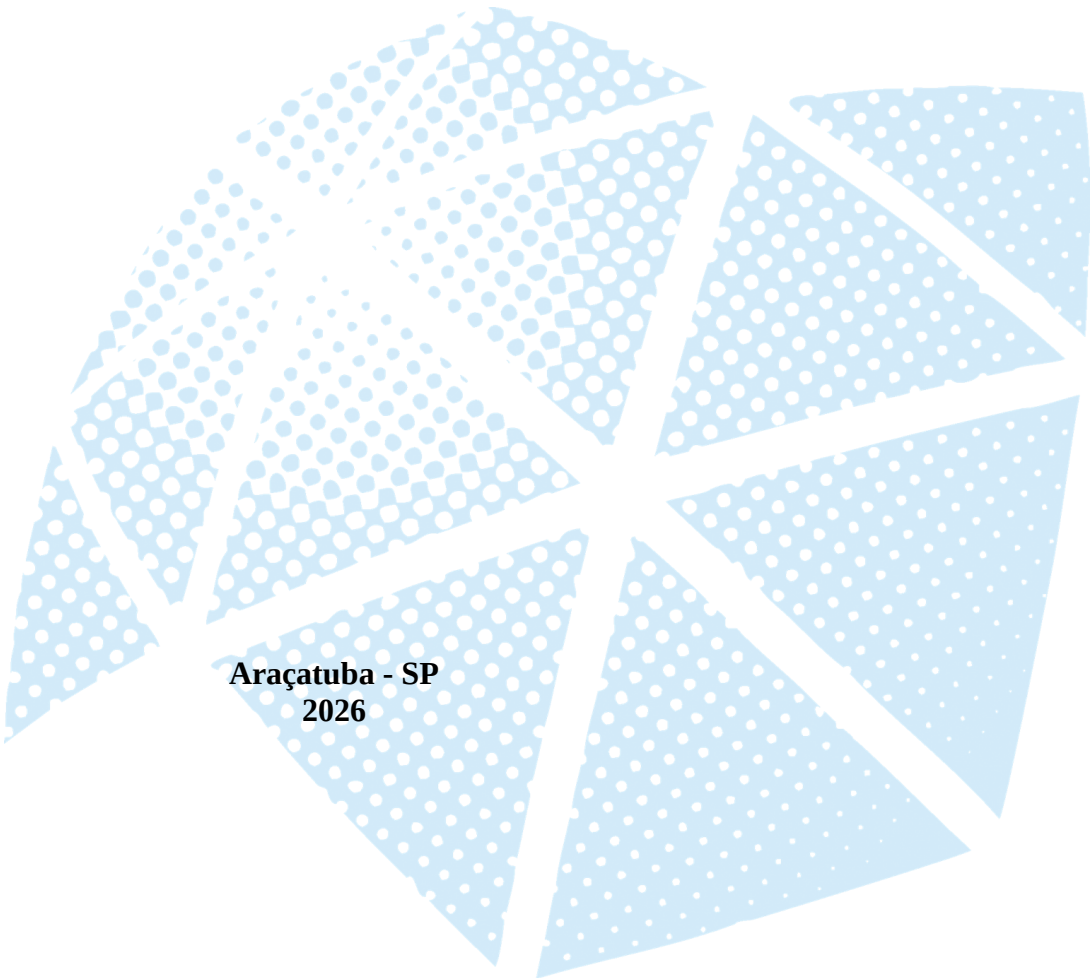
RESSALVA

Atendendo solicitação do(a) autor(a), o texto completo desta dissertação será disponibilizado somente a partir de 4/03/2028

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Araçatuba - Faculdade de Odontologia

MARIANA MARTINS GUERREIRO

Advances in Dental Implant Surfaces for Soft Tissue Integration and Therapeutic Function



Araçatuba - SP
2026

MARIANA MARTINS GUERREIRO

Advances in Dental Implant Surfaces for Soft Tissue Integration and Therapeutic Function

Dissertação apresentada à Universidade Estadual Paulista (UNESP), Faculdade de Odontologia de Araçatuba, para obtenção do título de Mestre em Odontologia.
Área de Concentração: Prótese Dentária

Orientador: Profª. Drª. Erica Dorigatti de Avila

Araçatuba - SP
2026

Catalogação na Publicação (CIP)

Diretoria Técnica de Biblioteca e Documentação – FOA / UNESP

Guerreiro, Mariana Martins.
G934s Smart/stimuli-responsive antimicrobial system for soft tissue integration. antibacterial coating validation in animal model / Mariana Martins Guerreiro. - Araçatuba, 2026
128 f. : il.

Dissertação (Mestrado) – Universidade Estadual Paulista (UNESP), Faculdade de Odontologia, Araçatuba
Orientadora: Profa. Érica Dorigatti de Avila

1. Peri-implantite 2. Sistemas de liberação de medicamentos 3. Polimetil metacrilato 4. Biofilmes 5. Experimentação animal I. T.

Black D3
CDD 617.6

Claudio Hideo Matsumoto CRB-8/5550

Dedico esta conquista para:

Meu Pai

Meu pai é a minha pessoa preferida no Mundo! Para uma menina, o amor de um pai é de extrema importância. Todas as vezes que eu já não acreditava mais em mim, ele esteve ao meu lado me mostrando o quanto sou forte e amada. Por todas as vezes que meu mundo desabou, meu pai esteve sempre me aconselhando. O meu pai nunca foi o tipo de pai que me privou dos desafios da vida e sim o que pegou em minha mão como quem diz: "Você nunca vai estar sozinha". Com isso, eu me tornei uma mulher independente, eu aprendi a enfrentar meus medos e a não desistir na primeira frustração. Segundo o espiritismo de Alan Kardec, antes de nosso nascimento, escolhemos a nossa família e quais desafios iremos enfrentar. Por muito tempo eu me questionei a respeito dessa premissa. Hoje, posso afirmar com toda a convicção no mundo, que eu escolheria mais infinitas vezes a minha família, somente por saber que o senhor será meu pai, eu repetiria tudo de novo. Meu pai não tem apenas o título da paternidade, ele é meu melhor amigo, é meu psicólogo, é minha mãe e pai ao mesmo tempo. E com todas essas funções, hoje eu sou o reflexo da sua educação. Tudo que eu faço é para retribuir um pouco de tudo que o senhor fez por mim e minha maior aspiração é te trazer orgulho

A minha pessoa preferida,

Claudio

Esta conquista não é minha, esta conquista é nossa!

Amo você!

AGRADECIMENTOS ESPECIAIS

Ao meu primeiro Pai,

Deus

Desde que mudei de São Carlos para Araçatuba eu escutei uma única música por esses quase 2 anos, principalmente o trecho abaixo:

Quando contei os meus sonhos para alguém, me disseram: "São grandes demais para você". Quando falei onde queria chegar, me disseram: "Pare por aqui, não vá além". Mas com Deus foi bem diferente, ele me disse: "Vá em frente, eu contigo estou". Desde então, eu nunca mais me limitei, descobri que os planos Dele para mim, são muito maiores do que os meus. Deus e Eu-Leandro Borges

E em todos os momentos de escuta, me pegava em lágrimas por sentir a Tua presença, obrigada por conversar comigo todos os dias!

