophil-Activating" OR "Chemokine CXCL8" OR "CXCL8, Chemokine" OR "Chemokines, CXCL8" OR "CXCL8 Chemokines" OR "Chemotactic Factor, Macrophage-Derived" OR "Chemotactic Factor, Macrophage Derived" OR "Macrophage-Derived Chemotactic Factor" OR "Chemotactic Factor, Neutrophil" OR "Neutrophil Chemotactic Factor" OR "Chemotactic Factor, Neutrophil, Monocyte-Derived" OR "CXCL8 Chemokine" OR "Chemokine, CXCL8" OR "IL-8" OR "AMCF-I" OR "Interleukin-23"[Mesh] OR "Interleukin-23" OR "Interleukin 23" OR "IL-23" OR "Matrix Metalloproteinase 3"[Mesh] OR "Matrix Metalloproteinase 3" OR "Metalloproteinase 3, Matrix" OR "MMP-3 Metalloproteinase" OR "MMP 3 Metalloproteinase" OR "Metalloproteinase, MMP-3" OR "MMP3 Metalloproteinase" OR "Metalloproteinase, MMP3" OR "Matrix Metalloproteinase 1"[Mesh] OR "Matrix Metalloproteinase 1" OR "Metalloproteinase 1, Matrix" OR "Matrix Metalloproteinase-1" OR "MMP1 Metalloproteinase" OR "Metalloproteinase, MMP1" OR "MMP-1 Metalloproteinase" OR "MMP 1 Metalloproteinase" OR "Metalloproteinase, MMP-1" OR "Pro-Matrix Metalloproteinase-1" OR "Metalloproteinase-1, Pro-Matrix" OR "Pro Matrix Metalloproteinase 1" OR "proMMP-1" OR "Promatrixmetalloproteinase-1" OR "Promatrixmetalloproteinase 1") OR ("Matrix Metalloproteinase 13"[Mesh] OR "Matrix Metalloproteinase 13" OR "Metalloproteinase 13, Matrix" OR "MMP13 Metalloproteinase" OR "Metalloproteinase, MMP13" OR "Matrix Metalloproteinase-13" OR "Metalloproteinase-13, Matrix" OR "MMP-13 Metalloproteinase" OR "MMP 13 Metalloproteinase" OR "Metalloproteinase, MMP-13" OR "Collagenase 3" OR "Collagenase-3" OR "Matrix Metalloproteinase 12"[Mesh] OR "Matrix Metalloproteinase 12" OR "Metalloproteinase 12, Matrix" OR "MMP12 Metalloproteinase" OR "Metalloproteinase, MMP12" OR "Metalloproteinase Elastase" OR "Elastase, Metalloproteinase" OR "MMP-12 Metalloproteinase" OR "MMP 12 Metalloproteinase" OR "Metalloproteinase, MMP-12" OR "Macrophage Metalloelastase" OR "Metalloelastase, Macrophage" OR "Macrophage-Specific Metalloelastase" OR "Macrophage Specific Metalloelastase" OR "Metalloelastase, Macrophage-Specific" OR "Matrix Metalloproteinase 8"[Mesh] OR "Matrix Metalloproteinase 8" OR "Metalloproteinase 8, Matrix" OR "Fibroblast Collagenase" OR "Collagenase, Fibroblast" OR "Matrix Metalloproteinase-8" OR "Metalloproteinase-8, Matrix" OR "MMP8 Metalloproteinase" OR "Metalloproteinase, MMP8" OR "Neutrophil Collagenase" OR "Collagenase, Neutrophil" OR "Collagenase-2" OR "Collagenase 2" OR "MMP-8 Metalloproteinase" OR "MMP 8 Metalloproteinase" OR "Metalloproteinase, MMP-8" OR "Leukocyte Elastase"[Mesh] OR "Leukocyte Elastase" OR "Elastase, Leukocyte" OR "Polymorphonuclear Leukocyte Elastase" OR "Elastase, Polymorphonuclear Leukocyte" OR "Leukocyte Elastase, Polymorphonuclear" OR "Neutrophil Elastase" OR "Elastase, Neutrophil" OR "PMN Elastase" OR "Elastase, PMN" OR "Granulocyte Elastase" OR "Elastase, Granulocyte" OR "Lysosomal Elastase" OR "Elastase, Lysosomal"))

Table S2. Biomarkers data reported in early stages of healing (0, at day of implant installation till 4 weeks) (n=26 studies).

Abbreviations: CL, conventional loading; IL, immediate loading; CU, Concentration Unit; CA, Collagenase activity; NL, Nitrite Level; NC, Nitrite Concentration, CG, control group; AbG, Antibiotic Group; LED, Light-Emitting Diode Photomodulation;, T2DM, Glycemic-Controlled Type 2 Diabetes Mellitus; OP, Osteopenia; FP, Fapped surgery; FS, Flapless surgery; HA, HA- coated nano-surface; S, Smooth surface; R, rough surface; PS, piezosurgery; D, drilling; BC,T2DM Better control; PC, T2DM Poor Control; Ma, Machined; Ox, Oxidized; La, Laser modified; RT, Reduced Torque; CT, Conventional Torque.

Study/ Groups/ CU	Biomarkers	0	1 day	2 days	7 days	10 days	2 weeks	3weeks	4 weeks	6 weeks
Nomura et al., 2005	TIMP-1				30.83(18.19)		15.43(13.07)		0.62(0.84)	
- ng/sample	MMP-1				2.19(3.21)		.		.	
- X 10^{-1} units/sample	MMP-8				2.93(2.60)		1.96(1.45)		0.93(0.75)	
	TIMP-1/MMP-1¹MMP-8				8.80(8.72)		45.91(114.77)		25.30(71.69)	
	CA, Active				4.2(2.1)		2.0(1.0)		2.8(2.0)	
	CA, APMA-activatable				6.1(2.4)		3.4(1.2)		3.8(1.9)	
Tozum et al. 2005*	Total NL: H								0.1486/0.1411	
- CL/IL (nmol)	Total NL: I								0.1474/0.1576	
Tozun et al., 2007a	Total NL								0.1408(0.034)/0.1578(0.053)	
- CL/IL; All impants (AI)	NC								0.4136(0.226)/0.3711(0.225)	
- nmol; nmol/µl	Total NL: AI								0.1478(0.046)	
	NC: AI								0.3980(0.2267)	
Tozun et al., 2007b	Volume						0.594(0.305)/ 0.727(0.505)		0.435(0.194)/0.503(0.283)	
- CL/IL										
- µl; nmol	Total NL						0.149(0.026)/ 0.145(0.04)		0.147(0.034)/0.161(0.055)	
Khoury et al., 2008	IL-1β	297.0(41-667/ 278.0(87-681)			428(149-1,767)/ 446(213 - 882)					
- CG/AbG (pg/ml)⁺	IL-8	2,2368.0(1,624-5,024)/ 1,079.0(248-2,960)			4,674(1,952-15,668)/ 3,400(695-9,040)					
Güncü et al., 2008	Volume								0.258(0.120)/0.368(0.310)	
- CL/IL										
- µl; nmol	Total NL								0.121(0.013)/0.129(0.036)	

Güncü et al., 2009	Total NL							0.118(0.014)/0.122(0.034)		
- CL/IL										
- nmol; nmol/µl	NC							0.611(0.471)/0.467(0.239)		
Gruber et al., 2010[+]	Neutophil elastase	256.7(302.7)			124.26(230.3)	201.6 (242.5)				
- U/µl	IL-1β	9.9(13.5)			5.4(6.5)	8.4(9.6)				
	IL-1β - S		1272.5(417.2)/107.2(105.7)			477.4(176.9)/225.5(225.2)		659.3(226.3)/1140.1(1041.7)		
	IL-1β R		976.6(427.3)/314.9(287.8)			438.1(168.7)/1341.1(1341.0)		438.9(151.5)/251.8(126.7)		
	TNF-α - S		87.0(43.3)/59.4(29.4)			11.9(7.3)/12.5(4.2)		13.7(5.8)/17.7(11.9)		
	TNF-α - R		49.9(30.7)/99.4(56.8)			20.2(7.5)/67.5(40.9)		13.2(4.8)/11.6(7.8)		
Slotte et al., 2012	OC - S		3.7(1.4)/1.5(0.4)			1.1(0.3)/1.0(0.2)		3.5(1.0)/1.9(0.5)		
- CL/IL (S, smooth; R, rough)	OC - R		1.7(1.4)/2.6(1.2)			9.2(2.9)/0.8(0.3)		3.1(0.9)/1.5(0.6)		
- Mean (SE)	ALP - S		14.5(4.3)/22.7(0.8)			7.1(1.4)/20.0(11.5)		22.3(15.8)/11.3(9.7)		
-Relative gene expression	ALP - R		13.5(3.4)/32.8(15.0)			15.9(4.9)/7.0(3.5)		8.8(3.1)/13.0(10.4)		
	CatK - S		3.6(1.3)/2.1(0.4)			20.1(17.7)/11.9(10.3)		2.1(0.8)/36.0(34.5)		
	CatK - R		2.1(0.2)/2.2(0.8)			16.9(12.2)/0.6(0.2)		5.3(2.3)/1.3(0.5)		
	TRAP - S		1.6(0.7)/1.5(0.7)			1.9(0.5)/6.5(0.4)		4.9(3.0)/11.4(9.0)		
	TRAP - R		3.1(1.0)/2.6(1.0)			15.8(8.0)/3.1(0.8)		2.6(0.6)/1.7(0.3)		
	IL-1β			13.0(3.0)		8.0(1.0)	12.0(2.0)		11.0(2.0)	
	IL-6			28.0(6.0)		9.0(3.0)	1(0.2)		1.0(0.3)	
Emecen-Huja et al., 2013	IL-8			168.0(36.0)		58.0(10.0)	48.0(7.0)		46.0(8.0)	
- ng/ml	MIP-1β			9(2.0)		7.0(1.0)	6.0(1.0)		6.0(1.0)	
	VEGF			25.0(3.0)		24.0(3.0)	37.0(5.0)		42.0(6.0)	
	TGF-α	1.0(2.2)/0.2(0.2)		2.8(5.1)/4.9(1.6)		5.4(6.5)/73.0(78.4)		16.7(19.6)/45.0(21.4)		
Prati et al., 2013	OPN	3.9(0.9)/3.2(1.2)		7.9(2.1)/ 12.7(4.0)		11.8(3.8)/102.4(24.7)		34.1(4.5)/192.9(48.0)		
- CL/IL	OC	46.7(26.8)/55.5(78.5)		15.8(5.1)/ 24.6(11.8)		24.0(4.1)/343.0(300.7)		89.9(17.5)/411.5(325.9)		
- pg/ml	PTH	4.2(1.7)/3.0(0.9)		11.4(5.4)/ 16.3(4.7)		14.9(6.0)/132.0(45.6)		54.3(13.0)/157.7(36.6)		
	OPG	2.4(0.5)/6.3(2.4)		10.5(2.8)/25.5(4.1)		19.5(2.2)/355.5(50.1)		118.9(18.7)/287.6(53.2)		
Tsoukaki et al., 2013	MMP-8			5.35(0.49) /3.23(0.49)					5.70(0.49) / 3.76 (0.49)	
- FP (Flapped)/FL (Flapless)										
- ng/site (Mean,SE)	s-RANKL					0.05(0.15)/0.13(0.15)				

Nogueira-Filho et al., 2014* - pg/µl	IL-1α				9.79(2.12-16.91)
	IL-8				7.04(0.38-73.42)
	TGF-β1				0
	VEGF				79.8(10.65- 101.78)
Gokmenoglu et al., 2014 - CG/LED - pg/mL	IL-1β				2.9(1.4–9.3)/1.8(0.9–3.9)
	TGF				1025.8(465.8–2649.1)/ 635.3(296.6–1139.6)
	PGE2				2408.3(1259.1–5474.7)/ 1213.1(655.1–2461.8)
	NO				127.3(38.8–280.1)/ 44.1 (19.1–119.5)
Dolanmaz et al., 2015* - A1 (SLA)/ A2 (SLActive)/ B (SLA) - pg/µl	OPG				1393.44 /1196.72/262.29
	sRANKL				160.65/124.59/31.14
	BMP-2				377.57/302.8/63.55
	BMP-7				20.21/10.63/4.04
Dogan et al., 2015* - CG/T2DM - ng/ml	IL-1β				14.15/14.15
	TNF-α				1.91/0.7
Onuma et al., 2015 - CG/OP - pg/mL	RANKL	43.67(24.15)/ 41.02(52.66)			
	OPG	0/ 0.94(2.92)			
Mandić et al. 2015 - CG/LG -U/Sample	ALP	18.16(5.11)/ 21.53(6.65)	10.39(4.05)/1.26(4.64)	10.22(4.26)/9.36(4.23)	8.45(3.46)/1.96(8.34)
De Wild et al., 2015 - HA/S - Fold Induction; Fold Difference (HA/S)	IL-6	0.544/1.837 0.296			
	CCL3	0.825/1.212; 0.681			
	TGF-β2	1.222/0.740; 1.652			
	IL-1β	1.338/0.769; 1.739			
	MMP-8	0.870/1.169; 0.744			
	IL-8	1.609/0.652; 2.470			
Ghiraldini et al, 2015	OPG		27.8(31.1)/24.7(23.5)/18.8(14.8)		

- CG/BC/PC	OC			150.81(169.7)/92.1(86.7)/167.4(125.4)		
- pg/µl	OPN			159.3(220.3)/121.5(126.3)/167.4(176.2)		
	FGF			45.3(70.9)/20.3(16.8)/26.22(26.3)		
	TGF-β			20.6(21.6)/17.6(11.6)/29.0(23.0)		
Peker Tekdal et al., 2015	RANKL			60.87(54.9)/46.22(44.2)		56.03(67.9)/46.22(30.8)
- PS/Drilling	OPG			9.46(9.4)/5.37(3.9)		13.49(9.4)/11.15(14.3)
- pg/µl, Mean(SD)	RANKL/OPG			2.25(2.8)/2.68(2.1)		0.93(0.8)/1.72(1.5)
Tirachaimongkol et al, 2016	ALP (nM/µg protein)	230.0(238.0)	139.0(139.0)	157.0(293.0)	108.0(134.0)	166.0(434.0)
- Median (interquartile range)	OC (pg/µg protein)	7.0(15.0)	16.0(69.0)	15.0(81.0)	68.0(46.0)	37.0(79.0)
Elsyad et al., 2016						
- Magneto/Locator	IL-1β			161.32(73.86) /		
- pg/µl (Mean, SD)				164.01(87.20)		
Sayardoust et al., 2017a* - Ma/Ox/La - Relative gene expression	IL-6 - Non-smoker	0.59/0.63/0.85	0.19/0.34/0.30	0.08/0.10/0.07		0.20/0.20/0.06
	IL-6 - Smoker	0.71/0.91/0.52	0.28/0.16/0.19	0.32/0.36/0.04		0.06/0.24/0.00
	IL-8 - Non-smoker	1.00/0.86/0.81	0.44/0.40/0.49	0.15/0.25/0.15		0.05/0.07/0.11
	IL-8 - Smoker	1.23/0.95/0.91	0.46/0.74/0.59	0.09/0.16/0.21		0.06/0.06/0.04
	TNF-α- Non-smoker	0.44/0.40/0.49	0.55/0.53/0.47	0.24/0.23/0.22		0.12/0.10/0.20
	TNF-α- Smoker	0.46/0.74/0.59	0.42/0.38/0.39	0.22/0.12/0.19		0.19/0.13/0.08
	ALP- Non-smoker	1.04/0.98/1.15	1.07/1.02/0.82	0.25/0.58/0.44		0.30/2.24/2.36
	ALP - Smoker	1.07/0.98/1.12	0.74/0.66/1.15	0.61/0.48/0.98		0.68/0.75/0.28
	OC- Non-smoker	1.02/0.77/0.91	1.15/1.76/0.91	0.36/0.580.47		0.69/0.33/0.60
	OC - Smoker	3.65/5.16/2.42	4.01/4.04/3.87	4.95/2.80/2.55		2.36/1.76/0.52
	CatK- Non-smoker	0.98/1.48/0.90	12.05/2.21/1.80	9.18/6.15/5.00		4.18/13.61/20.49
	CatK - Smoker	0.98/1.56/0.98	4.43/1.39/3.11	10.82/11.07/16.23		8.44/3.20/7.21
	BMP-2- Non-smoker	1.07/0.52/0.41	6.46/2.34/3.49	4.89/2.36/3.90		5.14/5.19/3.57
	BMP-2 - Smoker	0.41/0.91/0.33	1.79/0.71/1.24	5.49/2.61/3.57		3.93/2.20/5.38
	VEGF- Non-smoker	1.01/0.99/1.20	0.46/0.47/0.60	0.16/0.18/0.18		0.12/0.13/0.12
	VEGF - Smoker	1.00/1.64/1.66	0.48/0.44/0.63	0.16/0.21/0.33		0.15/0.10/0.13
Bielemann et al., 2017	IL-1β		33.3(0.0-598.0)	21.7(3.6-522.8)		21.4(2.8-150.0)

- pg/µl (Median, range)	IL-6	141.5(0.0-931.6)	102.6(0.0-573.2)	75.7(0.0-325.7)
	TNF-α	26,2 (0.0-120.9)	35.7(0.0-116.0)	37.7(0.0-120.9)
	IL-10	61.0(0.0-562.8)	73.3(0.0-881.7)	144.9(0.0-973.1)
	VEGF- RT/CT	185,365.85/24,390.24	178,048.78/89,024.39	256,097.56/125,609.76
Verrastro Neto et al. 2017*	BMP-9 - RC/CT	0.01/153,658.54	0.01/156,097.56	0.01/214,634.15
- RT/CT	OPG -RC/CT	0.19/0.01	0.21/0.01	0.178/0.01
	TRAP - RC/CT	0.04/ 0.30	0.14/0.44	0.10/0.32
	Periostin - RC/CT	0.05/0.05	0.15/0.17	0.10/0.06
	PLGF - RC/CT	275,000.0/401,639.3	275,000.0/336,065.5	400,000.0/475,409.8

*Data presented graphically were re-measured based on the calibrated distances of the figures using the software Adobe Photoshop version 6.0.1 (Adobe Systems).