Obrigada Senhor pelas infinitas bençãos em minha vida!

A Ti toda honra e toda glória!

Professora Dra. Erica Dorigatti de Avila,

Eu caí de paraquedas na vida da Erica, e desde então me faltam palavras suficientes para agradecer por tê-la conhecido. Minha admiração por ela vai muito além da orientação acadêmica. Eu a admiro como exemplo de ética e profissionalismo, como orientadora excepcional, como mãe cuidadosa e como ser humano admirável. A Erica, como profissional, é a certeza de que tudo será conduzido com excelência, rigor e dedicação incansável. Como Erica, é alguém capaz de perceber, em uma simples conversa, aquilo que muitas vezes nem nós mesmos conseguimos enxergar. É alguém que identifica nossas qualidades, inclusive aquelas que desconhecemos, e que faz questão de nos lembrar delas nos momentos em que

mais precisamos. Sem dúvida, um dos momentos mais marcantes da minha trajetória no mestrado ocorreu minutos antes de uma apresentação que realizei em inglês (a primeira). Naquele instante, ela fez questão de se levantar, ir até mim e, com a serenidade que lhe é característica, simplesmente me orientar a respirar. Esse gesto, aparentemente simples, traduz de forma profunda quem ela é. Revela não apenas sua sensibilidade, mas também o cuidado genuíno com os detalhes de uma conversa às exigências de uma pesquisa. Você inspira em todas suas versões. Sua confiança e apoio ultrapassaram em muito a construção deste trabalho; foram essenciais para meu crescimento pessoal e profissional.

Com carinho e gratidão infinita, obrigada!

AGRADECIMENTOS

À **Universidade Estadual Paulista "Júlio de Mesquita Filho" - Faculdade de Odontologia de Araçatuba**. Ao **Programa de Pós-graduação em Odontologia**, na pessoa da coordenadora **Professora Dra. Roberta Okamoto**. Sou grata às oportunidades que o Programa ofereceu durante o curso de mestrado. Muito obrigada!

À **Professora Luana Carla Pires Verzola**, minha profunda e sincera gratidão. Foi por meio de sua confiança e oportunidade que pude ingressar no mestrado, marco decisivo na minha trajetória. Mais do que isso, foi em sua competência e dedicação que encontrei inspiração para desejar e construir meu próprio caminho na carreira acadêmica. A admiração que nutro por ela foi, sem dúvida, uma das forças que impulsionaram minhas escolhas. Reconheço, com absoluta sinceridade, que muitas das conquistas alcançadas até aqui só foram possíveis porque, em algum momento, ela acreditou em mim. Sem sua orientação inicial, incentivo e exemplo, este percurso simplesmente não teria sido o mesmo. Obrigada, professora!

As minhas amigas e companheiras de equipe, **Daniela Moreira Cunha** e **Amanda Paino Santana**. Minhas companheiras da "salinha". Agradeço por toda ajuda, apoio e principalmente pela amizade que criamos. Foram dois anos convivendo todos os dias, compartilhando e vibrando por cada conquista. Ainda bem que conheci vocês, muito obrigada! Eu estarei sempre aqui torcendo e comemorando junto com vocês. A **Marta Maria Alves Pererira**, muito obrigada por toda ajuda. Seus conhecimentos foram extremamente valiosos.

Aos Professores **Rafael Scaf de Molon, Gabriel Mulinari-Santos**, **Edilson Ervolino**, **Marlise Inês Klein**, **Leonardo Faverani**, **Paula Aboud Barbugli**, **Rodolfo Debone, Jamil Awad Shibli** por toda a ajuda prestada na confecção dos trabalhos. Aos Professores da Universidade de Berna, Suíça, **Marting Degen**, **Ludovica Parisi** e **Anton Sculean** por terem me recebido tão gentilmente em um país completamente novo. Obrigada por toda a ajuda prestada para a realização deste presente trabalho.

Ao **Professor Paulo Tambasco** por ter aceitado o nosso convite em compor a banca da defesa desta Dissertação, muito obrigada!

Agradeço a **Stefany Barbosa**, **Maria Eduarda Fiorentino Ferreira de Andrade**, **Rodrigo Isaias Lopes Pereira** e **Vitória Marega Marchetti** por toda ajuda prestada durante o trabalho.

Aos funcionários da biblioteca, **Ana Claudia Grieger Manzatti, Claúdio Hideo Matsumoto, Luís Claúdio Sedlacek, Denise Maeda** e **Maria Cláudia Benez**. Gostaria de agradecer em especial, **Ana Claudia Grieger Manzatti**, por toda ajuda e correção deste trabalho! Muito obrigada!

O presente trabalho foi realizado com apoio da **Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)**, Processo nº 2024/14871-8 e nº 2025/08591-5. Meus sinceros agradecimentos pelo apoio financeiro, que possibilitou a realização deste curso.

“Bem-aventurado o homem que acha sabedoria, e o homem que adquire conhecimento”

Provérbios 3: 13

RESUMO GERAL

GUERREIRO, M. M. **Avanços nas Superfícies de Implantes Dentários para Integração dos Tecidos Moles e Função Terapêutica**. 2026. Dissertação (Mestrado) - Faculdade de Odontologia, Universidade Estadual Paulista (UNESP), Araçatuba, 2026.

O sucesso a longo prazo dos implantes dentários depende do estabelecimento de uma interface estável entre o tecido mole peri-implantar, capaz de resistir a desafios microbianos e manter a homeostase do tecido. No entanto, alcançar uma integração previsível do tecido mole permanece um desafio, e estratégias eficazes de biomateriais devem integrar proteção antimicrobiana com modulação das respostas do hospedeiro para promover a fixação epitelial, maturação do tecido conjuntivo e resiliência do tecido. As abordagens preventivas concentram-se em melhorar a qualidade biológica da vedação peri-implantar para reduzir a colonização bacteriana e a suscetibilidade a doenças. Em contrapartida, os revestimentos responsivos concebidos para combater a peri-implantite representam intervenções terapêuticas para condições em que a infeção e a inflamação já estão estabelecidas, utilizando sistemas de administração de fármacos responsivos a estímulos para liberar agentes antimicrobianos e imunomoduladores em resposta a sinais patológicos. Estes sistemas também podem apoiar a recuperação dos tecidos, promovendo a remodelação da matriz extracelular, sugerindo uma sinergia entre o controle da infeção e a modulação regenerativa. Este trabalho aborda dois temas de investigação interligados. Primeiro, explora os mecanismos biológicos subjacentes à integração do implante com o tecido mole e fornece uma análise crítica das estratégias de biomateriais baseadas em revestimentos concebidas para melhorar a fixação do tecido, limitando a colonização bacteriana, com ênfase particular nos desafios translacionais atuais. Em segundo lugar, descreve o projeto, a fabricação e a avaliação in vivo de um revestimento inteligente de administração de medicamentos para superfícies de implantes com base na montagem camada por camada (LbL), incorporando complexos de tetraciclina-β-ciclodextrina juntamente com uma película de ácido polimetacrílico (PMAA) sensível ao pH. O revestimento multifuncional demonstrou atividade antimicrobiana sustentada, modulação de mediadores inflamatórios, remodelação aprimorada da matriz extracelular, aumento da deposição de colagéno e maturação acelerada dos tecidos moles em um modelo de infecção polimicrobiana relacionada a implantes. Coletivamente, este trabalho contribui para o desenvolvimento de superfícies de implantes de última geração que integram funcionalidades preventivas e terapêuticas para melhorar a saúde peri-implantar.

Palavras-chave: peri-implantite; sistemas de liberação de medicamentos; polimetil metacrilato; biofilmes; experimentação animal.

GENERAL ABSTRACT

GUERREIRO, M. M. **Advances in Dental Implant Surfaces for Soft Tissue Integration and Therapeutic Function**. 2026. Dissertação (Mestrado) - Faculdade de Odontologia, Universidade Estadual Paulista (UNESP), Araçatuba, 2026.

The long-term success of dental implants depends on the establishment of a stable peri-implant soft tissue interface capable of resisting microbial challenges and maintaining tissue homeostasis. However, achieving predictable soft tissue integration remains challenging, and effective biomaterial strategies must integrate antimicrobial protection with modulation of host responses to promote epithelial attachment, connective tissue maturation, and tissue resilience. Preventive approaches focus on enhancing the biological quality of the peri-implant seal to reduce bacterial colonization and disease susceptibility. In contrast, responsive coatings designed to combat peri-implantitis represent therapeutic interventions for conditions in which infection and inflammation are already established, using stimuli-responsive drug delivery systems to release antimicrobial and immunomodulatory agents in response to pathological cues. These systems may also support tissue recovery by promoting extracellular matrix remodeling, suggesting synergy between infection control and regenerative modulation. This thesis addresses two interconnected research themes. First, it explores the biological mechanisms underlying implant-soft tissue integration and provides a critical analysis of coating-based biomaterial strategies designed to enhance tissue attachment while limiting bacterial colonization, with particular emphasis on current translational challenges. Second, it describes the design, fabrication, and *in vivo* evaluation of a smart drug-delivery coating for implant surfaces based on layer-by-layer (LbL) assembly, incorporating tetracycline- β-cyclodextrin complexes together with a pH-responsive poly(methacrylic acid) (PMAA) film. The multifunctional coating demonstrated sustained antimicrobial activity, modulation of inflammatory mediators, enhanced extracellular matrix remodeling, increased collagen deposition, and accelerated soft tissue maturation in a polymicrobial implant-related infection model. Collectively, this work contributes to the development of next-generation implant surfaces integrating preventive and therapeutic functionalities for improved peri-implant health.

Keywords: peri-implantitis; drug delivery systems; polymethyl methacrylate; biofilms; animal experimentation.

LISTA DE FIGURAS

Capítulo 1

Figure 1 Healing dynamics of peri-implant soft tissues following implant placement. Schematic representation of the temporal biological events occurring at the peri-implant soft tissue interface. Immediately after implant placement, an acute inflammatory response is characterized by vascular dilation, edema, and infiltration of inflammatory cells within the connective tissue. During the early healing phase (1-3 days), oral keratinocytes and basal epithelial cells migrate toward the implant/abutment surface, forming a weak epithelial attachment mediated by hemidesmosomal protein complexes. Concurrently, immune cells regulate the inflammatory milieu. In the subsequent remodeling phase (1-2 weeks), gingival fibroblasts become predominant, synthesizing and organizing collagen fibers that align predominantly parallel to the implant surface, while vascular density progressively decreases. In the late healing stage (3-4 weeks), a mature peri-implant soft tissue seal is established, characterized by a stable epithelial barrier, a dense collagenous connective tissue with limited vascularization, and the absence of periodontal ligament, like fiber insertion, distinguishing peri-implant tissues from those surrounding natural teeth. Created in BioRender. de Avila, E. (n.d.) https://www.biorender.com and with ChatGPT. 31

Figure 2 Schematic illustration of the biological mechanisms linking implant abutment surface microarchitecture to peri-implant inflammation. Microstructural features such as surface topography and roughness can act as niches for microbial colonization. The formation of a dysbiotic biofilm initiates the activation of the innate immune system through complement cascade components (C3a/C5a), recruiting dendritic cells and neutrophils. Dendritic cells function as antigen-presenting cells, promoting Th17 cell activation and the release of pro-inflammatory cytokines (IL-17, IL-22). These mediators amplify inflammation and stimulate fibroblast-derived matrix metalloproteinases (MMPs), contributing to connective tissue degradation. Simultaneously, macrophages and Th1 cells secrete IFN-γ, TNF-α, and IL-1β, enhancing the inflammatory milieu and promoting osteoclast differentiation and activation, ultimately leading to bone resorption. This cascade illustrates how implant microstructured surfaces, although designed to enhance cell adhesion, may also sustain bacterial persistence and provoke excessive immune activation that jeopardizes peri-implant tissue stability. Created in BioRender. de Avila, E. (n.d.) https://www.biorender.com.

47

Figure 3 Schematic representation of the influence of surface wettability on biological interactions at implant interfaces. (A) On the left, the hydrophilic surface exhibits a low contact angle with water, reflecting high wettability that favors the adsorption of adhesive proteins in their native conformation. This environment facilitates integrin engagement and promotes extensive fibroblast spreading. Conversely, the hydrophobic surface, shown on the right, displays a high contact angle indicative of low wettability. Under these conditions, protein adsorption is limited and often denatured, while air entrapment at the interface further impairs cell adhesion. Consequently, integrin-mediated signaling is delayed, resulting in restricted cell spreading and weaker cell–substrate interactions. Created in BioRender. de Avila, E. (n.d.) https://www.biorender.com. (B) Soft tissue-implant interface on microrough titanium surfaces. Reproduced from Parisi et al., 2022 (PLOS ONE), under the Creative Commons Attribution (CC BY) license. The figure illustrates the intimate interface between human MG-63 cells and microrough titanium implant surfaces with normal or enhanced hydrophilicity. (a) Immunofluorescence staining for actin (red), vinculin (green), and nuclei (blue) shows the polygonal morphology of MG-63 cells cultured on titanium surfaces. Scale bar: 50 μm. (b) SEM images obtained before and after FIB cross-sectioning reveal cell morphology and the close cell–surface contact established between MG-63 cells (red) and the underlying titanium. Scale bar: 2 μm; inset scale bar: 0.2 μm. Cell thickness and the distance between the cell membrane and titanium surface were quantified to assess the degree of cell–surface intimacy. $p < 0.05$. Green dashed lines indicate the upper and lower cell boundaries, while white stars denote air bubbles entrapped at the cell–titanium interface. 54