[+]The Baseline values in the Khoury et al. (2008) and Gruber et al. (2010) studies are related to GCF collection from teeth.

Table S3. Biomarker data reported in late stages of healing and during the first year (n=25).

Abbreviations: CL, conventional loading; IL, immediate loading; CU, Concentration Unit; CA, Collagenase activity; NL, Nitrite Level; NC, Nitrite Concentration, CG, control group; AbG, Antibiotic Group; LED, Light-Emitting Diode Photomodulation;, T2DM, Glycemic-Controlled Type 2 Diabetes Mellitus; OP, Osteopenia; FP, Fapped surgery; FS, Flapless surgery; HA, HA- coated nano-surface; S, Smooth surface; R, rough surface; PS, piezosurgery; D, drilling; BC,T2DM Better control; PC, T2DM Poor Control; Ma, Machined; Ox, Oxidized; La, Laser modified; RT, Reduced Torque; CT, Conventional Torque.

Study/ Groups/ CU	Biomarkers	8 weeks	10 weeks	12 weeks	4 months	6 months	7 months	9 months	Over 12 months
Nomura et al., 2005	TIMP-1			0.25(0.41)					
- ng/sample	MMP-1			.					
- X 10-1 units/sample	MMP-8			1.00(1.03)					
	TIMP-1/MMP-1¹MMP-8			0.49(1.34)					
	CA, Active			2.8(1.2)					
	CA, APMA-activatable			3.7(1.3)					
Tozum et al. 2005*	Total NL: H			0.1411/0.1169		0.1244/0.1497		0.1832/0.1709	
- CL/IL (nmol)	Total NL: I			0.1669/0.1203		0.1601/0.1331		1.1897/1.1387	
Tozun et al., 2007a	Total NL			0.1455(0.049)/0.1112(0.021)		0.1279(0.032)/ 0.1391(0.024)		0.1392(0.06)/0.1649(0.043)	0.1028(0.019)/0.1086(0.023)
- CL/IL; All impants (AI)	NC			0.7716(0.526)/0.6310(0.422)		0.1279(0.127)/0.1391(0.024)		0.8712(0.885)/0.8506(0.623)	0.8651(0.522)/0.7653(0.740)
- nmol; nmol/µl	Total NL: AI			0.1309(0.042)		0.1334(0.028)		0.1566(0.053)	0.1034(0.019)
	NC: AI			0.7250(0.4840)		0.9582(0.9460)		0.8826(0.7600)	0.7522(0.6060)
Tozun et al., 2007b	Volume					0.218(0.165)/0.315(1.227)			
- CL/IL									
- µl; nmol	Total NL					0.174(0.072)/0.215(0.212)			
Güncü et al., 2008	Volume			0.184(0.123)/0.313(0.217)		0.284(0.159)/0.270(0.138)		0.153(0.088)/0.192(0.087)	0.184(0.122)/0.190(0.013)
- CL/IL									
- µl; nmol	Total NL			0.128 (0.014)/0.121(0.024)		0.106(0.017)/0.117(0.017)		0.115(0.028)/0.109(0.018)	0.062(0.065)/0.062(0.045)
Nowzari et al., 2008*	IL-1β			5580 (1732.62)		2871.42(1551.69)			633.33(897.53)
- copies/mL	TNF-α			5320 (672.30)		1471.42(708.55)			0
	HCMV			106 (237.02)		0			0

	INF-γ		0	0		0
Güncü et al., 2009	Total NL		0.127(0.013)/0.121(0.023)	0.097(0.034)/0.107(0.037)	0.105(0.042)/0.101(0.035)	0.061(0.056)/0.061(0.055)
- CL/IL						
- nmol; nmol/µl	NC		0.929(0.575)/0.611(0.445)	0.433(0.274)/0.604(0.633)	1.037(0.991)/0.693(0.432)	0.516(0.669)/0.401(0.397)
Boynuegri et al., 2012*	IL-1β		3.09(0.2)/ 7.73(0.26)/ 9.59(0.5)/ 11.65(0.57)	10.10(4.58)/19.17(9.59)/ 27.31(4.27)/32.19(13.29)	9.69(2.50)/16.08(3,85)/ 16.19(3.33)/ 25.26(8.85)	11.55(9.38)/34.02 (24.90)/ 28.76(19.06)/30.00(14.48)
- A// B// C// D						
- pg	TNF-α		12.13(9.5)/ 30.0(87.54)/ 18.36(19.34)/19.01(7.54)	51.47(29.18)/71.47(7.54)/ 64.59(19.34)/70.49(7.54)	28.76(0.98)/41.65(12,13)/ 41.32(7.87)/74.71(40.66)	64.79(57.05)/110.74(80.98)/ 66.78(42.30)/119.67(67.21)
Basegmez et al., 2012	PGE2		30.21(2.23)	29.58(2.79)	31.03(2.56)	51.67(3.96)
- nanogram/pocket	MMP-8		1.77(0.87)	2.21(1.14)	3.53(1.69)	4.29(1.87)
Slotte et al., 2012	IL-1β - S		675.6(382.4)/1453.0(1173.5)			
	IL-1β R		579.3(144.6)/294.0(99.6)			
- CL/IL (S, smooth; R, rough)	TNF-α - S		5.1(1.1)/12.1(6.7)			
- Mean (SE) relative gene expression	TNF-α - R		17.5 (8.2)/15.5(13.1)			
	OC - S		1.7(0.3)/4.8(2.6)			
	OC - R		43.6(29.6)/2.6(1.2)			
	ALP - S		13.7(9.9)/ 5.3(3.6)			
	ALP - R		27.2(9.9)/4.5(1.2)			
	CatK - S		7.0(3.9) 18.9(17.4)			
	CatK - R		26.4(22.9) 1.1(0.4)			
	TRAP - S		2.8(0.7)/3.5 (1.5)			
	TRAP - R		13.3(8.7)/1.2(0.2)			
Emecen-Huja et al., 2013	IL-1β	14.0(2.0)	13(3)			
- ng/ml	IL-6	0.5(0.1)	1(0.2)			
	IL-8	77.0(11.0)	52(8)			
	MIP-1β	7.0(1.0)	5(1)			
	VEGF	68.0(13.0)	45(6)			
Prati et al., 2013	TGF-α	78.8(45.3)/30.3(15.0)	66.5(61.9)/ 24.5(12.4)	21.0(7.1)/ 13.1(6.5)		
- CL/IL	OPN	106.1(11.4)/508.9(205.7)	115.9(16.4)/ 670.5(299.5)	300.6(216.0)/284(159.5)		
- pg/ml	OC	1318.6(245.1)/1015.5(696.6)	1253.1(52.1)/ 1089.4(362.7)	1187.9(249.3)/833.9(1053.6)		

	PTH	70.3(17.6)/551.8(252.2)	112.8(37.6)/ 615.3(368.0)	332.8(185.9)/347.7(229.3)			
	OPG	60.2(7.2)/1025.8(271.8)	95.9(24.2)/478.3(110.1)	195.6(42.8)/271.1(61.5)			
Tsoukaki et al., 2013 - FP/FL - ng/site (Mean,SE)	MMP-8		0.80(0.49)/ 0.99(0.49)				
	s-RANKL(ng/site)						
Nogueira-Filho et al., 2014* - pg/µl	IL-1α	7.50(1.77-11.28)		8.99(2.40-15.35)	5.66(1.67-8.51)	11.63(1.94-17.15)	12.53(2.12- 22.95)
	IL-8	8.72(0.39-82.65)		9.17(1.39-11.94)	7.67(1.62-8.85)	87.80(1.62-135.12)	93.07(1.62-1041.13)
	TGF-β1	0		0(0-802.74)	0(0-860.27)	101.20(0-910.96)	89.11(0-1180.82)
	VEGF	79.6(0.42-104.75)		505.48(88.54-767.71)	0	730.13(0.01-876.12)	261.64(0.02-794.52)
Gokmenoglu et al., 2014 - CG/LED - pg/mL	IL-1β	5.1(2.9–7.2)/3.6(2.1–4.6)					
	TGF-β	1339.0(732.8–2108.2)/ 1362.4(702.1–2468.4)					
	PGE2	2868.2(1827.0–4022.3)/ 2687.4(1493.6–4394.4)					
	NO	107.7(85.2–162.6)/ 101.3(45.5–184.3)					
Dolanmaz et al., 2015* - A1 (SLA)/ A2 (SLActive) / B (SLA) - pg/µl	OPG(pg/µl):		2180.32/2393.44/1327.89				
	sRANKL(pg/µl):		249.59/221.31/109.83				
	BMP-2		485.98/579.43/355.14				
	BMP-7		31.06/36.59/35.74				
Dogan et al., 2015* - CG/TG - ng/ml	IL-1β			14.15/14.15		14.15/14.15	
	TNF-α			2.35/1.7		1.41/0.69	
Onuma et al., 2015 - CG/OP - pg/ml	RANKL			15.58(21.64)/19.10(26.52)			
	OPG			743.1(1498)/902.3(2488)			
Ghiraldini et al, 2015 - CG/BC/PC - pg/µl	OPG		55.4(54.0)/63.8(140.9)/51.6(43.1)		78.4(09.0)/46.2(50.3)/66.0(76.8)		97.0(91.2)/69.8(87.3)/53.1(53.7)
	OC		278.5(521.0)/123.4(122.7)/121.7(81.9)		149.1(149.7)/113.2(131.5)/130.7(130.7)		143.3(123.4)/113.6(117.0)/69.7(53.8)
	OPN		165.2(286.1)/163.9(219.7)/156.4(230.1)		237.8(293.3)/136.0(179.8)/145.2(117.7)		388.8(390.2)/196.5(323.5)/121.0(133.4)

	FGF			51.8(93.7)/31.6(39.7)/54.7(46.2)	62.9(91.8)/41.9(64.7)/34.4(31.8)	62.9(74.9)/30.6(45.5)/25.0(23.3)
	TGF-β			28.5(31.6)/30.9(63.9)/33.9(26.8)	16.3(18.3)/29.9(42.7)/19.1(25.0)	36.0(50.0)/17.1(15.4)/18.1(16.6)
Peker Tekdal et al., 2015	RANKL	46.3(47.9)/64.53(90.5)		8.3(79.6)/54.4(41.7)	75.2(62.1)/63.73(32.1)	
- PS/Drilling	OPG	32.30(54.6)/27.27(32.7)		72.59(112.8)/48.43(55.1)	9.46(9.4)/41.23(52.1)	
- pg/µl, Mean(SD)	RANKL/OPG	1.34(2.6)/1.07(1.5)		1.71(5.2)/2.42(8.2)	0.76(0.7)/0.67(0.6)	
Tirachaimongkol et al, 2016	ALP	147.0(296.0)	157(201)	151(968)		
- Median (interquartile range)	OC (pg/µg protein)	94.0(292.0)	91(87)	59(94)		
Elsyad et al., 2016					205.61(18.22)/	240.87(22.34)/
- Magneto/Locator	IL-1β				154.55(75.15)	213.70(28.42)
- pg/µl (Mean, SD)						
Sayardoust et al., 2017b*	IL-6 - Non-smoker	0.78/0.56/0.69		0.97/0.84/0.45		
- Ma/Ox/Laser	IL-6 - Smoker	0.53/0.65/0.39		1.10/0.83/0.18		
- Relative gene expression.	IL-8 - Non-smoker	0.98/1.00/0.81		1.52/0.98/1.00		
	IL-8 - Smoker	0.30/0.98/0.90		0.78/0.83/0.58		
	TNF-α- Non-smoker	1.00/1.25/2.39		0.78/1.30/1.16		
	TNF-α- Smoker	0.49/1.12/0.49		0.71/0.27/0.40		
	ALP- Non-smoker	0.43/0.34/0.61		0.47/0.74/1.25		
	ALP - Smoker	0.47/1.97/1.30		0.87/1.25/0.58		
	OC- Non-smoker	0.97/0.85/2.52		1.68/1.48/2.00		
	OC – Smoker	0.50/0.50/0.85		0.26/0.73/0.92		
	CatK- Non-smoker:	1.00/0.78/0.68		0.37/0.71/0.75		
	CatK - Smoker:	0.17/0.15/0		0.22/1.29/0.08		
	BMP-2- Non-smoker	1.00/0.65/0.70		0.55/0.99/0.89		
	BMP-2 - Smoker:	0.80/0.69/0.80		0.95/0.37/1.22		
	VEGF- Non-smoker	1.00/1.52/1.87		1.00/1.42/1.65		
	VEGF - Smoker	1.26/1.08/0.98		0.84/0.80/0.73		
Bielemann et al., 2017	IL-1β	13.5(0.0-97.3)		32.0(3.6-747.6)		
- pg/µl (Median, range)	IL-6	40.7(0.0-784.6)		126.9(0.0-1050.4)		

	TNF-α	19.1(0.0-138.3)	21.9(0.0-87.3)	
	IL-10	153.7(0.0-968.6)	675.8(37.1-948.0)	
Verrastro Neto et al. 2017* - RT/CT	VEGF- RT/CT			239,024.39/151,219.51
	BMP-9 - RC/CT			0.01/417,073.17
	OPG -RC/CT			0.09/0.03
	TRAP - RC/CT			0.07/0.21
	Periostin - RC/CT			0.08/0.19
	PLGF - RC/CT			600000.0/1237704.9

*Data presented graphically were re-measured based on the calibrated distances of the figures using the software Adobe Photoshop version 6.0.1 (Adobe Systems).

Table S4. Measuring study quality bias according to Downs and Black, quality bias was Poor (P) if ≤14, Fair (F) if 15–19, Good (G) if 20 –25 or Excellent (E) if 26 –28.

	Reporting										External Validity			Internal Validity - Bias							Internal Validity - Confounding						Power	D&B Score n/28	D&B
Question	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**		
Nomura et al., 2000	1	1	1	1	0	1	0	0	1	0	0	0	1	0	0	1	1	1	1	1	1	1	0	0	0	0	0	14	**P**
Tözüm et al., 2005	1	1	1	1	0	1	1	1	1	0	0	0	1	0	0	1	1	1	1	1	1	1	1	0	0	0	0	17	**F**
Tözüm et al., 2007a	1	1	1	1	0	1	1	1	1	1	0	0	1	0	0	0	1	1	1	1	1	1	1	1	0	0	0	18	**F**
Tozum et al., 2007b	1	1	1	1	0	1	1	0	1	0	0	0	1	0	0	0	1	1	1	1	1	1	1	1	0	0	0	16	**F**
Khoury et al., 2008	1	1	1	1	0	1	1	1	1	1	1	0	1	0	0	0	1	1	1	1	1	1	0	0	0	0	0	17	**F**
Güncü et al, 2008	1	1	1	1	0	1	0	1	1	1	0	0	0	0	1	0	1	1	1	1	1	1	1	0	0	0	0	16	**F**
Nowzari et al., 2008	1	1	1	1	0	1	0	1	1	0	0	0	1	0	1	0	1	0	1	1	1	0	0	0	0	0	0	13	**P**
Guncu et al., 2009	1	1	1	1	0	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	1	0	17	**F**
Gruber et al., 2010	1	1	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	8	**P**
Boynuegri et al., 2012	1	1	1	1	0	1	1	0	1	0	1	0	1	0	0	0	1	1	1	1	1	1	1	0	0	0	0	16	**F**
Basegmez et al., 2012	1	1	1	1	0	1	1	1	1	1	1	0	0	0	1	0	1	1	1	1	1	0	0	0	0	1	0	17	**F**
Slotte et al., 2012	1	1	0	1	0	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	1	0	1	0	0	1	0	15	**F**
Emecen-Huja et al., 2013	1	1	1	1	0	1	1	1	1	1	1	0	1	0	0	0	1	1	1	1	1	1	0	0	0	1	1	19	**F**
Prati et al., 2013	1	1	1	1	0	1	1	0	1	1	1	0	1	0	0	0	1	1	1	1	1	1	1	0	1	1	0	19	**F**
Tsoukaki et al., 2013	1	1	1	1	0	1	1	1	1	1	1	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	23	**G**
Nogueira-Filho et al., 2014	1	1	1	1	0	1	1	0	1	1	0	0	1	0	0	0	1	1	1	1	1	1	0	0	0	0	1	16	**F**
Gokmenoglu et al., 2014	1	1	1	1	0	1	1	0	1	1	1	0	1	0	0	0	1	1	1	1	1	1	1	0	0	0	0	17	**F**
Dolanmaz et al., 2015	1	1	1	1	0	1	1	0	1	0	1	0	1	0	0	0	1	1	1	1	1	1	0	0	1	0	0	16	**F**
Dogan et al., 2015	1	1	1	1	0	1	1	1	1	1	1	0	1	0	1	0	1	1	1	1	1	1	0	0	0	1	0	19	**F**
Onuma et al., 2015	1	1	1	1	0	1	0	0	1	0	1	0	1	0	1	0	1	1	1	1	1	1	0	0	1	1	1	18	**F**
Mandić et al., 2015	1	1	1	1	0	1	1	1	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1	0	1	1	0	19	**F**
De Wild et al., 2015	1	1	0	1	0	0	0	1	1	1	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	1	0	14	**P**
Ghiraldini et al, 2016	1	1	1	1	1	1	1	1	1	0	1	0	1	0	1	0	1	1	1	1	1	1	0	0	1	1	0	20	**G**
Peker Tekdal et al., 2016	1	1	1	1	1	1	0	1	1	1	1	0	1	0	1	0	1	1	1	1	1	1	1	0	0	0	1	20	**G**
Tirachaimongkol et al.,2016	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	1	0	18	**F**
Elsyad et al., 2016	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	1	0	1	0	0	1	1	18	**F**
Sayardoust et al., 2017 a	1	1	1	1	1	1	0	1	1	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	0	17	**F**
Sayardoust et al., 2017 b	1	1	1	1	1	1	0	1	1	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	20	**G**
Verrastro et al., 2017	1	1	1	1	1	1	1	1	1	0	0	0	0	1	1	0	1	1	1	1	1	1	1	1	0	1	0	20	**G**
Bielemann et al., 2018	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	1	1	0	0	1	1	1	19	**F**

Capítulo 2

Artigo Original submetido ao periódico *Journal of Periodontal Research*

Healing differences of narrow diameter implants submitted to immediate and conventional loading in mandibular overdentures: a randomized clinical trial

Running title: Healing according to overdenture loading protocols

Amália Machado Bielemann[1], Raissa Micaella Marcello Machado[2], Alessandra Julie Schuster[3], Otacílio Luiz Chagas Júnior[4], Altair Antoninha Del Bel Cury[2], Fernanda Faot[5]

[1] MSc, PhD student, Graduate Program in Dentistry, School of Dentistry, Federal University of Pelotas, RS, Brazil.