Figure 4. Schematic illustration highlighting the biological and structural differences between the natural tooth–gingival interface and the peri-implant soft tissue attachment. In natural teeth, epithelial adhesion is mediated by a specialized basement membrane composed of glycoproteins that anchor HD complexes. This architecture supports a robust JE and a perpendicular orientation of collagen fibers, forming an effective biological seal against bacterial invasion. In contrast, implant transmucosal surfaces lack a native basement membrane, resulting in weaker epithelial adhesion and collagen fibers aligned parallel to the surface, providing minimal resistance to microbial penetration. To address this limitation, bioinspired surface coatings have been designed to replicate the biochemical and structural features of the natural basement membrane. These coatings incorporate bioactive molecules, such as laminin-derived peptides and glycoprotein ligands, to enhance epithelial attachment and promote the establishment of a functional soft tissue barrier. Such biomimetic interfaces

aim to reinforce epithelial adhesion, improve microbial defense, and support long-term peri-implant stability. Created in BioRender. de Avila, E. (n.d.) https://www.biorender.com. 62

Capítulo 2

Figure 1. Schematic representation of the laboratory workflow showing the key steps in the preparation of the PMAA-based coating on the drug delivery system and the development of the implant-related infection model. 92

Figure 2. FTIR spectra showing light absorption by TCβCD (A-B) and pH-responsive PMAA film (C). 99

Figure 3. Comparison of contact angle values between the mean of LbL/TCβCD and LbL/TCβCD/PMAA, with the control group (Ti). Statistical comparisons were performed by one-way analysis of variance (ANOVA) with Dunnett's multiple comparisons test, using GraphPad Prism version 10.10c (GraphPad Software, CA, USA). All data are plotted as mean ± standard deviation (SD). Significant difference between means (p* < 0.05). Ti vs. LbL/TCβCD p = 0.08; Ti vs. LbL/TCβCD/PMAA p = 0.12. Illustration created using the BioRender platform (https://www.biorender.com/). 100

Figure 4. Quantitative comparisons, regarding the number of viable colonies, between coated surfaces: LbL/TCβCD (light gray bar), LbL/TCβCD/PMAA (dark gray bar) and uncoated: Ti (black bar) as commercial control, after 1 (A) and 7 days (B). Each value represents the mean ± SD of 6 samples. Statistical differences are indicated as *p = 0.0001 and **p < 0.0001 (Dunnet test). Effect of LbL/TCβCD and LbL/TCβCD/PMAA coatings on polymicrobial biofilm formation after 1 (C) and 7 (D) days, via confocal laser fluorescence microscopy. Qualitative images with a 20x objective, in 2D and 3D, illustrate the distribution of live (green)/dead (red) microorganisms and the thickness of the biofilm through the fluorescence intensity, respectively. 102

Figure 5. Histological aspects (cellularity and structure pattern) of the connective tissue on (A, D) Ti, (B, E) LbL/TCβCD, (C, F) LbL/TCβCD/PMAA at (A,B,C) 1 and (D, E, F) 7 days postoperatively. Magnification: 200x. Scale bars: 100 μm. 104

Figure 6. Histological appearance (total collagen fibers and their maturity levels) of connective tissue in contact with contaminated Ti discs in Ti (B, F), LbL/TCBCD (C, G), and LbL/TCBCD/PMAA (D, H) groups at 1 and 7 days postoperatively. Magnification: 100x. Scale bars: 100 μm. Staining: Picrosirius red in polarized light microscopy. Quantitative data of total and mature collagen fibers in the connective tissue surrounding Ti, LbL/TCβCD, and LbL/TCβCD/PMAA surfaces at 1 and 7 days postoperatively. Statistical analyses were

performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test (GraphPad Prism v10.10c; GraphPad Software, CA, USA). Data are presented as mean ± standard deviation (SD), and statistically significant differences are indicated as *p < 0.0001 between Ti (1D) vs. LbL/TCBCD/PMAA (1D), Ti (7D) vs. LbL/TCBCD (7D), Ti (7D) vs. LbL/TCBCD/PMAA (7D), LbL/TCBCD (7D) vs. LbL/TCBCD/PMAA (7D) of total collagen fibers and Ti (1D) vs. LbL/TCBCD/PMAA (1D), Ti (7D) vs. LbL/TCBCD (7D), Ti (7D) vs. LbL/TCBCD/PMAA (7D), LbL/TCBCD (7D) vs. LbL/TCBCD/PMAA (7D) of mature collagen fibers ; ** p=0.0003 between Ti (1D) vs. LbL/TCBCD (1D) of total collagen fibers ; *** p=0.0007 between Ti (1D) vs. LbL/TCBCD (1D) of mature collagen fibers. 106

Figure 7. Immunomarking for TNF-α (B–D) and IL-1β (F–H) in connective tissue in contact with contaminated Ti discs, 1 day postoperatively, in Ti (B, F), LbL/TCβCD (C, G), and LbL/TCβCD/PMAA (D, H) groups. Magnification: 400x. Scale bars:50 μm. Immunohistochemical density (%) of the inflammatory and tissue remodeling markers TNF-α, IL-1β on Ti, LbL/TCβCD and LbL/TCβCD/PMAA groups at 1 and 7 days postoperatively. Data are presented as mean ± standard deviation. Comparisons among groups were performed using one-way ANOVA, followed by Tukey's multiple comparison post hoc test (GraphPad Prism v10.10c; GraphPad Software, CA, USA). *Statistically significant differences between Ti (1D) vs. LbL/TCβCD/PMAA (1D); Ti (1D) vs. LbL/TCβCD (1D); Ti (1D) vs. LbL/TCβCD/PMAA (1D); Ti (7D) vs. LbL/TCβCD (7D) of TNF-α, Ti (1D) vs. LbL/TCβCD (1D), Ti (1D) vs. LbL/TCβCD/PMAA (1D), Ti (7D) vs. LbL/TCβCD (7D), Ti (7D) vs. LbL/TCβCD/PMAA (7D) of IL-1β (p<0.0001); **statistically significant differences between LbL/TCβCD (7D) vs. LbL/TCβCD/PMAA (7D) (p=0.0007); ***statistically significant differences between LbL/TCβCD (7D) vs. LbL/TCβCD/PMAA of IL-1β (p=0.0023). 108

Figure 8. Immunomarking for IL-10 (B–D) and TGFβ1 (F–H) in connective tissue incontact with contaminated Ti discs, 7 days postoperatively, in Ti (B, F), LbL/TCβCD (C, G), and LbL/TCβCD/MAA (D, H) groups. Magnification: 400x. Scale bars:50 μm. Immunohistochemical density (%) of the inflammatory and tissue remodeling markers IL-10 (A), TGFβ1 (E) on Ti, LbL/TCβCD and LbL/TCβCD/PMAA groups at 1 and 7 days postoperatively. Data are presented as mean ± standard deviation. Comparisons among groups were performed using one-way ANOVA, followed by Tukey's multiple comparison post hoc test (GraphPad Prism v10.10c; GraphPad Software, CA, USA). *Statistically significant differences between Ti (1D) and LbL/TCβCD (1D) of IL-10 (p=0.0096); **statistically

significant differences between Ti (1D) vs. LbL/TCβCD/PMAA (1D) of IL-10; LbL/TCβCD (7D) vs. LbL/TCβCD/PMAA (7D) of TGFβ1 (p=0.0042); ***statistically significant differences between Ti (7D) vs. LbL/TCβCD (7D) of IL-1β (p=0.0002); # statistically significant differences between Ti (7D) vs. LbL/TCβCD/PMAA (7D) of IL-1β; Ti (1D) vs. LbL/TCβCD/PMAA (1D), Ti (7D) vs. LbL/TCβCD/PMAA (7D), Ti (7D) vs. LbL/TCβCD (7D) of TGFβ1 (p<0.0001); ## statistically significant differences between Ti (1D) vs. LbL/TCβCD (1D) of TGFβ1 (p=0.0023). 110