[2] PhD, Department of Prosthodontics and Periodontology, Piracicaba Dental School, State University of Campinas, Piracicaba, SP, Brazil.

[3] PhD student, Graduate Program in Dentistry, School of Dentistry, Federal University of Pelotas, RS, Brazil.

[4] PhD, Professor, Department of Oral and Maxillofacial Surgery and Maxillofacial Prosthodontics, School of Dentistry, Federal University of Pelotas, RS, Brazil.

[5] PhD, Professor, Department of Restorative Dentistry, School of Dentistry, Federal University of Pelotas, RS, Brazil.

Corresponding author:

Prof. Fernanda Faot, PhD, Associate Professor, School of Dentistry, Federal University of Pelotas. Address: Gonçalves Chaves Street 457; 96015-560; Pelotas, RS, Brazil; e-mail: fernanda.faot@gmail.com

Abstract

Background: Biological responses to different loading protocols during the bone healing phase in subjects with high edentulism time, rehabilitated with narrow diameter implants (NDI) to retain mandibular overdentures (MO) are still unavailable.

Objective: This randomized clinical trial compared the peri-implant health, implant stability and concentrations of pro- and anti-inflammatory cytokines in the peri-implant crevicular fluid (PICF) in mandibular edentulous patients under conventional (CL) and immediate loading (IML) during healing.

Methodology: Twenty totally edentulous patients received two NDI (2.9x10mm, Facility NeoPoros) placed in mandible anterior region and were randomly assigned to two loading protocols: CL (n=10) or IML (n=10). The following clinical outcomes were evaluated 1, 2, 4, 8, and 12 weeks after surgery: i) peri-implant tissue health (gingival index-GI, plaque index-PI, calculus-PC, probing depth-PD and bleeding on probing-BOP); ii) implant stability quotient (ISQ) and iii) IL-1β , IL-6, IL-10 and TNF- α levels in the PICF analyzed by ELISA.

Results: The CL group showed significantly higher PC scores at weeks 8 and 12. The IML group showed significantly higher GI from the first week onwards. The IML group presented higher BOP rates than CL at week 12. The ISQ values of the CL group were higher than those of the IML group, except at week 4. The IML group released significantly more TNF-α between week 1–4 and more IL-1β during week 4–12, while releasing less IL-6 until week 8, mainly at week 2 (47.6% less). The release of IL-10 was similar for both groups and increased progressively over time. At week 12, the IML group released 45.74% more IL-10 than the CL group. The survival rates were 95% and 90% for CL and IML, respectively.

Conclusion: Despite IML group had presented more predictable peri-implant health results, the implant stability and the inflammatory marker concentrations were more stable in the CL group.

Keywords: full edentulism, edentulous mandible, overdenture, immediate loading, implant, healing phase, inflammation, cytokines, implant stability, randomized controlled clinical trial.

1. Introduction

Successful rehabilitation with dental implants depends on the biological bone-implant osseointegration. This process in turn depends on the bone volume and the bone bed quality [1]. Edentulous areas with a long time of edentulism are characterized by limited height and bone thickness due to residual ridge resorption [2]. For these specific cases, narrow diameter implants (NDI) can enable rehabilitation of edentulous areas without surgical interventions that increase bone availability [3].

The NDI present a simpler and more conservative surgical approach, particularly when complex surgical interventions are contraindicated [4]. The systematic review by Klein et al. (2014) found that NDI with a diameter less than 3.0 mm have high survival rates ranging from 90.2% to 100%[4]. Furthermore, a meta-analysis of short- and long-term studies by Marcello-Machado et al. (2018) [5] found a survival rate of 98% and a success rate of 96%, showing that NDI are a reliable option as mandibular overdenture (MO) retainers for patients with limited bone thickness. In addition, this meta-analysis showed that NDI had a better long-term predictability when the conventional loading (CL) protocol was used.

Therefore, treatment with NDI has been indicated for the rehabilitation of totally edentulous patients, particularly those with atrophic jaws and prolonged edentulism time [6]. In addition, current evidence recommends clinicians to use MOs retained by two implants, both with conventional [6] and immediate loading (IML) protocols [7]. When using CL, after three months of osseointegration MO retained by NDI show a high predictability, are cost-effective, and capable of significantly improving users' quality of life in by promoting a high level of patient satisfaction in a short period [8,9].

The IML protocol also presents adequate predictability but is more dependent on intrinsic factors that are mainly related to the bone site characteristics [9]. Achieving high implant insertion torques during surgery is a prerequisite to adopt the IML protocol. Biologically, this is reflected in a greater mechanical bonding between the threads of the implant and the bone bed, resulting in high primary stability [10]. Previous studies [10–12] indicate that the biological response to mechanical forces that are evenly distributed on the bone bed immediately after implant installation can be observed between the 1st and 4th weeks. This loading results in sharp resorption of the old bone together with bone neoformation at the bone-implant interface, followed by the more gradual maturation of the new bone that result in a slower way to achieve secondary stability [10–12].

Prati et al. (2013) [13] performed a randomized clinical trial to monitor the osseointegration when rehabilitating with mandibular Brånemark full-arch prostheses in a population of totally edentulous patients with a mean age of 55.5 years. They reported that biologically, IML induced the greatest release of bone markers, osteoprotegerin (OPG), osteocalcin (OCN), osteopontin (OPN), and parathyroid hormone (PTH), triggering a faster response of the body to the replacement of old bone around the implant, and thus, promoting greater bone-to-implant contact (BIC). However, with respect to MO, only four studies assessed the early healing with the implants. Two studies used the CL protocol [14,15] and two [16,17], investigated the biological response to different components and loads. In the study by Elsyad et al. (2016) [16], IML implants with Locator® attachments had lower concentrations of IL-1β, visible plaque index (PI), and values for implant stability quotient (ISQ), both during early and late healing, compared with Magneto attachments. The randomized clinical trial by Acham et al. (2017) [17] found that IML presents high ISQ up to 12 months in comparison with CL. Thus, studies investigating the impact of different occlusal loading protocols during the bone healing phase in ridges with high edentulism time and rehabilitated with NDI are still scarce.

Systematic reviews[18,19] have shown that CL has more favorable success and survival rates than IML. However, when these studies are analyzed separately, significant differences between the types of loading are not observed. Although all three well-known loading protocols provide high survival rates, early and CL protocols are still better documented than IML and seem to result in fewer implant failures during the first year[18]. Furthermore, the patient selection for innovative IML protocols may be biased by selection of patients with few or no risk factors such as smoking, diabetes, or poor bone quality. This selection may result in success rates that may not be reproducible in everyday clinical practice, highlighting the importance of carefully conducted clinical studies that provide a high level of evidence for clinical decision-making. Finally, other factors like patient-centered benefits and disadvantages or the costs of prosthodontic aftercare may also be considered during clinical decision-making. According to Zygogiannis et al. (2016) [19], IML protocols used to support and retain mandibular overdentures opposed by a maxillary complete denture seem to be a viable alternative to CL. So far, no specific IML protocol outperformed CL in terms of clinical and prosthodontics outcomes. However, the 14 studies analyzed Zygogiannis et al. (2016) [19] indicate that this data should be interpreted cautiously, due to the differences in study designs, end point outcomes, and small sample sizes. In addition,

more data is needed to enable accurate recommendations regarding implant diameter, number of implants, and attachment system when using IML protocols. Finally, they also recommend to carefully monitor the establishment of primary stability and optimization of the biomechanical distribution of loads through appropriate prosthesis design and occlusal adjustment when IML is adopted [19].

More randomized clinical trials are needed to provide a better insight for the optimal management of fully edentulous mandibular cases with overdentures supported by IML implants [19]. In view of this, it is equally important to understand the possible biological responses to each type of occlusal loading during the bone healing phase in subjects with high edentulism time and rehabilitated with NDI. The null hypothesis to be tested in this randomized clinical trial is that there will be no differences in bone healing and peri-implant health and implant stability for implants subjected to immediate occlusal loading in comparison with conventional occlusal loading. In addition, this study aimed to investigate if patient characteristics as bone atrophy and bone type could also influence the clinical and biological outcomes during the healing process.

2. **Materials and Methods**

The design of this parallel, controlled, randomized clinical trial followed the guidelines of Consolidated Standards of Reporting Trials (CONSORT) guidelines for randomized controlled clinical trials [20].

2.1. Study overview

This study was performed during 12 weeks of follow-up for monitoring the healing of two narrow diameter implants inserted in the lower jaws of edentulous patients treated at the School of Dentistry of the Federal University of Pelotas, Brazil between June 2014 and June 2015. This study was approved by the institutional Committed Ethics board for human subjects (protocol 1.267.086). Totally edentulous patients wearing conventional complete dentures in both arches with at least 3 months of adaptation, presenting poor bone availability in the anterior region of the mandible, mandibular prosthesis reduced stability, and insufficient retention of the mandibular complete denture were included. Following the diagnosis of mandibular atrophy [21], the patients were recruited for treatment through rehabilitation with MO. The study exclusion criteria were as follows: history of radiotherapy in the head or neck region, previous history of oral implant insertion, patients who have had bisphosphonate

treatment in the past 12 months, smokers, severe diabetes (hyperglycemia or inadequate glycemic control), bleeding disorders (hemorrhagic diathesis, drug-induced anticoagulation), severe systemic diseases (rheumatoid arthritis, osteogenesis imperfecta) and compromised immune systems (HIV, immunosuppressive medications) [22].

The sample size was determined according to the data reported by Prati et al. 2013 [13] using the statistical program G*Power 3.1®. Due to the heterogeneity in the follow-up periods, we choose the results of the transforming growth factor alpha (TGF-α) to perform the calculation. This cytokine required the largest number of implants to obtain a high power of the statistics test, small effect sizes and significance to detect equality between parallel groups, especially during the early healing stages. For the calculation of sample size, the mean and standard deviation of the control group (conventional loading, 66.5 ± 61.9 pg/ml), and test group (immediate loading, 24.5 ± 12.4 pg/ml), were used with an effect size of 0.94, a 10% beta error, and 5% alpha error. Accordingly, the required sample size was 30 implants. To account for possible dropouts, 20% was added to the sample size; thus, a minimum of 36 implants or 18 patients were estimated required. For statistical analysis, the implant was considered as an experimental unit. Twenty-five patients were invited to participate, and after clarification about the treatment and risks, a total of 20 patients accepted to participate in the study and signed an informed consent form.

Digital panoramic radiographs were performed to measure the bone availability before surgical planning (Digital System Dentascan - Rotograph Plus, Del Medical Imaging Corp., USA). The images were scanned by the system sensors (12.7 × 30 cm dimensions) and processed using the DBSWin 4.5 software (Dürr Dental, Bietigheim-Bissingen, Germany). A single expert examiner (RMMM) performed the radiographic linear measurements to evaluate the mandibular bone height in the anterior and posterior region. The mandibular atrophy level was then determined following the methodology previous described by Marcello-Machado et al (2017) [23].

For randomization, a computer-generated list was created by a blinded investigator who was not involved in the screening, treatment, follow-up, data collection or analysis. Patients were allocated treatment by an independent, centralized online randomization service, using a 1:1 allocation ratio to the CL or IML group. For the CL group (n = 10), the MO loading was performed after 12 weeks of bone healing, while the IML group (n = 10) received immediate rehabilitation with MO retained by stud attachment system. This information was concealed in sealed envelopes, which were

opened immediately before surgical treatment. Neither the surgeon nor the patient was aware of the group assignment until right before the surgery. The insertion torque values greater than 30 Ncm have been adopted to determine immediate or late loading of overdentures with high predictability and success rates [18]. If sufficient primary stability was not reached in the participants allocated in the IML group, they were switched to the CL group. The flow chart of the study is shown in Figure 1.

2.2. Surgical procedure

According to previous methodology described by Bielemann et al. [15], a standardized one-stage surgical protocol performed by an experienced surgeon was followed in the placement of all implants. Full-thickness flaps were reflected to expose the bone ridge at the mandibular midline area, and osteotomies were guided by the position of the distal face of upper lateral incisives, approximately 5mm anterior to the mental foramina and a minimum inter-implant distance of 20 mm. Implant surgery drill sequence was followed to install two NDIs (ø2.9 - 10 mm Facility® NeoPoros, Neodent Osseointegrated Implants, Curitiba, Brazil). Bone quality was determined during osteotomies according to the subjective perception of an experienced surgeon based on the bone density (dense bone and extremely soft bone) [15]. All mandibles in our study were classified as bone types I or II. During implant placement, the insertion torque was recorded according to the surgical wrench with a fixed calibration of 32 Ncm. The insertion torque values greater than 30 Ncm have been adopted to determine immediate or late loading of overdentures with high predictability and success rates [18]. If sufficient primary stability was not reached in the participants allocated in the IML group, they were switched to the CL group. Finally, the mucoperiosteal flaps were adapted around the neck of the non-submerged healing cap in CL group and around the Equator® abutment in IML group (Neodent Osseointegrated Implants, Curitiba, Brazil). Afterwards, in the CL group, the mandibular complete denture was relined using a soft intermediate liner (Trusoft®, Bossworth Company, USA) that was replaced monthly until the end of the bone healing period. For IML group, the mandibular overdentures were immediately loaded after surgery by an experienced prosthodontist (A.M.B.) connecting the O-ring attachments (the female part of the Equator abutment) intraorally using self-curing denture acrylic resin (VIPI Flash®, VIPI industry, São Paulo, Brazil) to transfer the system to the internal surface of the prosthesis.

Postoperative medication included amoxicillin 500 mg three times a day for 7 days, ibuprofen 600 mg three times a day for 3 days, and paracetamol 500 mg four times a day as needed. Nylon sutures (Procare®; Lamedid Ltda, Barueri-SP, Brazil) were removed 10 days after surgery. Complete denture care instructions were provided for all patients and reinforced during each follow-up.

2.3. Implant Stability Analysis

The primary implant stability quotient (ISQ) was determined using magnetic resonance frequency device (Osstell® - Integration Diagnostics AB, Göteborg, Sweden). A Smartpeg™ type A3 was connected manually into the internal connection of the healing caps or the equator abutments, and the ISQ probe was held perpendicular to the Smartpeg™. ISQ value was measured in four different directions (mesial, distal, buccal, and lingual) performed during implant placement (baseline) and 1, 2, 4, 8, and 12 weeks afterwards. The analyses were performed in triplicate by the same examiner (A.M.B).

2.4. Clinical Monitoring and Data Sample Collection

Clinical examination of implants was performed at four regions per implant (mesial, distal, buccal and lingual) following a previous study by Bielemann et al. (2018)[15]. A single calibrated examiner (A.M.B) assessed plaque index (PI) score, the presence of calculus and gingival index (GI) score at 1, 2, 4, 8, and 12 weeks. The probing depth (PD) and the bleeding on probing index (BOP) [15] measurements were performed at 2, 4, 8, and 12 weeks post-implantation. Panoramic radiographs with standardized settings were taken during the implant surgery and abutment connection.

2.5. Peri-implant crevicular fluid (PICF) collection and analysis

The collection of PICF was performed at 1, 2, 4, 8 and 12 weeks post-implantation by the same operator (AMB). The plaque was removed, and each implant was dried for 10 seconds with compressed air and isolated with a cotton roll. Two standardized paper strips (Periopaper™, Proflow, Amityville, NY, EUA) were inserted separately for 40 seconds at the medial and distal surface of each implant. The paper strips were then placed in a single Eppendorf vial containing 100µl phosphate-buffered saline and stored at −80 °C. Interleukin (IL-)1 beta (IL-1β), IL-6, IL-10, and tumor necrosis factor alpha (TNF-α) were quantified by enzyme-linked immunosorbent assay (ELISA) kits following the procedures recommended by the manufacturer (Duoset kit®;

R&D, Minneapolis, MN, USA). The standard solution and samples were added to wells, which had been pre-coated with specific monoclonal capture antibodies. After 3 hours, polyclonal antibodies conjugated with horseradish peroxidase were added to each well and incubated for 1 hour. A substrate solution containing hydrogen peroxidase and chromogen was added and allowed to react for 20 minutes. The cytokine levels were assessed by a micro-ELISA reader (Ultramark®, Bio-Rad, CA, USA) at 450 nm and normalized to the abundance of standard solution. All analyses were performed by a blinded technician, and the results were expressed in pg/µl.