Figure 9. Immunomarking for MMP2 (B–D) and MMP9 (F–H) in connective tissue in contact with contaminated Ti discs, 1 day postoperatively, in Ti (B, F), LbL/TCBCD (C, G), and LbL/TCBCD/PMAA (D, H) groups. Magnification: 400x. Scale bars: 50 μm. Immunohistochemical density (%) of the inflammatory and tissue remodeling markers MMP2 (A) and MMP9 (E) on Ti, LbL/TCβCD and LbL/TCβCD/PMAA groups at 1 and 7 days postoperatively. Data are presented as mean ± standard deviation. Comparisons among groups were performed using one-way ANOVA, followed by Tukey's multiple comparison post hoc test (GraphPad Prism v10.10c; GraphPad Software, CA, USA). *Statistically significant differences between Ti (1D) vs. LbL/TCβCD (1D), Ti (1D) vs. LbL/TCβCD/PMAA (1D), Ti (7D) vs. LbL/TCβCD (7D), Ti (7D) vs. LbL/TCβCD/PMAA (7D) of MMP2; Ti (1D) vs. LbL/TCβCD (1D), Ti (1D) vs. LbL/TCβCD/PMAA (1D), Ti (7D) vs. LbL/TCβCD (7D), Ti (7D) vs. LbL/TCβCD/PMAA (7D) of MMP9 (p<0.0001); **statistically significant differences between LbL/TCβCD (1D) vs. LbL/TCβCD/PMAA (1D) of MMP2 (p=0.0006); ***statistically significant differences between LbL/TCβCD (7D) vs. LbL/TCβCD/PMAA (7D) of MMP2 (p=0.003); # statistically significant differences between LbL/TCβCD (1D) vs. LbL/TCβCD/PMAA (1D) of MMP9 (p=0.0013). 112

Figure 10. Immunomarking for TIMP-1 (B–D) and TIMP-2 (F–H) in connective tissue in contact with contaminated Ti discs, 7 days postoperatively, in Ti (B, F), LbL/TCBCD (C, G), and LbL/TCBCD/PMAA (D, H) groups. Magnification: 400x. Scale bars: 50 μm. Immunohistochemical density (%) of the inflammatory and tissue remodeling markers TIMP-1 (A) and TIMP-2 (E) on Ti, LbL/TCβCD and LbL/TCβCD/PMAA groups at 1 and 7 days postoperatively. Data are presented as mean ± standard deviation. Comparisons among groups were performed using one-way ANOVA, followed by Tukey's multiple comparison post hoc test (GraphPad Prism v10.10c; GraphPad Software, CA, USA). *Statistically significant differences between Ti (7D) vs. LbL/TCβCD/PMAA (7D) of TIMP-1 (p<0.0034); **Statistically significant differences between Ti (7D) vs. LbL/TCβCD/PMAA (7D) of

TIMP-2 ($p<0.0007$); ***Statistically significant differences between Ti (7D) vs. LbL/TCβCD (7D) of TIMP-2 ($p<0.0001$). 114

Figure 11. Gene expression profile of extracellular matrix–related markers in human gingival fibroblasts cultured on Ti, LbL/PMAA, and LbL/TCβCD/PMAA surfaces in different time points. mRNA levels of *COL1A1* (B), *COL3A1* (C), *FN1* (D), *ELN* (E), and *P21*/CDKN1A (F) were quantified by qPCR after 1, 7, and 14 days of culture on uncoated Ti, (LbL/PMAA) and (LbL/TCβCD/PMAA). Data are presented as mean ± SD of three independent experiments. Statistical analysis was performed using one-way ANOVA, followed by Tukey's post hoc test to compare differences among groups at each time point. *Statistically significant differences between Ti (1D) vs. LbL/PMAA (1D), Ti (1D) vs. LbL/TCβCD/PMAA (1D) of *COL1A1;* Ti (7D) vs. LbL/PMAA (7D), LbL/PMAA (7D) vs. LbL/TCβCD/PMAA (7D) of *COL3A1,* Ti (1D) vs. LbL/PMAA (1D), Ti (1D) vs. LbL/TCβCD/PMAA (1D) of *FN1,* LbL/PMAA (1D) vs. LbL/TCβCD/PMAA (1D) of *ELN* ($p=0.03$); **Statistically significant differences between Ti (7D) vs. LbL/PMAA (7D), LbL/PMAA (7D) vs. LbL/TCβCD/PMAA (7D) of *COL1A1,* Ti (14D) vs. LbL/PMAA (14D), Ti (14D) vs. LbL/TCβCD/PMAA (14D) of *COL3A1;* Ti (14D) vs. LbL/PMAA (14D), Ti (14D) vs. LbL/TCβCD/PMAA (14D) of ELN; Ti (1D) vs. LbL/PMAA (1D), LbL/PMAA (1D) vs. LbL/TCβCD/PMAA (1D) ($p=0.006$); ***Statistically significant differences between Ti (14D) vs. LbL/PMAA (14D), Ti (14D) vs. LbL/TCβCD/PMAA (14D) of *COL1A1;* Ti (1D) vs. LbL/PMAA (1D), Ti (1D) vs. LbL/TCβCD/PMAA (1D) of *ELN* ($p<0.0006$); ****Statistically significant differences between LbL/PMAA (14D) vs. LbL/TCβCD/PMAA (14D) *COL1A1* ($p<0.0008$). 116

Figure 12. Immunofluorescence images showing vimentin (green) and nuclei (blue) staining of human gingival fibroblasts (hGFs) cultured on (A) uncoated Ti, (B) LbL/PMAA, and (C) LbL/TCβCD/PMAA substrates 3 days post-seeding. 118

LISTA DE TABELAS

Capítulo 1

Table 1. Summary of key clinical, *in vivo*, and *in vitro* studies evaluating transmucosal implant/abutment materials and surface modifications highlighting study design, advantages and limitations, and main outcomes related to peri-implant soft tissue integration. 41

Table 2. Summary of key *in vitro* and *in vivo* studies on bioactive and biological coatings for peri-implant soft tissue integration. 59

LISTA DE ABREVIATURAS E SIGLAS

%	Percent
°C	Degrees celsius
µL	Microliter
2D	2-dimensional
3D	3-dimensional
AA	Acrylic acid
AFM	Atomic force microscopy
Ag	Silver ions
Allyl	Allylanine
BIC	Bone-implant contact
BOP	Bleeding on probing
CDKN1A	P21
CFU	Colony forming unit
CH	Methylene
cm	Centimeter
COL1A1	Collagen type I
COL3A1	Collagen type III
COL4A1	Collagen type IV
DMEM	Dulbecco's Morified eagle medium
ECM	Extracellular matrix
ELN	Elastin
FBS	Fetab bovine serum
FN	Fibronectin
FTIR	Fourier transform infrared spectroscopy
g	grams
GL13K	(GKIIKLKA-SLKLL-NH_2)
GO	Graphene oxide
H&E	Hematoxylin and eosin
HD	Hemidesmosome
HGF	Fibroblasts
IFN- γ	Interferon-gamma
IL-10	Interleukin-10