2.6. Implant success and survival

The success of the implants was evaluated according to the clinical criteria proposed by Misch et al. (2008) [24] and Papaspyridakos et al. (2012) [25] as follows: no pain or tenderness upon function, no clinically implant mobility, radiographic marginal bone loss <1.5 from initial surgery, no infections, dysesthesia or exudates history. If the implants remained *in situ* but did not meet the criteria for success, they were categorized as the survival group.

2.7. Data management and analysis

The data were tabulated independently by two researchers (RMMM and AJS), and then compared. The Shapiro-Wilk test indicated that PD and the ISQ data followed a normal distribution. The variables PI, CP, GI, and BOP were dichotomized; scores 0 and 1 were denominated as absence and scores 2 and 3 as presence. For the comparisons between groups of dichotomous variables, the chi-square test was used, and for the comparisons over time (intra-groups), the McNemar test was used. For the continuous clinical variables, PD and ISQ, the T-test was used to verify the possible differences between groups and the paired T-test for intra-group comparisons (over time). The concentration of inflammatory markers between the groups was compared by the Mann-Whitney test and, over time (intra-groups), by Wilcoxon's matched pairs signed-rank test. The Pearson correlation (R_p) test was used to verify the possible relationships between the variables: sex, age, edentulism time, atrophy, bone type, GI, PI, CP, PD, BOP, and ISQ. The Spearman correlation (R_s) test was used to verify the relationships between the variables: IL-1β, TNF-α, IL-6, and IL-10. The results of the correlations were stratified to be interpreted as follows: i) very high positive / negative (0.90–1.00), ii) high positive / negative (0.70–0.90), iii) moderate positive / negative

(0.50–0.70), and iv) low positive / negative (0.30 - 0.50), v) without correlation (0.00–0.30)[26]. Kaplan-Meier survival analysis was used to calculate the survival rate of implants for each group. The level of significance was set at 5%. All analyses were performed using SPSS Version 22 software (IBM SPSS Statistics 22).

3. Results

Table 1 presents the patient's characteristics and implants according to the type of loading. Twenty patients with a mean age of 66.9 ± 6.61 years and mandibular edentulism time of 23.2 ± 13.22 years participated in this study. The CL group consisted of 7 women and 3 men with a mean age of 67.0 ± 3.24 years, and the IML group consisted of 5 women and 5 men with a mean age of 66.8 ± 8.92 years. Mandibular atrophy was diagnosed in 11 patients; 5 patients in the CL group and 6 patients in the IML group. The most prevalent bone type in the IML group was bone type I (14 implants, 70%) and in the CL group was bone type II (14 implants, 70%), with a significant difference in prevalence between the groups ($p = 0.011$). A total of 40 NDIs was installed in the anterior region of the mandible, presenting a 90% survival and success rate in the IML group, in which two NDIs failed at week 9 in a single patient. This rate was 90% for the CL group, in which an NDI failed at week 8 and one at week 12. The Kaplan-Meier survival curve is shown in figure 2. The lost NDIs were replaced by larger diameter implants (3.5 x 9.0 mm - Titamax Cone Morse Implant - Neodent Implants Osseointegrated, Curitiba, Brazil), which presented a 100% success rate.

Table 2 presents the analysis of the variables related to peri-implant health and Figure 3 the record of implant stability, according to each group and throughout the evaluated times since its installation. For PI, a significant difference between groups was not observed throughout the follow-up period ($p > 0.05$). The presence of CP was observed in the IML group only at week 4, while in the CL group at weeks −8 and −12, 30% ($p = 0.035$) and 20% ($p = 0.039$) respectively. GI in the IML group was 50% higher than the CL group at week 1 ($p = 0.006$), and 30% lower at week 8 ($p = 0.017$). PD of the IML group was a mean of 21.49% lower than the CL group in all the evaluated periods, being significant this difference in weeks −2 ($p = 0.040$), −4 ($p = 0.027$), −8 ($p = 0.038$) and −12 ($p = 0.004$). BOP of the IML group was 28.9% higher at week 12 compared to the CL group ($p = 0.044$). The IML group presented a mean of 8.95% lower ISQ than the CL group from week 1 to week 12 (w1: $p = 0.013$; w2: $p = 0.007$; w8: $p = 0.012$; w12: $p = 0.021$). The IML intra-group analysis in the first week showed 90% of IG presence that was significantly higher to all the evaluated periods ($p \leq 0.0001$), and a significant progressive decrease from week 2 until week 12. Baseline ISQ value (T = 0) was 12% higher than all follow-up periods, with a significant reduction up to week 12 (w1: $p = 0.016$; w2: $p = 0.002$; w4: $p = 0.0001$; w8: $p = 0.001$; w12: $p \leq 0.0001$). The CL intra-group analysis, the GI presented significant reduction of

27.5%, being higher at week 1 when compared with weeks 4 (p = 0.008) and −12 (0.016). The mean ISQ of the CL group was8.49% higher at the baseline and weeks 1 and 2 compared with week −8 (p = 0.021; p = 0.012), respectively.

Figure 4 shows the concentration of peri-implant inflammatory markers according to each group over the evaluated times. In the IML group, a 40.75% higher TNF-α concentration was observed compared with the CL group at week−1 (38.75%, p = 0.019), −2 (34.26%, p≤0.0001), and −4 (49.59% p = 0.003). The IL-1β concentration was higher in the IML than the CL group at all periods, and from week 4, this significant difference was a mean of 57.78% higher for the IML group, significant at weeks −4 (43.45%; p = 0.003), −8 (66.18%; p = 0.045), and −12 (63.80%; p = 0.001). IML group presented 53.94% lower IL-6 concentration than CL group until week 8, this difference being significant only at week 2 (47.6%; p = 0.0037). The IL-10 concentration was similar between groups up to week 8, at week 12 the IML group had 45.74% higher concentration than the CL group (p = 0.003). According to the IML intra-group analysis, the TNF-α concentration at week 8 was 51.96% higher than the previous weeks (p = 0.05) and in week 12 was 69.5% higher than weeks 1 and 2 (p = 0.003; p = 0.001). For IL-1β there was a reduction of 30.15% from week 1 through week 8 and for IL-6 this reduction was 61.05% (p = 0.05). However, at week 12, a peak at 55.9% concentration of IL-1β and 75.84% of IL-6 was observed in relation to the previous weeks (p = 0.05). For IL-10, week 8 had a 52.5% higher concentration than at weeks 1 and 2 (p = 0.002; p = 0.026), and week 12 had 85.86% higher concentration than all follow-up periods (p = 0.05). In the intra-group analysis for the CL group, the TNF-α concentration remained stable in all periods, with a mean of 35.0 pg/µl. On the other hand, IL-1β concentrations were higher in weeks 1, 2, and 12 than week−4 (w 1-4: p = 0.014; w 2-4: p = 0.008; w 4-12: p = 0.044) and −8 (w 1-8: p = 0.006; w 2-8: p = 0.008; w 8-12: p = 0.011). The IL-6 concentration showed a peak at week 1 in relation to weeks −2 (p = 0.017), −4 (p = 0.002), and −8 (p = 0.004). The IL-10 concentration in the CL group increased significantly over time (p = 0.05), the concentration at week 12 was 83.43% higher than at week 1 (p < 0.001).

The analysis of the correlations between the demographic variables showed that the IML group had a high positive correlation edentulism time and atrophy (p < 0.001; R_p= 0.923); in the CL group, low positive correlation was found between edentulism time and age (p = 0.033; R_p = 0.478) and bone type and atrophy (p = 0.002; R_p = 0.655). Table 3 presents the results of the correlations between the ISQ of each group and the following outcomes: demographic characteristic, clinical parameters,

and cytokine concentrations over time. The IML group did not show a positive correlation with the ISQ. In contrast, in the CL group, there was a high positive correlation between ISQ and GI at week 2 ($p < 0.001$; $R_p = 0.724$), moderate positive correlations between ISQ and IL-6 at week 4 ($p = 0.005$; $R_s = 0.603$), and atrophy at week 12 ($p = 0.004$, $R_p = 0.625$). The IML group showed moderate negative correlations between ISQ and age at weeks 1 ($p = 0.031$; $R_p = -0.483$), 4 ($p < 0.001$; $R_p = -0.607$), and 8 ($p = 0.003$; $R_p = -0.622$), with PD at week 8 ($p = 0.002$; $R_p = -0.644$) and TNF-α at week 4 ($p = 0.007$; $R_s = -0.584$).

Table 4 shows the correlations between the cytokines concentration in the PICF and the peri-implant health monitoring variables over time for both groups. The most significant correlations were found in the IML group that revealed in the early stages of healing, a high negative correlation between IL-10 and IL-6 at weeks 2 ($p = 0.001$; $R_s = -0.791$) and −4 ($p = 0.046$; $R_s = -0.540$); and a high positive correlation between IL-1β and IL-6 at week 1 ($p = 0.001$; $R_s = 0.785$). At week 8, a high positive correlation was observed between IL-10 and IL-1β ($p = 0.010$; $R_s = 0.664$), IL-10 and TNF-α ($p = 0.002$; $R_s = 0.758$), and between TNF-α and IL-1β ($p < 0.001$; $R_s = 0.861$). For the CL group, only moderate significant correlations were found as follow: positive correlation between IL-10 and PI at week 1 ($p = 0.018$; $R_s = 0.521$); IL-1β and IL-6 at week −1 ($p = 0.017$, $R_s = 0.526$), −2 ($p = 0.033$, $R_s = 0.478$), and −12 ($p = 0.029$, $R_s = 0.501$); and a negative correlation between IL-10 and TNF-α at week 2 ($p = 0.003$; $R_s = -0.632$). For the clinical parameters, the CL group had a moderate positive correlation between GI and PD at week−4 ($p = 0.003$; $R_p = 0.623$) and a high positive at week −12 ($p < 0.001$; $R_p = 0.814$).

4. **Discussion**

The forces of compression and tension that act on the bone after immediate loading of the implants cause intense bone remodeling, due to the greater bone mineral content and bone-implant contact [27]. There is no consensus regarding the loading protocol that should be used as a function of factors such as age, edentulism time, and bone availability. The present study investigated the inflammatory response profile during bone healing and the peri-implant soft tissue healing in patients that received NDIs and presented long mandibular edentulism time (23.20 ± 13.22 years) and low bone availability (23.77 ± 3.74 mm) subjected to two types of loading, CL or IML.

Both the groups had elevated GI scores in the first week. The IML group showed 36.36% more inflammation than the CL group ($p = 0.006$). Although the

surgical technique was the same for both groups, the IML group experiences a longer prosthetic phase after surgery. The latter may be more damaging to the peri-implant soft tissues, due to the need for immediate capture of the prosthetic abutments with self-curing acrylic resin [28]. In addition, literature has shown that this material presents a higher cytotoxicity than resilient liner [28], because poly methyl methacrylate (PMMA) can induce allergic reactions, cell injury, and production of pro-inflammatory cytokines through oxidative stress [29]. Tözüm et al. (2007) [30] rehabilitated 34 patients with MO subjected to conventional and immediate loading and found similar GI scores after 30 days of healing. Previous studies [13,17] also show that GI and PD decrease over time for both types of loading, as observed in this study. However, the IML group had a mean PD of 2.61 mm, which was significantly lower ($p = 0.05$) than the CL group at all times (mean PD 3.31 mm). Thus, we suggest that the prosthetic stability caused by the immediate loading of the overdentures can provide a better conditioning of the peri-implant tissues.

Systematic reviews [9,19] regarding the use of resonance frequency analysis for the primary stability check has shown that there is no difference in ISQ values between the two types of occlusal loading. Zygogiannis et al. (2016) [19] also found no difference between the two types of loading for implants retaining MO. In addition, we emphasize that patients in our study that received NDIs with either protocol presented acceptable primary stability, with ISQ values after the installation of approximately 55.28 ± 4.19. However, contrary to the previous studies, we observed that the secondary ISQ of the IML group was approximately 9% lower than the CL group in all follow-ups ($p = 0.05$). A similar result was found by Scepanovic et al. (2015) [31]; in a study with MO retained by 4 NDI subjected to IML, they found a reduction in ISQ values during establishment of secondary stability until the 6th week of follow-up. In contrast, Acham et al. (2017)[17] recently reported that for patients with MO retained by 5 implants, the IML group obtained higher ISQ than the CL group during 6 months of follow-up.

Osseointegration of implants with immediate loading involves a concomitant evolution of bone neoformation, an active process of reabsorption of the remaining bone that can result in a prolonged time to consolidate the osseointegration. A higher bone-implant interface juxtaposition during implant insertion, evidenced by a high primary ISQ, will be associated with more active bone resorption relative to bone neoformation [10,11]. This process directly affects the secondary stability, which is in agreement with the lower secondary stability of the IML group in our study. Elsyad et al. (2016) [16] performed a study with MO retained by two implants with locator and

magnet components. They attributed the decreased ISQ values to continuous bone remodeling occurring after loading, which decreases bone-implant anchorage because of increased micromotions affecting the bone-implant stability. In our study, most implants (70%) in the IML group were inserted in bone type I, a more corticalized bone. We expected to achieve a better mechanical locking, evidenced by a higher primary ISQ. However, our study showed no correlation between the bone type and ISQ, as previously described in other studies [32,33].

Insertion of implants with reduced osseocompression and tension can act as an osteogenic stimulus to the peri-implant cells [27], stimulating osseointegration in implants with a lower initial contact surface [11]. Berglundh et al. (2013) followed *in vivo* bone formation around implants adopting a wound chamber methodology that required a surgical site preparation using light torque forces; only a minimal pressure was exerted to the lateral bony walls of the implant bed at the pitch region. During the establishment phase, there is a delicate balance between bone resorption in the contact regions between the titanium body and the mineralized bone and the bone formation in the contact-free areas[10]. The dynamic healing process described by these authors showed that in implants with a large contact-free surface between the bone and implant (similar to CL protocols), the osseointegration seems to be established faster than for implants inserted with a large contact surface (similar to IML conditions). In the latter, the rate of the osseointegration is also influenced by the magnitude of the press-fit and the resulting bone necrosis. Similarly, an *in vivo* study by Kim et al. (2008) [34] showed that after 10 weeks of loading, CL group implants had a significantly higher bone implant contact (BIC, 46% higher than implants of the IML group). Therefore, we partially attribute the more favorable secondary ISQ results for the CL group in this study to the aforementioned factors that contribute to an early bone maturation compared to the IML group.

Romanos et al. (2016) [27] also points out that the osteoid matrix may act as a shock absorber at the bone-implant interface, reducing shear or compressive stresses. In the long-term, moderate tensions can act as a stimulus for osteogenic peri-implant cells ensuring success in rehabilitation with IML. However, in our study, most of the implants (60%) of the IML group were inserted in atrophic jaws, which had a positive correlation with edentulism time. Thus, in addition to the more unfavorable secondary stability achievement by the IML group, we also suggest that this atrophic bone is incapable of responding quickly to the loads generated by the exerted mechanical forces. The latter could at least in part explain the lower secondary stability of the IML

group. These results may also justify the biological responses in the IML group. Biological bone atrophy is associated with immobilization of the long-term receptor bone bed, which may affect the vascular support of the bone [35]. These characteristics could delay the formation of a connective tissue rich in vascular units and fibroblasts in direct contact with the surface of the implant, which normally forms during the second week of bone healing [36]. This event marks the beginning of vascularization and the organization of the model for the new bone formation [10]. Bielemann et al. (2017) followed the healing of two NDIs as MO retainers subjected to CL in a similar population. Their results indicate that populations with a high edentulism time seem to need an extended time to achieve adequate secondary stability.

Some studies have investigated the biology of osseointegration of implants supporting MO and the influence of loading on the release of inflammatory biomarkers[14,16,30,37,38]. The results from Tozum et al. [30,37,38] showed that immediate loading provided higher release of nitric oxide, a pro-inflammatory marker involved in bone remodeling. The remodeling process is initially mediated by pro-inflammatory cytokines released by M1-type macrophages, which act during immediate post-injury repair and osteoclastogenesis [39]. In our study, the IML group presented a more exacerbated inflammatory reaction than the CL group, releasing approximately 40% more TNF-α until week 4 (week 1, $p = 0.019$; week 2, $p < 0.0001$; week 4, $p = 0.003$), and after the 4th week until the final phase of osseointegration, the IL-1β concentration was approximately 50% higher than the CL group ($p = 0.05$). In the study by Slotte et al. (2012) [40], both loading protocols had a higher TNF-α expression in the first week and then decreased gradually until week 12. However, considering only implants with smooth surface abutments, the IL-1β release by the IML group was similar to that observed in our study. The increase in activity of IL-1β and TNF-α was also described in peri-implantitis sites and reflects the osteoclastogenesis and alveolar bone resorption[41]. Therefore, as IML group showed the most pronounced pro-inflammatory activity, according to the IL-1β and TNF-α concentrations, and because of the positive correlation between them until the 8th week, we hypothesize that IML is associated with a continuous and accentuated process of bone remodeling due to the mechanical stress to which the implants are subjected. However, studies have also shown that mechanical stimuli motivate pre-osteoblasts to develop bone matrix proteins (osteocalcin) [13,42]. This eventually results in a significantly higher bone density between the threads of the implant in the IML group (11.6% higher than the CL group) [27,35].

IL-1β is the main factor responsible for the activation of lymphocytes that trigger the acute responses and processes to preserve and restore post-injury homeostasis or infection [43]. As expected and evidenced in previous studies [15,32], the initial IL-1β activity was high in both groups, and this is interpreted as a response to the surgical trauma. Because of this, we suggest that IL-1β cannot be used to detect imbalances in the bone metabolism [15]. Nonetheless, IL-1β plays an important role in the protection of tissues against iatrogenic factors, such as plaque accumulation and trauma, and its activity sometimes increases even after one year of scarring due to the presence of plaque [16]. This role was evident at week 12 where there was a peak in IL-1β concentration in both groups. Moreover, in the CL group, this increase was positively correlated with the GI at weeks 8 and 12, when there was a higher presence of calculus. Even with the reinforcement of oral hygiene during each return visit, the soft reliner used to fill the prosthesis in the CL group may have suffered degradation after 12 weeks. Previous studies pointed out the need to exchange the temporary reline material when it is unsatisfactory [9,44]. Another contributing factor could be the lack of fine motoric control in the elderly sample population, which hinders adequate hygienic care for the prosthetic abutments [15,16].

IL-6 is a pro-inflammatory cytokine secreted by osteoblasts, osteoclasts, and stromal cells. It appears to be an important regulator, not only of bone remodeling and stimulating osteoclastic bone resorption, but also for promoting osteoblasts formation under conditions of high bone turnover [45]. The CL group released twice as much IL-6 as the IML group until week 8, but this difference was only statistically significant at week 2 (47.6%, $p = 0.037$). As already shown in the study by Bielemann et al. (2017) [15], the prosthetic instability in the CL group until functional loading can cause a quick IL-6 release due to trauma, remaining elevated for days. The high IL-6 concentration has also been associated with the inhibition of both TNF-α production and IL-1β release [45]. The latter accounts for the lower production of both cytokines in the CL group observed in our study. In addition, we observed a positive correlation between IL-6 and IL-1β in both loading protocols at week 1. Loi et al. (2016) [46] emphasize that IL-1β stimulates the production of IL-6 by osteoblasts assisting bone healing and local angiogenesis. At week 12, the IML group released 23.54% more IL-6 than the CL group and had a 30% higher BOP than the CL group ($p = 0.05$). Thus, it is evident that IL-6 is a reliable marker of the severity of the lesion in acute inflammatory response to surgery, trauma, and infection. Iatrogenic factors occurring in any interval between healing and osseointegration may stimulate peri-implant soft tissue responses, which

in turn directly stimulate the IL-6 release. This enables predicting the regulation of cellular responses with the observation of temporal changes in the concentration of IL-6 [15,45].

M2-type macrophages are responsible for modulating and closing the inflammatory response and are crucial for tissue remodeling and repair [46,47]. However, there was a negative correlation between IL-10 and TNF-α in the initial healing period for both groups, and between IL-10 and IL-6 in the IML group. These results may indicate that IL-10 is suppressed by the release of pro-inflammatory markers during the early stages of bone healing. During osseointegration, IL-10 is responsible for promoting bone formation, reducing or inhibiting the differentiation and activity of osteoclasts and osteoblasts, and increasing collagen synthesis [46]. The concentration of IL-10 was similar for both groups up to week 8, and there was a progressive increase in the concentration over time, as observed by Bielemann et al. (2017) [15]. At week 12, there was a statistically significant difference between the groups: the IML group released 45.74% more IL-10 than the CL group ($p = 0.003$). We suggest that this is related to the need to inhibit the more active remodeling process in the IML group. This also evidences the anti-inflammatory action of IL-10 and its action as a suppressor in the osteoclastic differentiation, finalizing the bone resorption process [48]. At week 8, there was a positive correlation between IL-10 and TNF-α and IL-1β biomarkers in both groups, evidencing the inhibiting role of IL-10 in the final phase of osseointegration, reducing the pro-inflammatory response of the other cytokines [47].

Finally, this was the first randomized clinical study that evaluated the biology of osseointegration of NDI implants as MO retainers subjected to different loading protocols. Although the sample of this study was small, it was adequate to provide statistically significant results. Our study found a survival rate of 95% for the CL group and 90% for the IML group, resembling the 95.11% survival rate described in the systematic review by Lemos et al. (2017) [49]. Thus, we observe that it is possible to achieve a high predictability of osseointegration of NDIs as MO retainers for the rehabilitation of mandibles with high edentulism time, irrespective of the loading protocol adopted. The survival and success rates were similar in both groups, as already described in the literature [17]. Nonetheless, clinically and physiologically distinct results were found, showing that the IML group presented more predictable peri-implant health results, while the implant stability and the inflammatory marker concentrations were more stable in the CL group. Therefore, studies that compare biologically different implant loading protocols are still necessary to further investigate

the different healing profile that each loading protocol generates. Furthermore, investigating new bone markers to investigate bone resorption kinetics would be useful. Finally, the normal baseline levels of the inflammatory markers during healing are not yet established in the literature. This information is essential for reliable short- and long-term monitoring of osseointegration.

5. Conclusion

Our peri-implant clinical health results revealed that IML promotes conditioning of the peri-implant tissues, because of the stability provided by the prosthesis. However, IML also generated a more intense inflammatory response until the end of osseointegration compared to CL, as evidenced by the higher concentrations of TNF-α and IL-1β, and clinical parameters. Furthermore, the secondary ISQ and the more stable concentration of pro- and anti-inflammatory cytokines in the CL group indicate more stable biological results. Thus, we recommend adopting conventional loading of narrow diameter implants for rehabilitation of patients with high edentulism time and low bone availability.

References

1. Davies JES. Understanding peri-implant endosseous healing. *J Dent Educ*. 2003;67(8):932-949.
2. Pan S, Dagenais M, Thomason JM, et al. Does mandibular edentulous bone height affect prosthetic treatment success? *J Dent*. 2010;38(11):899-907.
3. Ortega-Oller I, Suárez F, Galindo-Moreno P, et al. The Influence of Implant Diameter on Its Survival: A Meta-Analysis Based on Prospective Clinical Trials. *J Periodontol*. 2014;85(4):569-580.
4. Klein MO, Schiegnitz E, Al-Nawas B. Systematic review on success of narrow-diameter dental implants. *Int J Oral Maxillofac Implants*. 2014;29 Suppl:43-54.
5. Marcello-Machado RM, Faot F, Schuster AJ, Nascimento GG, Del Bel Cury AA, Drive G. Mini-implants and narrow diameter implants as mandibular overdenture retainers: A systematic review and meta-analysis of clinical and radiographic outcomes Running. *J Oral Rehabil*. 2018;45(2):161-183.
6. Heydecke G, Rompre PH, Grandmont P De, et al. Impact of implant support for mandibular dentures on satisfaction, oral and general health-related quality of life: A meta-analysis of randomized- controlled trials. *Clin Oral Implants Res*. 2009;20(6):533-544.
7. Emami E, Cerutti-Kopplin D, Menassa MM, et al. Does immediate loading affect clinical and patient-centered outcomes of mandibular 2-unsplinted-implant overdenture? A 2-year within-case analysis. *J Dent*. 2016;50:30-36.
8. Schuster AJ, Marcello-Machado RMRM, Bielemann AM, et al. Short-term quality of life change perceived by patients after transition to mandibular overdentures. *Braz Oral Res*. 2017;31(0):1-9.
9. De Bruyn H, Raes S, Ostman P-O, Cosyn J. Immediate loading in partially and completely edentulous jaws: a review of the literature with clinical guidelines. *Periodontol 2000*. 2014;66(1):153-187.
10. Berglundh T, Abrahamsson I, Lang NP, Lindhe J. De novo alveolar bone formation adjacent to endosseous implants. *Clin Oral Implants Res*. 2003;14(3):251-262.
11. Duyck J, Roesems R, Cardoso M V., Ogawa T, De Villa Camargos G, Vandamme K. Effect of insertion torque on titanium implant osseointegration: an animal experimental study. *Clin Oral Implants Res*. 2015;26(2):191-196.
12. Javed F, Ahmed HB, Crespi R, Romanos GE. Role of primary stability for successful osseointegration of dental implants: Factors of influence and evaluation. *Interv Med Appl Sci*. 2013;5(4):162-167.

13. Prati AJ, Casati MZ, Ribeiro FV, et al. Release of Bone Markers in Immediately Loaded and Nonloaded Dental Implants. *J Dent Res*. 2013;92(12_suppl):161S-167S.
14. Boynueğri AD, Yalim M, Nemli SK, et al. Effect of different localizations of microgap on clinical parameters and inflammatory cytokines in peri-implant crevicular fluid: a prospective comparative study. *Clin Oral Investig*. 2012;16(2):353-361.
15. Bielemann AM, Marcello-Machado RM, Leite FRM, et al. Comparison between inflammation-related markers in peri-implant crevicular fluid and clinical parameters during osseointegration in edentulous jaws. *Clin Oral Investig*. 2018;22(1):531-543.
16. Elsyad MA, Mahanna FF, Elshahat MA, Elshoukouki AH. Locators versus magnetic attachment effect on peri-implant tissue health of immediate loaded two implants retaining a mandibular overdenture: a 1-year randomised trial. *J Oral Rehabil*. 2016;43(4):297-305.
17. Acham S, Rugani P, Truschnegg A, Wildburger A, Wegscheider WA, Jakse N. Immediate loading of four interforaminal implants supporting a locator-retained mandibular overdenture in the elderly. Results of a 3-year randomized, controlled, prospective clinical study. *Clin Implant Dent Relat Res*. 2017;19(5):895-900.
18. Schimmel M, Srinivasan M, Herrmann FR, Müller F. Loading protocols for implant-supported overdentures in the edentulous jaw: a systematic review and meta-analysis. *Int J Oral Maxillofac Implants*. 2014;29 Suppl(Supplement):271-286.
19. Zygogiannis K, Wismeijer D, Aartman I, Osman R. A Systematic Review on Immediate Loading of Implants Used to Support Overdentures Opposed by Conventional Prostheses: Factors That Might Influence Clinical Outcomes. *Int J Oral Maxillofac Implants*. 2016;31(1):63-72.
20. Moher D, Schulz KF, Altman DG. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomised trials. *Clin Oral Investig*. 2003;7(1):2-7.
21. Kapur KK. A clinical evaluation of denture adhesives. *J Prosthet Dent*. 1967;18(6):550-558.
22. Bornstein MM, Cionca N, Mombelli A. Systemic Conditions and Treatments as Risks for Implant Therapy. *Int J Oral Maxillofac Implant*. 2009;24:12-27.
23. Marcello-Machado RMRM, Bielemann AMAM, Nascimento GG, et al. Masticatory function parameters in patients with varying degree of mandibular bone resorption. *J Prosthodont Res*. 2016;61(3):315-323.
24. Misch CE, Perel ML, Wang H-LL, et al. Implant success, survival, and failure: the International Congress of Oral Implantologists (ICOI) Pisa Consensus Conference.

Implant Dent. 2008;17(1):5-15.

25. Papaspyridakos P, Chen CJ, Singh M, Weber HP, Gallucci GO. Success criteria in implant dentistry: a systematic review. *J Dent Res*. 2012;91(3):242-248.
26. Mukaka MM. Statistics corner: A guide to appropriate use of correlation coefficient in medical research. *Malawi Med J*. 2012;24(3):69-71.
27. Romanos G. Biomolecular Cell-Signaling Mechanisms and Dental Implants: A Review on the Regulatory Molecular Biologic Patterns Under Functional and Immediate Loading. *Int J Oral Maxillofac Implants*. 2016;31(4):939-951.
28. De Andrade Lima Chaves C, Machado AL, Vergani CE, De Souza RF, Giampaolo ET. Cytotoxicity of denture base and hard chairside reline materials: A systematic review. *J Prosthet Dent*. 2012;107(2):114-127.
29. Nishimiya H, Yamada M, Ueda T, Sakurai K. N-acetyl cysteine alleviates inflammatory reaction of oral epithelial cells to poly (methyl methacrylate) extract. *Acta Odontol Scand*. 2015;73(8):616-625.
30. Tözüm TF, Lser Türkyılmaz I ·, Yamalık N, et al. The effect of delayed versus early loading on nitric oxide metabolism around dental implants: an 18-month comparative follow-up study. *Int J Oral Maxillofac Implants*. 2007;22(1):53-62.
31. Scepanovic M, Todorovic A, Markovic A, et al. Immediately loaded mini dental implants as overdenture retainers: 1-Year cohort study of implant stability and peri-implant marginal bone level. *Ann Anat*. 2015;199:85-91.
32. Gokmenoglu C, Ozmeric N, Erguder I, Elgun S. The effect of light-emitting diode photobiomodulation on implant stability and biochemical markers in peri-implant crevicular fluid. *Photomed Laser Surg*. 2014;32(3):138-145.
33. Fu M-W, Fu E, Lin F-G, Chang W-J, Hsieh Y-D, Shen E-C. Correlation Between Resonance Frequency Analysis and Bone Quality Assessments at Dental Implant Recipient Sites. *Int J Oral Maxillofac Implants*. 2017;32(1):180-187.
34. Kim SH, Choi BH, Li J, et al. Peri-implant bone reactions at delayed and immediately loaded implants: an experimental study. *Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology*. 2008;105(2):144-148.
35. Romanos GE. Wound healing in immediately loaded implants. *Periodontol 2000*. 2015;68(1):153-167.
36. Trindade R, Albrektsson T, Wennerberg A. Current concepts for the biological basis of dental implants: foreign body equilibrium and osseointegration dynamics. *Oral Maxillofac Surg Clin North Am*. 2015;27(2):175-183.
37. Tözüm TF, Turkyilmaz I, Yamalik N, Karabulut E, Eratalay K. Analysis of the Potential

Association of Implant Stability, Laboratory, and Image-Based Measures Used to Assess Osteotomy Sites: Early Versus Delayed Loading. *J Periodontol*. 2007;78(9):1675-1682.

38. Tözüm TF, Türkyilmaz I, Yamalik N. Analysis of the Possible Impact of Inflammation Severity and Early and Delayed Loading on. *Int J oral Maxillofac Implant*. 2005;(20):547-556.
39. Rakic M, Nikolic-Jakoba N, Struillou X, et al. Receptor activator of nuclear factor kappa B (RANK) as a determinant of peri-implantitis. *Vojnosanit Pregl*. 2013;70(4):346-351.
40. Slotte C, Lennerås M, Göthberg C, et al. Gene expression of inflammation and bone healing in peri-implant crevicular fluid after placement and loading of dental implants. A kinetic clinical pilot study using quantitative real-time PCR. *Clin Implant Dent Relat Res*. 2012;14(5):723-736.
41. Hall J, Pehrson N-G, Ekestubbe A, Jemt T, Friberg B. A controlled, cross-sectional exploratory study on markers for the plasminogen system and inflammation in crevicular fluid samples from healthy, mucositis and peri-implantitis sites. *Eur J Oral Implantol*. 2015;8(2):153-166.
42. Sato R, Matsuzaka K, Kokubu E, Inoue T. Immediate loading after implant placement following tooth extraction up-regulates cellular activity in the dog mandible. *Clin Oral Implants Res*. 2011;22(12):1372-1378.
43. Freshney NW, Rawlinson L, Guesdon FF, et al. Interleukin-1 activates a novel protein kinase cascade that results in the phosphorylation of hsp27. *Cell*. 1994;78(6):1039-1049.
44. Attard NJ, Zarb GA, Laporte A. Long-term treatment costs associated with implant-supported mandibular prostheses in edentulous patients. *Int J Prosthodont*. 2005;18(2):117-123.
45. Jawa RS, Anillo S, Huntoon K, Baumann H, Kulaylat M. Analytic review: Interleukin-6 in surgery, trauma, and critical care: part I: basic science. *J Intensive Care Med*. 2011;26(1):3-12.
46. Loi F, Córdova LA, Pajarinen J, Lin T hua, Yao Z, Goodman SB. Inflammation, fracture and bone repair. *Bone*. 2016;86:119-130.
47. Gu Q, Yang H, Shi Q. Macrophages and bone inflammation. *J Orthop Transl*. 2017;10:86-93.
48. Zhang Q, Chen B, Yan F, et al. Interleukin-10 inhibits bone resorption: a potential therapeutic strategy in periodontitis and other bone loss diseases. *Biomed Res Int*. 2014;2014:284836.

49. Lemos CAA, Verri FR, Batista VE de S, et al. Complete overdentures retained by mini implants: A systematic review. *J Dent*. 2017;57:4-13.

Table 1. Patient characteristics according to immediate (IML) and conventional loading (CL).

	IML (n=10)	**CL (n=10)**	**Overall (n=20)**
Gender (Female/Male)	5/5	7/3	12/8
Age (years)	66.80 ± 8.92	67.00 ± 3.24	66.90 ± 6.61
Mandibular Edentulism Time (years)	23.50 ± 15.20	22.90 ± 11.67	23.20 ± 13.22
Mandibular Body Length (mm)	107.09 ± 10.01	111.87 ± 8.62	109.48 ± 9.22
Bone height in the anterior region (mm)	23.88 ± 4.75	23.67 ± 2.64	23.77 ± 3.74
Bone height in the posterior region (mm)	14.29± 3.66	15.42± 2.73	14.85 ± 3.22
Superior height of the foramina (mm)	3.47 ± 3.72	4.42 ± 2.58	3.95 ± 3.03
Bone Type (Type I / Type II)*	14 / 6	6 / 14	20 / 20
Bone Atrophy (Yes/No)$^{+}$	6 / 4	5 / 5	11 / 9

* Bone Type values refer to number of implants

$^{+}$Gender and Bone Atrophy values refer to number of patients

Table 2. Results of peri-implant health outcomes according to intragroup and intergroup comparisons at different healing periods. IML, immediate loading; CL, conventional loading.