IL-17	Interleukin-17
IL-1β	Interleukin-1β
IL-22	Interleukin-22
IL-6	Interleukin-6
JE	Junctional epithelium
KMW	Peri-implant keratinized mucosa width
LamLG3	Laminin-derived peptides
LbL	Layer by layer
LTAP	Low-temperature atmospheric plasma
MAA	Acrylic acid
mg	Milligram
min	Minute
mL	Milliliter
mM	Molar mass
mm	Millimeter
MMPs	Metalloproteinases
Nm	Nanometers
OD	Optical density
PAA	Poly(acrylic acid)
PBS	Phosphate-buffered saline
PD	Probing depth
PDA	Polydopamine
PDGF	Platelet-derived growth factor
PEEK	Polyetheretherketone
PI	Plaque Index
PLL	Poly(l-lysine)
PMAA	Polymethyl methacrylate
SD	Standard deviation
SHI-FSMS	Modified rich medium
TC	Tetracycline
TEM	Transmission electron microscopy
TGF- β	Transforming growth factor-beta
Th17	T helper 17 cells

Ti	Titanium
TiO_2	Titanium dioxide
TNF- α	Tumor necrosis factor-alpha
UV	Ultraviolet
UVC	Ultraviolet C
VEGF	Vascular endothelial growth factor
YTZP	Yttria-stabilized zirconia
Zn	Zinc
Zn/CS/Gel	Zinc, chitosan and gelatin
βCD	Beta cyclodextrin

SUMÁRIO

1 CAPÍTULO 1 – SOFT TISSUE INTEGRATION AT DENTAL IMPLANT INTERFACES: BIOLOGICAL PRINCIPLES, BIOMATERIAL STRATEGIES, AND CLINICAL IMPLICATIONS 23
1.1 Introduction 25
1.2 Healing Dynamics of Peri-Implant Soft Tissues Following Implant Placement 25
1.3 Soft Tissue Integration in Implantology: Definition, Criteria, and Biological Basis 31
1.4 Whats Does Soft Tissue Integration Mean around dental implansts Dentistry? 33
1.5 Strategies for Achieving Soft Tissue Integration in Dental Implants 34
1.5.1 Biological behavior of soft tissues in response to different implant materials 34
1.5.1.1 Ti-based materials 34
1.5.1.2 Ceramic-based materials 37
1.5.1.3 Polymeric Materials 38
1.5.2 Biomaterial surface design to support soft tissue integration at the implant interface 43
1.5.2.1 Soft Tissue Integration through Mechanical Retention 43
1.5.2.2 Soft tissue integration through wettability 49
1.5.3 Bioactive coatings to stimulate soft tissue integration at the implant interface 53
1.6 Coating Technologies for Soft Tissue Integration: Current Challenges, Translational Barriers, and Future Directions 61
1.7 Conclusion 64
1.8 References 65
2 CAPÍTULO 2 – SMART COATINGS INTEGRATING ANTIMICROBIAL AND REMODELING FUNCTIONS FOR IMPLANT INTERFACES 80
2.1 Introduction 81
2.2 Material and Methods 83
2.2.1 Titanium disc preparation 83
2.2.2 Preparation, Processing, and Characterization of Materials 84
2.2.3 Construction of the LbL/TCβCD/PMAA Multilayer System 84
2.2.4 Physicochemical Properties of PMAA 85
2.2.5 Saliva Collection and Polymicrobial Community Standardization 86
2.2.6 *In Vivo* infection model 86
2.2.6.1 Pilot Study Design 87
2.2.6.2 In Vivo Study Design 87

2.2.6.3 Histopathological, histochemical and immunohistochemistry analysis of samples 89
2.2.7 *In vitro* Experiment 91
2.2.7.1 *Cell Culture* 91
2.2.7.2 RNA extraction, cDNA synthesis, and quantitative real-time PCR analysis 92
2.2.8 Statistical Analysis 93
2.3 Results 93
2.3.1 Successful production of TCβCD and PMAA 93
2.3.2 LbL/TCβCD/PMAA system maintains the hydrophilicity of the surface 95
2.3.3 LbL/TCβCD/PMAA system reduced polymicrobial biofilm formation 95
2.3.4 LbL/TCβCD/MAA promoted a milder inflammatory response and more mature connective tissue 97
2.3.5 LbL/TCβCD/MAA promoted collagen remodeling with increased proportion mature collagen fibers 100
2.3.6 LbL/TCβCD/PMAA system modulated the immune response by reducing early inflammation and promoting tissue remodeling 102
2.3.7 qRT-PCR analysis 105
2.3.8 Immunofluorescence (IF) analysis **Erro! Indicador não definido.**
2.4 Discussion 108
2.5 Conclusion **Erro! Indicador não definido.**
2.6 References 112
ANEXOS 116

Funding information

The State of Sao Paulo Research Foundation (FAPESP-Brazil) grant numbers: # 2024/14871-8 and #2025/08591-5 to M.M.G.; #2021/09434-0 and 2018/20719-3 to E.D.A; #2023/15750-7 to R.S.M.; #2025/07203-1 to D.M.C.; #2024/14872-4 to M.E.F.F.A.; #2025/02912-4 to A.P.S; National Council for Scientific and Technological Development, Brazil (CNPq # 314479/2023–6 and # 408905/2025-5) to J.A.S

1.8 References

1. Bentour E, Papamanoli E, Karoussis IK. The Decision Between Tooth Retention or Replacement with Implants: A Continuing Dilemma. *Dent J (Basel)* 2025;13.

2. Berglundh T, Armitage G, Araujo MG, et al. Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Clin Periodontol* 2018;45 Suppl 20:S286–S291.

3. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biol* 2016;14:e1002533.

4. Bandara H, Panduwawala CP, Samaranayake LP. Biodiversity of the human oral mycobiome in health and disease. *Oral Dis* 2019;25:363–371.

5. Deo PN, Deshmukh R. Oral microbiome: Unveiling the fundamentals. *J Oral Maxillofac Pathol* 2019;23:122–128.

6. Radaic A, Kapila YL. The oralome and its dysbiosis: New insights into oral microbiome-host interactions. *Comput Struct Biotechnol J* 2021;19:1335–1360.

7. Berguer R. Sharps safety in the operating room. *AORN J* 2011;94:4–6.

8. Diemert LM, Bondy SJ, Brown KS, Manske S. Young adult smoking cessation: predictors of quit attempts and abstinence. *Am J Public Health* 2013;103:449–453.

9. Di Spirito F, Giordano F, Di Palo MP, et al. Microbiota of Peri-Implant Healthy Tissues, Peri-Implant Mucositis, and Peri-Implantitis: A Comprehensive Review. *Microorganisms* 2024;12.

10. Shahjouei S, Ansari S, Pourmotabbed T, Zand R. Potential Roles of Adropin in Central Nervous System: Review of Current Literature. *Front Mol Biosci* 2016;3:25.