Week	**1**		**2**		**4**		**8**		**12**	
	IML	**CL**	**IML**	**CL**	**IML**	**CL**	**IML**	**CL**	**IML**	**CL**
					% Presence					
PI	70.0 AD	60.0 AB	50.0 AB	40.0 A	70.0 AC	70.0 BC	30.0 BC	30.0 A	33.3 BD	60.0 AC
Calculus	0 A	0 A	0 A	0 A	5.0 A	10.0 A	0 *A	30.0 *A	0 *A	20.0 *A
GI	90.0 *A	40.0 *A	30.0 B	35.0 A	0 C	15.0 B	0 *C	30.0 *AB	0 BC	10.0 B
BOP	.	.	15.0 AB	25.0 A	0 A	15.0 A	10.0 AB	20 A	38.9 B	10.0 *A
					mm Mean(SD)					
PD	.	.	3.38 ± 0.96 *A	4.02 ± 0.96 *A	2.70± 0.84 *B	3.41± 1.08 *B	2.36 ± 0.86 *BC	3.01± 1.03 *BC	2.00± 0.72 *C	2.80± 0.78 *C

*Asterisks show the differences between IML and CL groups at each period (Chi-square Test and T-test, $p<0.05$). Letters show the significant differences found for intragroup comparisons (McNemar Test and Paired T-test, $p<0.05$).

Table 3. Correlation between implants stability quotient (ISQ) and patients' characteristics, clinical parameters and cytokines concentration (pg/µl), according to each evaluation period for IML and CL groups. R_p values are related to Pearson correlation coefficients, and R_s values are related to Spearman correlation.

	ISQ									
	Week 1		Week 2		Week 4		Week 8		Week 12	
	IML	CL	IML	CL	IML	CL	IML	CL	IML	CL
Age (years)	p= 0.031 R_p= -0.483				p < 0.001 R_p= -0.607		p= 0.003 R_p= -0.622			
Mandible Edentulism Time (years)				p= 0.024 R_p= 0.504						
Bone Atrophy (Y/N)								p= 0.026 R_p= 0.498		p= 0.004 R_p= 0.625
PI (Y/N)		p= 0.035 R_p= 0.475								
GI (Y/N)	p= 0.036 R_p= 0.471			p < 0.001 R_p= -0.724						
PD (mm)							p= 0.002 R_p= -0.644			
TNF-α (pg/µl)					p= 0.007 R_s= -0.584					
IL-6 (pg/µl)						p = 0.005 R_s= 0.603	p= 0.011 R_s= 0.653			

No correlations between ISQ and IL-1β and IL-10 were observed.

Table 4. Correlation between cytokines concentration (pg/µl) and clinical parameters according to each evaluation period for IML and CL groups. R_p values are related to Pearson correlation coefficients, and R_s values are related to Spearman correlation.

	Week 1		Week 2		Week 4		Week 8		Week 12	
	IML	**CL**	**IML**	**CL**	**IML**	**CL**	**IML**	**CL**	**IML**	**CL**
	IL-10		**IL-10**		**IL-10**		**IL-10**		**IL-10**	
IL-1β							p= 0.010 R_s= 0.664	p= 0.013 R_s= 0.547		
TNF-α	p= 0.006 R_s= -0.698			p= 0.003 R_s= -0.632			p= 0.002 R_s= 0.758	p= 0.004 R_s= 0.614		
IL-6			p= 0.001 R_s=-0.791		p= 0.046 R_s= -0.540					
PI		p= 0.018 R_s= 0.521								
	TNF-α		**TNF-α**		**TNF-α**		**TNF-α**		**TNF-α**	
IL-1β	p= 0.037 R_s= 0.468		p= 0.014 R_s= 0.542		p < 0.001 R_s= 0.716		p < 0.001 R_s= 0.861	p= 0.005 R_s= 0.603		
IL-6										p= 0.001 R_s= -0.691
PI					p= 0.005 R_s= -0.604					
	IL-1β		**IL-1β**		**IL-1β**		**IL-1β**		**IL-1β**	
IL-6	p= 0.001 R_s= 0.785	p= 0.017 R_s= 0.526		p= 0.033 R_s= 0.478						p= 0.029 R_s=0.501
PI				p= 0.004 R_s= -0.618	p= 0.005 R_s= -0.607					
GI								p= 0.017 R_s= 0.525		p= 0.019 R_s= 0.532
	IL-6		**IL-6**		**IL-6**		**IL-6**		**IL-6**	
PI						p= 0.001 R_s= -0.666	p= 0.044 R_s= -0.544		p= 0.010 R_s= 0.707	
PD									p= 0.005 R_s= -0.748	
			GI		**GI**		**GI**		**GI**	
PD						p= 0.003 R_p= 0.623				p < 0.001 R_p= 0.814

Figure 1: CONSORT flow diagram.

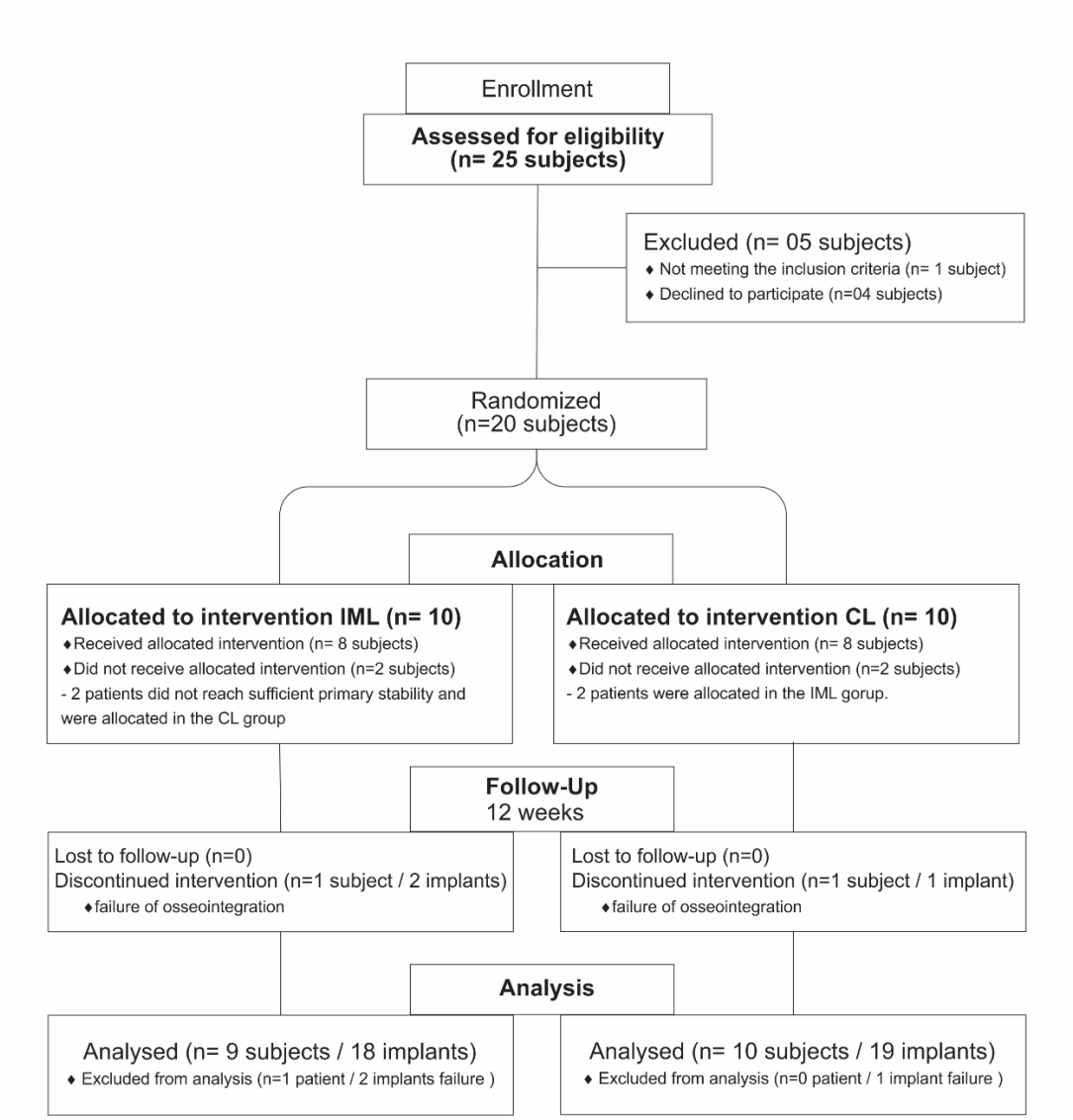


Figure 2: Kaplan-Meier survival curve of narrow diameter implants according to IML and CL groups during 12 weeks of follow-up.

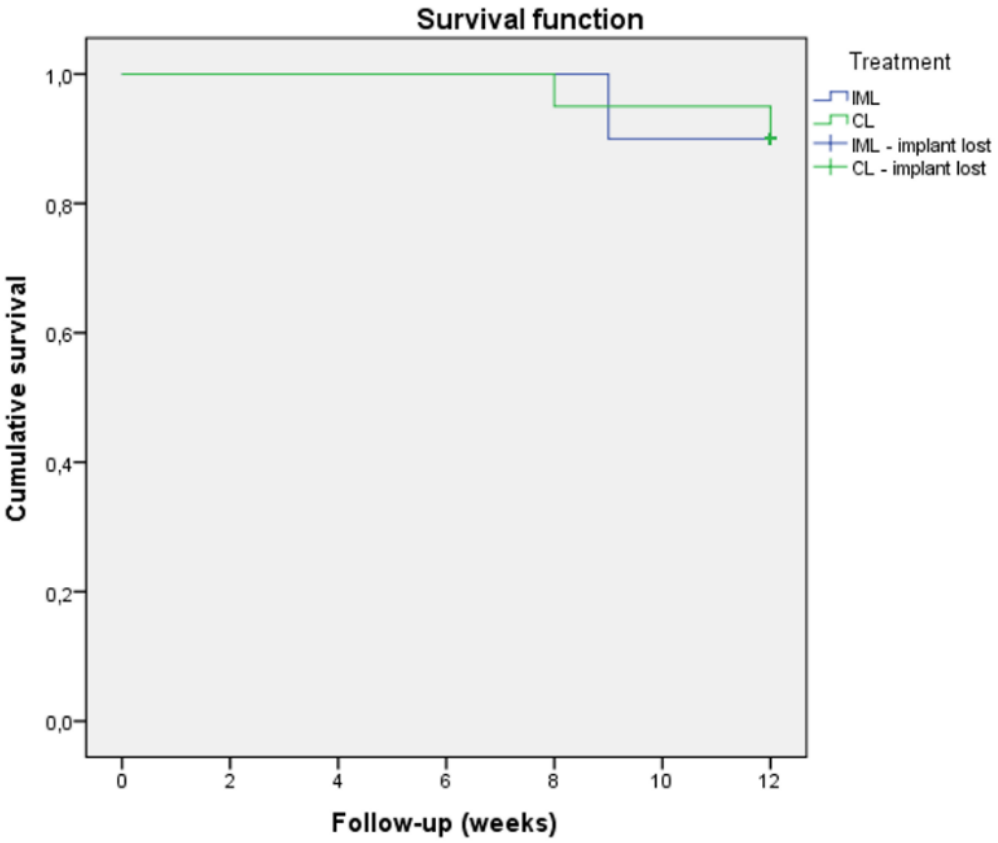


Figure 3: Implant stability (ISQ values) for IML and CL groups during the evaluation periods. Asterisk (*) indicates a statistically significant difference between the groups (T test, $p < .05$); lowercase letters indicate a statistically significant difference among the periods of evaluation within CL group and uppercase letters within IML group (Paired T test, $p < .05$).

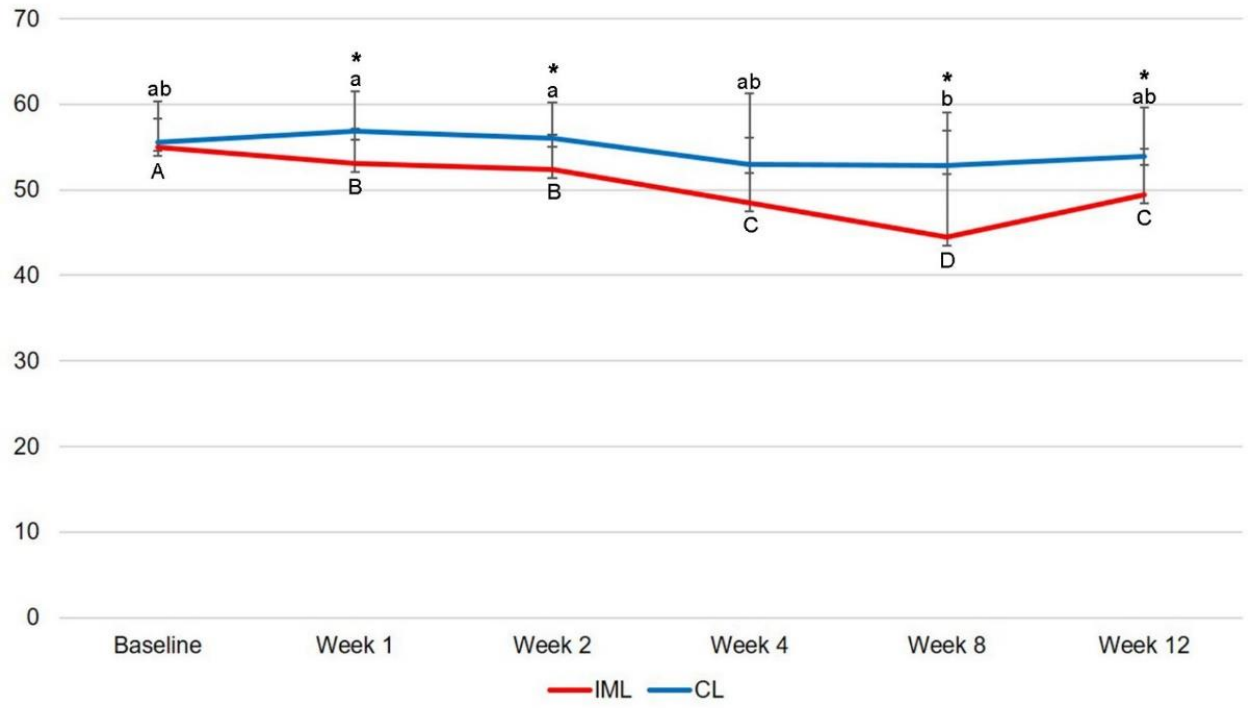
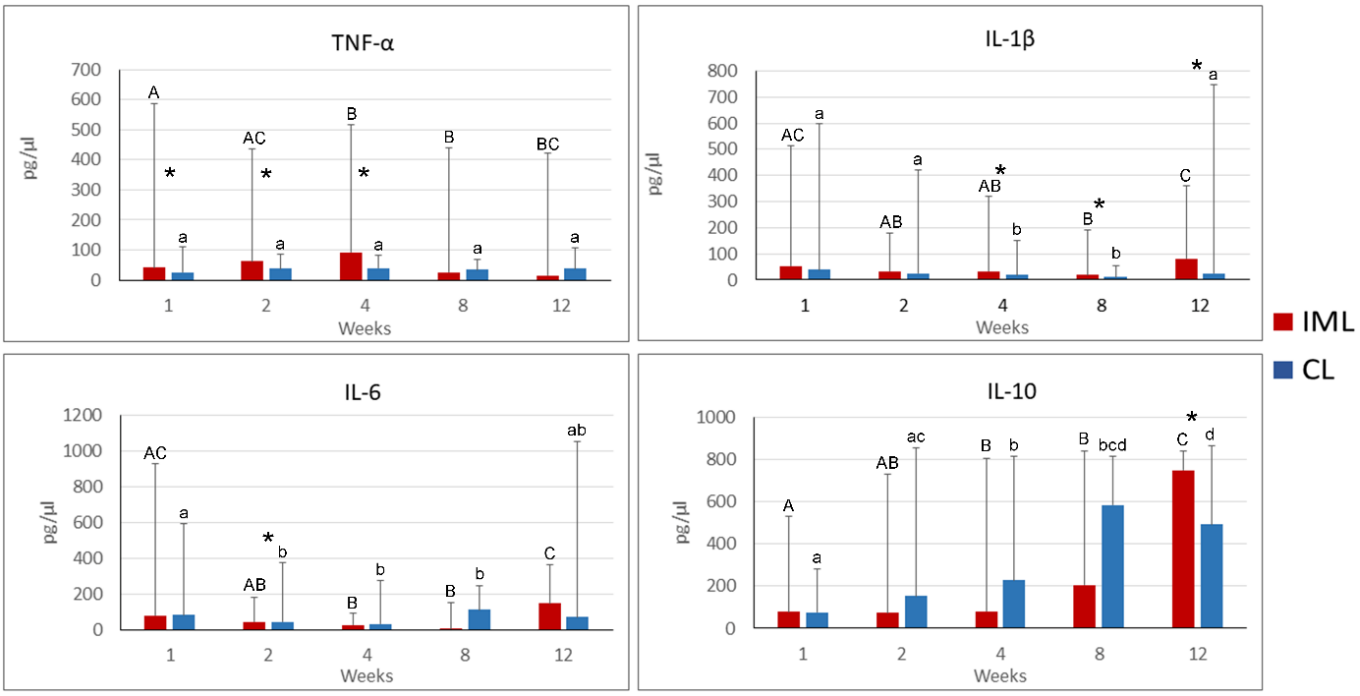


Figure 4: Comparisons between the median and ranges of the inflammatory markers concentration (pg/µl) according to the intragroup and intergroup comparisons at different periods for IML and CL. Asterisk (*) indicates a statistically significant difference between groups (Mann-Whitney Test, p<0.05); Lowercase letters indicate a statistically significant difference among the periods of evaluation within CL group and uppercase letters within IML group (Wilcoxon Test, p<0.05).