11. Tsukioka T, Izumi N, Komatsu H, et al. Elevation of Neutrophil-to-Lymphocyte Ratio Is a Significant Poor Prognostic Factor in Completely Resected Centrally Located Lung Squamous Cell Carcinoma. *In Vivo* 2022;36:2303–2307.

12. Wang X, Chen Y, Yuan W, et al. MicroRNA-155-5p is a key regulator of allergic inflammation, modulating the epithelial barrier by targeting PKIalpha. *Cell Death Dis* 2019;10:884.

13. Bozzi S, Vesentini S, Santus M, et al. Fluid dynamics characterization and thrombogenicity assessment of a levitating centrifugal pump with different impeller designs. *Med Eng Phys* 2020;83:26–33.

14. Laleman I, Lambert F. Implant connection and abutment selection as a predisposing and/or precipitating factor for peri-implant diseases: A review. *Clin Implant Dent Relat Res* 2023;25:723–733.

15. Herrera D, Berglundh T, Schwarz F, et al. Prevention and treatment of peri-implant diseases-The EFP S3 level clinical practice guideline. *J Clin Periodontol* 2023;50 Suppl 26:4–76.

16. Jia SS, Zhao HQ, Song YL, Sun Y, Wang GW, Wang XJ. Challenges of Soft Tissue Integration Around Dental Implants and Strategies Based on Material Surface Modification. *Adv Mater Interfaces* 2025;12.

17. Li WW, Carter MJ, Mashiach E, Guthrie SD. Vascular assessment of wound healing: a clinical review. *Int Wound J* 2017;14:460–469.

18. Fischer NG, Moussa DG, Skoe EP, De Jong DA, Aparicio C. Keratinocyte-Specific Peptide-Based Surfaces for Hemidesmosome Upregulation and Prevention of Bacterial Colonization. *ACS Biomater Sci Eng* 2020;6:4929–4939.

19. Koidou VP, Argyris PP, Skoe EP, et al. Peptide coatings enhance keratinocyte attachment towards improving the peri-implant mucosal seal. *Biomater Sci* 2018;6:1936–1945.

20. Stricker A, Bergfeldt T, Fretwurst T, et al. Impurities in commercial titanium dental implants - A mass and optical emission spectrometry elemental analysis. *Dent Mater* 2022;38:1395–1403.

21. Becker J, John G, Becker K, Mainusch S, Diedrichs G, Schwarz F. Clinical performance of two-piece zirconia implants in the posterior mandible and maxilla: a prospective cohort study over 2 years. *Clin Oral Implants Res* 2017;28:29–35.

22. Schunemann FH, Galarraga-Vinueza ME, Magini R, et al. Zirconia surface modifications for implant dentistry. *Mater Sci Eng C Mater Biol Appl* 2019;98:1294–1305.

23. Shaw TJ, Martin P. Wound repair at a glance. *J Cell Sci* 2009;122:3209–3213.

24. Wilkinson HN, Hardman MJ. Wound healing: cellular mechanisms and pathological outcomes. *Open Biol* 2020;10:200223.

25. Abrahamsson I, Berglundh T, Glantz PO, Lindhe J. The mucosal attachment at different abutments. An experimental study in dogs. *J Clin Periodontol* 1998;25:721–727.

26. Sculean A, Gruber R, Bosshardt DD. Soft tissue wound healing around teeth and dental implants. *J Clin Periodontol* 2014;41 Suppl 15:S6–22.

27. Roessler S, Born R, Scharnweber D, Worch H, Sewing A, Dard M. Biomimetic coatings functionalized with adhesion peptides for dental implants. *J Mater Sci Mater Med* 2001;12:871–877.

28. Shineh G, Janghour LM, Xia Y, et al. Biomolecule-functionalized dental implant surfaces: Towards augmenting soft tissue integration. *Bioact Mater* 2025;53:540–590.

29. Yang Z, Liu M, Yang Y, et al. Biofunctionalization of zirconia with cell-adhesion peptides via polydopamine crosslinking for soft tissue engineering: effects on the biological behaviors of human gingival fibroblasts and oral bacteria. *RSC Adv* 2020;10:6200–6212.

30. Zhao BH, Tian WM, Feng HL, Lee IS, Cui FZ. Effects of RGD peptide grafting to titanium dental implants on the adhesion of human gingival fibroblasts and epithelial cells. *Curr Appl Phys* 2005;5:407–410.

31. Werner S, Huck O, Frisch B, et al. The effect of microstructured surfaces and laminin-derived peptide coatings on soft tissue interactions with titanium dental implants. *Biomaterials* 2009;30:2291–2301.

32. Berglundh T, Lindhe J, Ericsson I, Marinello CP, Liljenberg B, Thomsen P. The soft tissue barrier at implants and teeth. *Clin Oral Implants Res* 1991;2:81–90.

33. Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound Healing: A Cellular Perspective. *Physiol Rev* 2019;99:665–706.

34. Smith SA, Travers RJ, Morrissey JH. How it all starts: Initiation of the clotting cascade. *Crit Rev Biochem Mol Biol* 2015;50:326–336.

35. Berglundh T, Abrahamsson I, Welander M, Lang NP, Lindhe J. Morphogenesis of the peri-implant mucosa: an experimental study in dogs. *Clin Oral Implants Res* 2007;18:1–8.

36. De Molon RS, Pereira MMA, De Avila ED. Implant surface features as key role on cell behavior. *Biocell* 2022;46:1151–1156.

37. de Molon RS, Vernal R, Oliveira GE, et al. Inflammatory bone loss and signaling pathways in periodontitis: mechanistic insights and emerging therapeutic strategies. *Bone Res* 2026;14:1.

38. Vignoletti F, Nunez J, Sanz M. Soft tissue wound healing at teeth, dental implants and the edentulous ridge when using barrier membranes, growth and differentiation factors and soft tissue substitutes. *J Clin Periodontol* 2014;41 Suppl 15:S23–35.

39. Darby IA, Laverdet B, Bonte F, Desmouliere A. Fibroblasts and myofibroblasts in wound healing. *Clin Cosmet Investig Dermatol* 2014;7:301–311.

40. Moon IS, Berglundh T, Abrahamsson I, Linder E, Lindhe J. The barrier between the keratinized mucosa and the dental implant. An experimental study in the dog. *J Clin Periodontol* 1999;26:658–663.

41. Cho YD, Kim KH, Lee YM, Ku Y, Seol YJ. Periodontal Wound Healing and Tissue Regeneration: A Narrative Review. *Pharmaceuticals (Basel)* 2021;14.

42. Grzesik WJ, Narayanan AS. Cementum and periodontal wound healing and regeneration. *Crit Rev Oral Biol Med* 2002;13:474–484.

43. Sone ED, McCulloch CA. Periodontal regeneration: Lessons from the periodontal ligament-cementum junction in diverse animal models. *Front Dent Med* 2023;4:1124968.

44. Deng Z, Liang J, Fang N, Li X. Integration of collagen fibers in connective tissue with dental implant in the transmucosal region. *Int J Biol Macromol* 2022;208:833–843.

45. Gomes NA, Garcia VG, Mulinari-Santos G, et al. Expression and localisation of amelotin, laminin and protein secreted by follicular dendritic cells after ligature-induced experimental periodontitis in rats. *Br Dent J* 2025.