APPENDIX B - CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	1
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	2
	2b	Specific objectives or hypotheses	2-3
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	4
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	4
Participants	4a	Eligibility criteria for participants	4
	4b	Settings and locations where the data were collected	4
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	4-7
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	Figure 1
Sample size	7a	How sample size was determined	4
	7b	When applicable, explanation of any interim analyses and stopping guidelines	-
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	5
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	55
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	5
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	5
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	-
	11b	If relevant, description of the similarity of interventions	-
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	7

	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	7
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	8
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	4
	14b	Why the trial ended or was stopped	-
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Table 1
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	-
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Table 2
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	Table 3 Table 4
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	-
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	15
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	11-15
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	11-1
Other information			
Registration	23	Registration number and name of trial registry	-
Protocol	24	Where the full trial protocol can be accessed, if available	-
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	-

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

Discussão Geral e Recomendações

A reabilitação oral por meio de implantes dentários é uma realidade na prática clínica, e o sucesso da osseointegração do conjunto osso-implante é comprovado através dos resultados obtidos por estudos *in vitro, in vivo* e clínicos. Contudo, falhas são recorrentes e podem estar associadas a peri-implantite e mucosite, adicionalmente há falhas decorrentes do insucesso da osseointegração sem relação com agentes patógenos, as quais ainda não são bem explicadas, mas que podem estar associadas a resposta biológica e imunológica do hospedeiro. Uma das formas de realizar o monitoramento peri-implantar, na tentativa de se diagnosticar processos de falhas precoces, é através do mapeamento dos biomarcadores inflamatórios presentes neste micro-ambiente

O capítulo 1 desta tese, por meio de uma revisão sistemática, realizou um mapeamento dos estudos clínicos que investigaram os biomarcadores presentes no fluido peri-implantar antes do carregamento dos implantes. Os resultados desses estudos foram sintetizados e associados com os eventos biológicos já conhecidos, afim de demonstrar quais os biomarcadores já quantificados e associados com as fases de osseointegração. É evidente que o sistema imune é o responsável pelo reconhecimento do implante, consequentemente pela osseointegração, desencadeando as respostas imuno-dirigidas deste processo (TRINDADE et al., 2018). Estando, esse, diretamente influenciado pelas carcaterísticas do hospedeiro, em que, as respostas podem ser diferentes para cada distúrbio crônico e/ou sistêmico, como diabetes, osteopenia, tabagismo, terapia medicamentosa e atrofia dos ossos. Adicionalmente, fatores locais também podem desencadear uma reação inflamatória modificada, decorrentes de cirurgias iatrogênicas, do superaquecimento durante a perfuração, de fragmentos liberados da superfície do implante, do torque de inserção, e de micro-movimentos sobre os implantes.

De acordo com a revisão de Noronha Oliveira e colaboradores (2018), as citocinas pró-inflamatórias, a infiltração de células inflamatórias e a ativação de osteoclastos nos tecidos peri-implantares são estimuladas pela presença de partículas metálicas e íons. Evidenciando que, cada etapa, estimula a liberação de fatores relacionados à cicatrização com potencial para serem utilizados como biomarcadores clínicos. Os resultados de MA e colaboradores (2018), destacaram que, fatores de confundimendo como a nanoestrutura da superfície do implante, a resposta inflamatória dos macrófagos e a diferenciação osteogênica das células-tronco mesenquimais ósseas (CTMOs), influenciam a osseointegração de implantes. Além

disso, os efeitos retro-reguladores das CTMOs na diferenciação osteoclástica dos macrófagos e do sistema e meio de cultura, em função da superfície do implante, podem fornecer uma abordagem prospectiva para melhorar a osseointegração do implante via regulação imune.

A revisão sistemática proposta no estudo 1, identificou 52 biomarcadores investigados durante 16 semanas de osseointegração após a inserção do implante. Relantando resultados de estudos com pacientes que receberam implantes com e sem carregamento oclusal e, estudos de pacientes com complicações sistêmicas e grupos controle saudáveis. Identificou-se alguns fatores de confusão, os quais, podem influenciar a resposta do osso hospedeiro, pela mediação da liberação de citocinas pró- e anti-inflamatórias, quimiocinas, fatores de crescimento, moduladores de células T e biomarcadores ósseos da angiogênese. Esses fatores incluem idade, diabetes, tabagismo, higiene bucal, características do sítio ósseo, administração de antibióticos pré- e pós-operatórios, além de aspectos cirúrgicos, como técnica de retalho, protocolos de perfuração e torque de inserção. Alguns estudos (BIELEMANN et al., 2018; PRATI et al., 2013; SAYARDOUST et al., 2017; SAYARDOUST; OMAR; THOMSEN, 2017; TSOUKAKI et al., 2013; VERRASTRO NETO et al., 2017) adaptaram seu desenho experimental e seleção de pacientes, para investigar a influência desses fatores na liberação de citocinas durante os estágios iniciais e tardios da osseointegração.

Durante a pimeira fase de cicatrização, entre 0 e 14 dias, sugere-se que a coleta de FCPI durante as primeiras 24 horas não seja ideal, devido a contaminação das amostras com sangue advindo dos tecidos peri-implantares, assim como, o infiltrado inflamatório deste tecido (WANG; ZHANG; MIRON, 2016). Quanto a administração de antibióticos, a profilaxia com amoxicilina afetou os parâmetros clínicos, mas não os biológicos, IL-1β e IL-8, sugerindo que o tratamento não suprime a inflamação durante a osseointegração (CHRCANOVIC; ALBREKTSSON; WENNERBERG, 2014; KHOURY et al., 2008). No entanto, a administração de clorexidina para a manutenção da higiene pode afetar os parâmetros clínicos periodontais e o conteúdo de FCPI (KHOURY et al., 2008). Durante a primeira semana as altas concentrações dos biomarcadores: ALP (MANDIC et al., 2015), MMP-8 (TAKASHI NOMURA et al., 2000; TSOUKAKI et al., 2013), IL-6 e IL-8 (BIELEMANN et al., 2018; EMECEN-HUJA et al., 2013; KHOURY et al., 2008), refletem a resposta inflamatória inicial devido ao trauma cirúrgico.

Ao longo da fase intermediária de cicatrização, entre 14 e 30 dias, sugere-se que o carregamento imediato (CI) induza a liberação precoce de OPG e OPN, enquanto para o carregamento convencional (CC), ocorra no período tardio e pós-osseointegração. A concentração dos biomarcadores pró-inflamatórios, IL-1β e IL-6, em implantes com CC reduziram ao longo do período inicial de cicatrização, da semana 1 até a 12 (BIELEMANN et al., 2018; EMECEN-HUJA et al., 2013; SLOTTE et al., 2012), já para o CI, o carregamento funcional dos implantes ocasionou o aumento de IL-1β (ELSYAD et al., 2016; SLOTTE et al., 2012). Esses achados sugerem que o carregamento oclusal estimula a liberação de fatores pró-inflamatórios, favorecendo a remodelação óssea e a neoformação ocorram simultaneamente com a carga funcional dos implantes (RAGHAVENDRA et al., 2005).

Durante a cicatrização tardia, após 30 dias, níveis reduzidos de TRAP em implantes inseridos com baixo torque de inserção (VERRASTRO NETO et al., 2017), sugerem um impacto positivo na resposta do hospedeiro local em torno desses implantes. Em adição, a liberação de TRAP aos 30 dias, teve correlação possitiva com a estabilidade primária (SLOTTE et al., 2012), indicando que a TRAP é um biomarcador de reabsorção óssea envolvido no recrutamento e função de osteoclastos (HALL et al., 2015). Já aos 90 dias, o CC apresentou aumento da expressão de ALP, correlação negativa entre a ALP e cicatrização dos tecidos moles peri-implantares (SLOTTE et al., 2012), e correlação positiva entre a ALP e o ISQ (TIRACHAIMONGKOL et al., 2016). Esses resultados evidenciam que a expressão de ALP e subseqüente neogênese óssea, está relacionada às células ósseas pré-existentes ao redor do implantes (STUCKI et al., 2001). Indicando que a maturação óssea ocorre antes do aumento de ALP, e que esse, poderia ser um biomarcador geral do metabolismo ósseo pois, desempenha um papel importante na progressão da mineralização da matriz osteóide (PLAGNAT et al., 2002).

Adicionalmente os fatores confundidores como diabetes, demonstraram que nesses pacientes, com controle glicêmico comprometido, há um perfil distinto de fatores relacionados ao tecido ósseo que podem prejudicar o processo de reparo ósseo. Biomarcadores osteogênicos e/ou de mineralização óssea foram reprimidos em pacientes com diabetes pouco controlada, uma vez que as menores concentrações de OPN foram encontradas em 12 meses (GHIRALDINI et al., 2016). Pacientes com osteopenia não apresentaram diferenças significativas nos níveis de fatores relacionados à osteoclastogênese (sRANKL e OPG) em implantes com CI,

sugerindo que a remodelação óssea no ambiente peri-implantar não é afetada pela osteopenia (ONUMA et al., 2015). Já em pacientes fumantes, observou-se que o fumo tem um efeito precoce sobre a osseointegração, a qual é dependente das propriedades da superfície do implante e da resposta local do hospedeiro. As falhas ocorridas nesses pacientes também podem ser atribuídas a uma estrutura e composição alteradas do osso hospedeiro (SAYARDOUST et al., 2017; SAYARDOUST; OMAR; THOMSEN, 2017). O fumo também, foi capaz de influenciar a liberação de TNF-α, IL-1β, IL-6 e IL-10, em que, a IL-10 foi a citocina mais afetada, uma vez que pacientes não fumantes apresentaram uma maior liberação dessa interleucina durante as 12 semanas de osseointegração (BIELEMANN et al., 2018).

Por fim, acrescentamos que devido a heterogeinidade metodológica dos estudos incluídos na RS, não foi possível agrupar os dados afim de realizar uma meta-análise das concentrações de biomarcadores presentes no FCPI. Os resultados dos estudos selecionados também, não possibilitaram o agrupamento por tempo biológico ou tipo de biomarcador, inviabilizando definir quais biomarcadores são mais relevantes em cada fase da osseointegração. Devido a isso, a estrutura de apresentação deste estudo é um instrumento de consulta facilitador para pesquisadores e clínicos utilitizem-no para compreender o prognóstico de falha dos implantes, assim como, do diagnóstico precoce das doenças peri-implantares com base na suscetibilidade do paciente. Até o momento, os parâmetros clínicos de monitoramento da saúde peri-implantar ainda são o padrão ouro para ser executado na prática clínica. Para o futuro, quando definidos os biomarcadores adequados para o monitoramento peri-implantar, assim como, seus valores referenciais, a coleta de FCPI será uma ferramenta de diagnóstico precoce a ser utilizada na prática clínica.

O capítulo 2, apresentou um estudo clínico randomizado que acompanhou o a cicatrização de IDRs como retentores de overdentures mandibulares (OM) submetidas ao CC e ao CI em pacientes com elevado tempo de edentulismo a baixa disponibilidade óssea. Sabe-se que as forças de compressão e tensão que atuam no osso após o carregamento imediato dos implantes proporcionam intensa remodelação óssea, devido ao maior conteúdo de osso mineral e contato osso-implante (ROMANOS, 2016; ROMANOS et al., 2002, 2003). Diferentemente, forças menores desencadeiam rápida neoformação óssea devido a menor área de contato entre o osso remanescente-implante (DUYCK et al., 2015). Ao nos deparamos com a ausência de um consenso na literatura, sobre qual o protocolo de carregamento deve

ser utilizado levando em consideração populações com características específicas, como idade, tempo de edentulismo e disponibilidade óssea, o estudo 2 foi delineado. Este ensaio clínico randomizadado investigou o perfil da resposta inflamatória durante a cicatrização óssea e da cicatrização dos tecidos moles peri-implantares de IDRs, instalados em pacientes com elevado tempo de edentulismo, 23.20(13.22) anos, e baixa disponibilidade óssea 23.77(±3.74) mm, submetidos a dois tipos de carregamentos, CI e CC. No total 40 implantes de diâmetro reduzido (IDRs) (2.9 x 10.0mm – Facility Neoporos - Neodent, Curitiba, Brazil) foram instalados na região anterior de mandíbula, apresentando taxa de sucesso e sobrevivência de 90% para ambos grupos. As falhas reportadas ocorreram na fase de ossointegraçao e não foram possíveis de serem diagnosticadas por índices clínicos de insucesso nem pela presença de trauma. Os IDRs perdidos foram substituídos por implantes de diâmetro maior (3.5x9.0mm - Titamax Cone Morse Implant - Neodent Implants Osseointegrated, Curitiba, Brasil), os quais apresentaram 100% de taxa de sucesso. Assim, independentemente do protocolo de carregamento adotado, foi possível alcançar uma alta previsibilidade de osseointegração de IDRs como retentores de OM para a reabilitação de mandíbulas com alto tempo de edentulismo.

Os resultados em curto prazo mostraram que implantes submetidos ao carregamento imediato apresentaram um melhor condicionamento aos tecidos peri-implantares, devido aos melhores resultados clínicos, porém a estabilidade secundária deste grupo foi 9% inferior à do grupo CC, salientando que o CI pode necessitar de um tempo relativamente mais longo para reestabelecer a osseointegração. Entretanto o grupo CI, apresentou resposta inflamatória mais exacerbada e instável ao leito ósseo, evidente pela maior liberação de TNF-α e IL-1β, e na semana 12 pela produção exacerbada de IL-10, afim de conter e suspender a resposta pró-inflamatória. Assim, os resultados biológicos para o CC foram mais estáveis e seguros para a reabilitação com OM retidas por IDRs, evidenciado pelo ISQ e pela liberação mais estável de citocinas anti- e pró-inflamatórias.

Poucos estudos investigam a biologia da osseointegração de implantes como suporte de overdentures mandibulares (BOYNUEĞRI et al., 2012; ELSYAD et al., 2016; TÖZÜM et al., 2007a, 2007b; TÖZÜM; TÜRKYILMAZ; YAMALIK, 2005), bem como, a influência do carregamento na liberação de biomarcadores inflamatórios. Destacamos que, esse foi o primeiro estudo clínico randomizado que avaliou a biologia de osseointegração de IDRs como retentores de OM submetidos a diferentes

carregamentos oclusais, e apesar do tamanho amostral ser pequeno, este se mostrou adequada para prover resultados estatisticamente significantes. Entretanto, ainda há a necessidade do desenvolvimento de estudos que comparem biologicamente diferentes protocolos de carregamento oclusal de implantes. Assim, inúmeras dúvidas clínicas puderam ser levantadas relativas : i) ao diferente perfil de cicatrização que cada protocolo de carregamento desempenha, ii) a biomarcadores inflamatórios ósseos que ainda não foram quantificados e estudados detalhadamente, afim de, se investigar as diferenças na cinética de reabsorção óssea frente as forças mastigatórias, e iii) a inexistência da descrição de níveis de normalidade dos biomarcadores inflamatórios durante a cicatrização. Uma vez que um maior número de estudos clínicos controlados e randomizados estejam disponíveis nesta temática, será possível o desenvolvimento de uma ferramenta de diagnóstico baseada na coleta de FCPI. Assim, propiciando no futuro, o monitoramento mais confiável da saúde peri-implantar e, também, ao longo prazo da osseointegração.

Por fim, esta tese apresentou um mapeamento da osseointegração a partir dos resultados de estudos clínicos prévios e elucidou que ainda há necessidade de investigações mais profundas para compreenção das respostas imuno-dirigidas referentes a cicatrização peri-implantar. Em adição, o perfil de cicatrização em de uma parcela da população que necessita de maior previsibilidade no tratamento reabilitador, mostrou que ainda há necessidade de mais estudos que ilustrem a resposta imuno-inflamatória peri-implantar desencadeada pelos diferentes tipos de carregamentos oclusais para OM implanto-retidas.

Até o momento, os parâmetros clínicos de monitoramento da saúde peri-implantar ainda são o padrão ouro para ser adotado na prática clínica. Futuramente, quando definidos os biomarcadores adequados para o monitoramento peri-implantar assim como seus valores de referências, a coleta de FCPI será uma ferramenta de diagnóstico precoce a ser utilizada na na prática clínica.

Adicionalmente, sugere-se que para reabilitação de pacientes com elevado tempo de edentulismo e baixa disponibilidade óssea seja adotado o carregamento oclusal convencional. Implantes de diâmetro reduzido, em especial do sistema Facility-Equator, quando utilizados com carregamento oclusal convencional apresentaram resultados biológicos mais estáveis e seguros na reabilitação com overdentures mandibulares implnto-retidas evidenciados pela superioridade da estabilidade secundária (ISQ) e pela concentração mais estável de citocinas pró- e anti-inflamatórias no fluído peri-implantar durante o período de osseointegração.

ALBREKTSSON, T. et al. Osteoinduction, osteoconduction and osseointegration. **European Spine Journal**, v. 10, n. SUPPL. 2, p. S96–S101, 1 out. 2001.

ALBREKTSSON, T. et al. Initial and long-term crestal bone responses to modern dental implants. **Periodontology 2000**, v. 73, n. 1, p. 41–50, fev. 2017.

BIELEMANN, A. M. et al. Comparison between inflammation-related markers in peri-implant crevicular fluid and clinical parameters during osseointegration in edentulous jaws. **Clinical oral investigations**, v. 22, n. 1, p. 531–543, 14 jan. 2018.

BORNSTEIN, M. M.; CIONCA, N.; MOMBELLI, A. Systemic Conditions and Treatments as Risks for Implant Therapy. **International Journal of Oral & Maxillofacial Implants**, v. 24, p. 12–27, 2009.

BOYNUEĞRI, A. D. et al. Effect of different localizations of microgap on clinical parameters and inflammatory cytokines in peri-implant crevicular fluid: a prospective comparative study. **Clinical oral investigations**, v. 16, n. 2, p. 353–61, abr. 2012.

BRASIL, M. DA S. S. **SB Brasil 2010: Pesquisa Nacional de Saúde Bucal: resultados principais**. Brasília-DF: SVS, 2012.

CHANG, P.-C. C.; LANG, N. P.; GIANNOBILE, W. V. Evaluation of functional dynamics during osseointegration and regeneration associated with oral implants. **Clinical Oral Implants Research**, v. 21, n. 1, p. 1–12, jan. 2010.

CHO, S.-C. et al. Immediate loading of narrow-diameter implants with overdentures in severely atrophic mandibles. **Practical Procedures & Aesthetic Dentistry : PPAD**, v. 19, n. 3, p. 167–74, abr. 2007.

CHRCANOVIC, B. R.; ALBREKTSSON, T.; WENNERBERG, A. Reasons for failures of oral implants. **Journal of Oral Rehabilitation**, v. 41, n. 6, p. 443–476, jun. 2014.

DUARTE, P. M. et al. Could cytokine levels in the peri-implant crevicular fluid be used to distinguish between healthy implants and implants with peri-implantitis? A systematic review. **Journal of periodontal research**, v. 51, n. 6, p. 689–698, dez. 2016.

DUYCK, J. et al. Effect of insertion torque on titanium implant osseointegration: an animal experimental study. **Clinical Oral Implants Research**, v. 26, n. 2, p. 191–196, 2015.

ELSYAD, M. A. et al. Locators versus magnetic attachment effect on peri-implant tissue health of immediate loaded two implants retaining a mandibular overdenture: a 1-year randomised trial. **Journal of Oral Rehabilitation**, v. 43, n. 4, p. 297–305, abr. 2016.

EMECEN-HUJA, P. et al. Peri-implant versus periodontal wound healing. **Journal of Clinical Periodontology**, v. 40, n. 8, p. 816–824, ago. 2013.

FAOT, F. et al. Can Peri-Implant Crevicular Fluid Assist in the Diagnosis of Peri-Implantitis? A Systematic Review and Meta-Analysis. **Journal of Periodontology**, v. 86, n. 5, p. 631–645, maio 2015.

FERRARIO, V. F. et al. Quantification of translational and gliding components in human temporomandibular joint during mouth opening. **Archives of Oral Biology**, v. 50, n. 5, p. 507–515, 2005.

GHIRALDINI, B. et al. Influence of Glycemic Control on Peri-Implant Bone Healing: 12-Month Outcomes of Local Release of Bone-Related Factors and Implant Stabilization in Type 2 Diabetics. **Clinical Implant Dentistry and Related Research**, v. 18, n. 4, p. 801–809, ago. 2016.

GREENSTEIN, G.; CAVALLARO, J. Implant Insertion Torque: Its Role in Achieving Primary Stability of Restorable Dental Implants. **Compendium of continuing education in dentistry**, v. 38, n. 2, p. 88–95; quiz 96, fev. 2017.

HALL, J. et al. A controlled, cross-sectional exploratory study on markers for the plasminogen system and inflammation in crevicular fluid samples from healthy, mucositis and peri-implantitis sites. **European journal of oral implantology**, v. 8, n. 2, p. 153–66, 2015.

HOF, M. et al. Impact of Insertion Torque and Implant Neck Design on Peri-Implant Bone Level: A Randomized Split-Mouth Trial. **Clinical Implant Dentistry and Related Research**, v. 16, n. 5, p. 668–674, 2014.

IBGE, I. B. DE G. E E. **Projeção da população do Brasil por sexo e idade 1980-2050: revisão 2008**. [s.l: s.n.]. v. 24

IBGE, I. B. DE G. E E. **Pesquisa Nacional de Saúde 2013 - Percepção do estado de saúde, estilos de vida e doenças crônicas**. [s.l: s.n.]. v. 41

IBGE, I. B. DE G. E E. **Pesquisa Nacional por Amostra de Domicílios Contínua: Características gerais dos moradores 2012-2016**. [s.l: s.n.].

ISIDOR, F. Influence of forces on peri-implant bone. **Clinical Oral Implants Research**, v. 17, n. SUPPL. 2, p. 8–18, 2006.

KHOURY, S. B. et al. Early Wound Healing Following One-Stage Dental Implant Placement With and Without Antibiotic Prophylaxis: A Pilot Study. **Journal of Periodontology**, v. 79, n. 10, p. 1904–1912, out. 2008.

LIM, H.-S.; PARK, Y.-H.; KIM, S.-K. Relationship between Serum Inflammatory Marker and Bone Mineral Density in Healthy Adults. **Journal of Bone Metabolism**, v. 23, p. 27–33, 2016.

MA, Q.-L. et al. Bone mesenchymal stem cell secretion of sRANKL/OPG/M-CSF in response to macrophage-mediated inflammatory response influences osteogenesis on nanostructured Ti surfaces. **Biomaterials**, v. 154, p. 234–247, fev. 2018.

MANDIC, B. et al. Influence of postoperative low-level laser therapy on the osseointegration of self-tapping implants in the posterior maxilla: A 6-week split-mouth clinical study. **Vojnosanitetski pregled**, v. 72, n. 3, p. 233–240, mar. 2015.

MARCELLO-MACHADO, R. M. et al. Mini-implants and narrow diameter implants as mandibular overdenture retainers: A systematic review and meta-analysis of clinical

and radiographic outcomes. **Journal of Oral Rehabilitation**, v. 45, n. 2, p. 161–183, fev. 2018.

MONJE, A. et al. Sensitivity of Resonance Frequency Analysis for Detecting Early Implant Failure: A Case-Control Study. **The International Journal of Oral & Maxillofacial Implants**, v. 29, n. 2, p. 456–461, 2014.

NAERT, I. et al. A 10-year randomized clinical trial on the influence of splinted and unsplinted oral implants retaining mandibular overdentures: peri-implant outcome. **The International Journal of Oral & Maxillofacial Implants**, v. 19, n. 5, p. 695–702, 2004.

NORONHA OLIVEIRA, M. et al. Can degradation products released from dental implants affect peri-implant tissues? **Journal of Periodontal Research**, v. 53, n. 1, p. 1–11, 2 fev. 2018.

ONUMA, T. et al. Levels of Osteoclastogenesis-Related Factors in the Peri-Implant Crevicular Fluid and Clinical Parameters of Immediately Loaded Implants in Patients with Osteopenia: A Short-Term Report. **The International Journal of Oral & Maxillofacial Implants**, v. 30, n. 6, p. 1431–1436, nov. 2015.

PLAGNAT, D. et al. Elastase, alpha2-macroglobulin and alkaline phosphatase in crevicular fluid from implants with and without periimplantitis. **Clinical Oral Implants Research**, v. 13, n. 3, p. 227–233, 2002.

PRATI, A. J. J. et al. Release of Bone Markers in Immediately Loaded and Nonloaded Dental Implants. **Journal of Dental Research**, v. 92, n. 12_suppl, p. 161S–167S, 24 dez. 2013.

RAGHAVENDRA, S. et al. Early wound healing around endosseous implants: a review of the literature. **The International Journal of Oral & Maxillofacial Implants**, v. 20, n. 3, p. 425–31, 2005.

ROMANOS, G. Biomolecular Cell-Signaling Mechanisms and Dental Implants: A Review on the Regulatory Molecular Biologic Patterns Under Functional and Immediate Loading. **The International Journal of Oral & Maxillofacial Implants**, v. 31, n. 4, p. 939–951, ago. 2016.

ROMANOS, G. E. et al. Histologic and histomorphometric evaluation of peri-implant bone subjected to immediate loading: an experimental study with Macaca fascicularis. **The International Journal of Oral & Maxillofacial Implants**, v. 17, n. 1, p. 44–51, 2002.

ROMANOS, G. E. et al. Bone-implant interface around titanium implants under different loading conditions: a histomorphometrical analysis in the Macaca fascicularis monkey. **Journal of Periodontology**, v. 74, n. 10, p. 1483–90, out. 2003.

SAYARDOUST, S. et al. Clinical, radiological, and gene expression analyses in smokers and non-smokers, Part 2: RCT on the late healing phase of osseointegration. **Clinical Implant Dentistry and Related Research**, v. 19, n. 5, p. 901–915, out. 2017.

SAYARDOUST, S.; OMAR, O.; THOMSEN, P. Gene expression in peri-implant crevicular fluid of smokers and nonsmokers. 1. The early phase of osseointegration. **Clinical Implant Dentistry and Related Research**, v. 19, n. 4, p. 681–693, ago. 2017.

SCHIMMEL, M. et al. Loading protocols for implant-supported overdentures in the edentulous jaw: a systematic review and meta-analysis. **The International Journal of Oral & Maxillofacial Implants**, v. 29 Suppl, n. Supplement, p. 271–86, 2014.

SLOTTE, C. et al. Gene expression of inflammation and bone healing in peri-implant crevicular fluid after placement and loading of dental implants. A kinetic clinical pilot study using quantitative real-time PCR. **Clinical Implant Dentistry and Related Research**, v. 14, n. 5, p. 723–36, out. 2012.

STOW, J. L. et al. Cytokine secretion in macrophages and other cells: pathways and mediators. **Immunobiology**, v. 214, n. 7, p. 601–612, 2009.

STUCKI, U. et al. Temporal and local appearance of alkaline phosphatase activity in early stages of guided bone regeneration. A descriptive histochemical study in humans. **Clinical Oral Implants Research**, v. 12, n. 2, p. 121–127, 2001.

TAKASHI NOMURA, H. et al. Tissue inhibitor of metalloproteinases-1, matrix metalloproteinases-1 and -8, and collagenase activity levels in peri-implant crevicular fluid after implantation. **Clinical Oral Implants Research**, v. 11, n. 5, p. 430–40, out. 2000.

TALLGREN, A. The continuing reduction of the residual alveolar ridges in complete denture wearers: a mixed-longitudinal study covering 25 years. **The Journal of Prosthetic Dentistry**, v. 27, n. 2, p. 120–32, fev. 1972.

THOMASON, J. M. et al. Mandibular two implant-supported overdentures as the first choice standard of care for edentulous patients--the York Consensus Statement. **British Dental Journal**, v. 207, n. 4, p. 185–186, 2009.

TIRACHAIMONGKOL, C. et al. Relation between the stability of dental implants and two biological markers during the healing period: a prospective clinical study. **International Journal of Implant Dentistry**, v. 2, n. 1, p. 27, 8 dez. 2016.

TÖZÜM, T. F. et al. The effect of delayed versus early loading on nitric oxide metabolism around dental implants: an 18-month comparative follow-up study. **The International Journal of Oral & Maxillofacial Implants**, v. 22, n. 1, p. 53–62, 2007a.

TÖZÜM, T. F. et al. Analysis of the Potential Association of Implant Stability, Laboratory, and Image-Based Measures Used to Assess Osteotomy Sites: Early Versus Delayed Loading. **Journal of Periodontology**, v. 78, n. 9, p. 1675–1682, set. 2007b.

TÖZÜM, T. F.; TÜRKYILMAZ, I.; YAMALIK, N. Analysis of the Possible Impact of Inflammation Severity and Early and Delayed Loading on. **International Journal of Oral and Maxillofacial Implants**, n. 20, p. 547–556, 2005.

TRINDADE, R. et al. Foreign Body Reaction to Biomaterials: On Mechanisms for Buildup and Breakdown of Osseointegration. **Clinical implant dentistry and related research**, v. 18, n. 1, p. 192–203, fev. 2016.

TRINDADE, R. et al. Osseointegration and foreign body reaction: Titanium implants activate the immune system and suppress bone resorption during the first 4 weeks after implantation. **Clinical Implant Dentistry and Related Research**, v. 20, n. 1, p. 82–91, 28 fev. 2018.

TRINDADE, R.; ALBREKTSSON, T.; WENNERBERG, A. Current Concepts for the Biological Basis of Dental Implants. **Oral and Maxillofacial Surgery Clinics of North America**, v. 27, n. 2, p. 175–183, maio 2015.

TSOUKAKI, M. et al. Clinical, radiographic, microbiological, and immunological outcomes of flapped vs. flapless dental implants: a prospective randomized controlled clinical trial. **Clinical Oral Implants Research**, v. 24, n. 9, p. 969–76, set. 2013.

VERRASTRO NETO, A. et al. The impact of different torques for the insertion of immediately loaded implants on the peri-implant levels of angiogenesis- and bone-related markers. **International Journal of Oral and Maxillofacial Surgery**, n. November, p. 1–7, nov. 2017.

WANG, Y.; ZHANG, Y.; MIRON, R. J. Health, Maintenance, and Recovery of Soft Tissues around Implants. **Clinical Implant Dentistry and Related Research**, v. 18, n. 3, p. 618–634, jun. 2016.

WEI, L. et al. The effects of dopamine receptor 2 expression on B cells on bone metabolism and TNF-α levels in rheumatoid arthritis. **BMC Musculoskeletal Disorders**, v. 17, n. 1, p. 352, 19 dez. 2016.

WORLD HEALTH ORGANIZATION, W. **World Report on Ageing and Health.** Geneva, Switzerland: WHO Press, 2015.

ZYGOGIANNIS, K. et al. A Systematic Review on Immediate Loading of Implants Used to Support Overdentures Opposed by Conventional Prostheses: Factors That Might Influence Clinical Outcomes. **The International Journal of Oral & Maxillofacial Implants**, v. 31, n. 1, p. 63–72, jan. 2016.

Parecer do Comitê de Ética

FACULDADE DE ODONTOLOGIA DA UNIVERSIDADE FEDERAL DE

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: EFEITO DE OVERDENTURES MANDIBULARES NA EVOLUÇÃO DA FUNÇÃO MASTIGATÓRIA DE DESDENTADOS TOTAIS COM ATROFIA ÓSSEA

Pesquisador: Fernanda Faot

Área Temática:

Versão: 2

CAAE: 47353215.4.0000.5318

Instituição Proponente: Faculdade de Odontologia da Universidade Federal de Pelotas/ FO-UFPel

Patrocinador Principal: MINISTERIO DA CIENCIA, TECNOLOGIA E INOVACAO

DADOS DO PARECER

Número do Parecer: 1.267.086

Apresentação do Projeto:

Em virtude do aumento da expectativa de vida das populações em envelhecimento dos países em desenvolvimento, tem resultado no aumento da necessidade e substituição de próteses totais. O principal problema que acomete esta população é o processo de reabsorção óssea fisiológica, mais severa na mandíbula, resultando em problemas cada vez mais frequentes de retenção e estabilidade das próteses totais. Neste sentido, as “overdentures” implantosuportadas proporcionam um grande benefício a esses pacientes, aumentando a estabilidade e retenção e surtindo efeitos diretos na “performance” mastigatória, controle neuromuscular, e na qualidade de vida. Porém o custo efetivo desta intervenção bem como a severidade da atrofia óssea decorrente do tempo de edentulismo tem dificultado o acesso dos pacientes a esta modalidade de tratamento.

Objetivo da Pesquisa:

o objetivo deste estudo é avaliar a evolução da função mastigatória de pacientes com atrofia óssea mandibular severa antes e após a reabilitação com “overdentures” implantosuportadas, ancoradas em implantes de pequeno diâmetro.

Endereço: Rua Gonçalves Chaves, 457
Bairro: Centro **CEP:** 96.015-560
UF: RS **Município:** PELOTAS
Telefone: (53)3222-4439 **Fax:** (53)3222-4439 **E-mail:** cep.fop@gmail.com

FACULDADE DE ODONTOLOGIA DA UNIVERSIDADE FEDERAL DE

Avaliação dos Riscos e Benefícios:

Riscos e desconfortos mínimos. Benefícios incluem propor intervenções clínicas reabilitadoras que auxiliam na prevenção do processo de reabsorção óssea.

Comentários e Considerações sobre a Pesquisa:

Os pesquisadores atenderam todas as solicitações do parecer anterior de forma satisfatória.

Considerações sobre os Termos de apresentação obrigatória:

Todos os termos foram apresentados de forma adequada.

Recomendações:

Nenhuma

Conclusões ou Pendências e Lista de Inadequações:

Nenhuma pendência

Considerações Finais a critério do CEP:

APÓS ANALISE DA RESPOSTA E ESCLARECIMENTO AO PARECER NO1.201.436, O PROTOCOLO REAPRESENTADO FOI APROVADO.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_512188.pdf	30/09/2015 14:00:45		Aceito
Outros	resposta_parecer.pdf	30/09/2015 13:59:50	Fernanda Faot	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_Resposta.pdf	30/09/2015 13:56:21	Fernanda Faot	Aceito
Outros	Proposta de emenda.pdf	17/07/2015 17:50:29		Aceito
Folha de Rosto	folhaDeRosto final.pdf	03/07/2015 09:03:16		Aceito
Projeto Detalhado / Brochura Investigador	Projeto Final-Emenda CEP 2015.pdf	03/07/2015 09:01:31		Aceito
Outros	carta deresponsabilidade.pdf	03/07/2015 08:59:43		Aceito
Outros	carta de apresentação.pdf	03/07/2015 08:59:27		Aceito
Parecer Anterior	aprovação comitê de ética.jpg	13/05/2015		Aceito

Endereço: Rua Gonçalves Chaves, 457
Bairro: Centro **CEP:** 96.015-560
UF: RS **Município:** PELOTAS
Telefone: (53)3222-4439 **Fax:** (53)3222-4439 **E-mail:** cep.fop@gmail.com

FACULDADE DE ODONTOLOGIA DA UNIVERSIDADE FEDERAL DE

Continuação do Parecer: 1.267.086

Parecer Anterior	aprovação comitê de ética.jpg	10:49:01		Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

PELOTAS, 07 de Outubro de 2015

Assinado por:
Renato Waldemarin
(Coordenador)

Endereço: Rua Gonçalves Chaves, 457
Bairro: Centro **CEP:** 96.015-560
UF: RS **Município:** PELOTAS
Telefone: (53)3222-4439 **Fax:** (53)3222-4439 **E-mail:** cep.fop@gmail.com