46. Theodoro LH, Garcia VG, Ervolino E, Holcroft J, McCulloch CA, Ganss B. Role of junctional epithelium in maintaining dento-gingival adhesion and periodontal health. *Front Dent Med* 2023;4:1144537.

47. Gibbs S, Roffel S, Meyer M, Gasser A. Biology of soft tissue repair: gingival epithelium in wound healing and attachment to the tooth and abutment surface. *Eur Cell Mater* 2019;38:63–78.

48. Alexander R, Liu X. Soft tissue integration around dental implants: A pressing priority. *Biomaterials* 2026;324:123491.

49. Fischer NG, Kobe AC, Dai J, et al. Tapping basement membrane motifs: Oral junctional epithelium for surface-mediated soft tissue attachment to prevent failure of percutaneous devices. *Acta Biomater* 2022;141:70–88.

50. Walko G, Castanon MJ, Wiche G. Molecular architecture and function of the hemidesmosome. *Cell Tissue Res* 2015;360:363–378.

51. Deng Z, Yu L, Sun Y, et al. Titanium surface modified with nanostructure mimicking the surface of natural cementum up-regulated epithelial expression of integrin beta4 and enhanced the attachment of the transmucosal gingiva. *Dent Mater* 2025;41:1201–1212.

52. Stewart RL, O'Connor KL. Clinical significance of the integrin alpha6beta4 in human malignancies. *Lab Invest* 2015;95:976–986.

53. Tomasi C, Tessarolo F, Caola I, Wennstrom J, Nollo G, Berglundh T. Morphogenesis of peri-implant mucosa revisited: an experimental study in humans. *Clin Oral Implants Res* 2014;25:997–1003.

54. Jin S, Yu Y, Zhang T, et al. Surface modification strategies to reinforce the soft tissue seal at transmucosal region of dental implants. *Bioact Mater* 2024;42:404–432.

55. Liegeois L, Borie M, Lecloux G, Van Hede D, Lambert F. Influence of Implant Component Materials on Peri-Implant Soft Tissue Healing: A Comparative Histological and Immunohistochemical Study in Humans. *Clin Oral Implants Res* 2025;36:397–409.

56. Bellon B, Pippenger B, Stahli A, Degen M, Parisi L. Cementum and enamel surface mimicry influences soft tissue cell behavior. *J Periodontal Res* 2025;60:64–76.

57. Tete S, Mastrangelo F, Bianchi A, Zizzari V, Scarano A. Collagen fiber orientation around machined titanium and zirconia dental implant necks: an animal study. *Int J Oral Maxillofac Implants* 2009;24:52–58.

58. Atsuta I, Ayukawa Y, Kondo R, et al. Soft tissue sealing around dental implants based on histological interpretation. *J Prosthodont Res* 2016;60:3–11.

59. Renvert S, Persson GR, Pirih FQ, Camargo PM. Peri-implant health, peri-implant mucositis, and peri-implantitis: Case definitions and diagnostic considerations. *J Periodontol* 2018;89 Suppl 1:S304–S312.

60. Farag MM. Recent trends on biomaterials for tissue regeneration applications: review. *J Mater Sci* 2023;58:527–558.

61. Silva RCS, Agrelli A, Andrade AN, et al. Titanium Dental Implants: An Overview of Applied Nanobiotechnology to Improve Biocompatibility and Prevent Infections. *Materials (Basel)* 2022;15.

62. Parisi L, Toffoli A, Ghezzi B, Mozzoni B, Lumetti S, Macaluso GM. A glance on the role of fibronectin in controlling cell response at biomaterial interface. *Jpn Dent Sci Rev* 2020;56:50–55.

63. Baus-Dominguez M, Maza-Solano S, Vazquez-Pachon C, et al. Behaviour of the Peri-Implant Soft Tissue with Different Rehabilitation Materials on Implants. *Polymers (Basel)* 2023;15.

64. Sanz M, Klinge B, Alcoforado G, et al. Biological aspects: Summary and consensus statements of group 2. The 5(th) EAO Consensus Conference 2018. *Clin Oral Implants Res* 2018;29 Suppl 18:152–156.

65. Pesce P, Del Fabbro M, Menini M, et al. Effects of abutment materials on peri-implant soft tissue health and stability: A network meta-analysis. *J Prosthodont Res* 2023;67:506–517.

66. Willis J, Li S, Crean SJ, Barrak FN. Is titanium alloy Ti-6Al-4 V cytotoxic to gingival fibroblasts-A systematic review. *Clin Exp Dent Res* 2021;7:1037–1044.

67. Dalal A, Pawar V, McAllister K, Weaver C, Hallab NJ. Orthopedic implant cobalt-alloy particles produce greater toxicity and inflammatory cytokines than titanium alloy and zirconium alloy-based particles in vitro, in human osteoblasts, fibroblasts, and macrophages. *J Biomed Mater Res A* 2012;100:2147–2158.

68. Osman RB, Swain MV. A Critical Review of Dental Implant Materials with an Emphasis on Titanium versus Zirconia. *Materials (Basel)* 2015;8:932–958.

69. Lee J, Hurson S, Tadros H, Schupbach P, Susin C, Wikesjo UM. Crestal remodelling and osseointegration at surface-modified commercially pure titanium and titanium alloy implants in a canine model. *J Clin Periodontol* 2012;39:781–788.

70. El-Hadad S, Safwat EM, Sharaf NF. In-vitro and in-vivo, cytotoxicity evaluation of cast functionally graded biomaterials for dental implantology. *Mater Sci Eng C Mater Biol Appl* 2018;93:987–995.

71. Corvino E, Pesce P, Mura R, Marcano E, Canullo L. Influence of Modified Titanium Abutment Surface on Peri-implant Soft Tissue Behavior: A Systematic Review of In Vitro Studies. *Int J Oral Maxillofac Implants* 2020;35:503–519.

72. Ou P, Hao C, Liu J, He R, Wang B, Ruan J. Cytocompatibility of Ti-xZr alloys as dental implant materials. *J Mater Sci Mater Med* 2021;32:50.

73. Gomez-Florit M, Ramis JM, Xing R, et al. Differential response of human gingival fibroblasts to titanium- and titanium-zirconium-modified surfaces. *J Periodontal Res* 2014;49:425–436.

74. Cai H, Chen J, Li C, Wang J, Wan Q, Liang X. Quantitative discoloration assessment of peri-implant soft tissue around zirconia and other abutments with different colours: A systematic review and meta-analysis. *J Dent* 2018;70:110–117.

75. Cosgarea R, Gasparik C, Dudea D, Culic B, Dannewitz B, Sculean A. Peri-implant soft tissue colour around titanium and zirconia abutments: a prospective randomized controlled clinical study. *Clin Oral Implants Res* 2015;26:537–544.

76. Ekfeldt A, Furst B, Carlsson GE. Zirconia abutments for single-tooth implant restorations: a retrospective and clinical follow-up study. *Clin Oral Implants Res* 2011;22:1308–1314.

77. Hanawa T. Zirconia versus titanium in dentistry: A review. *Dent Mater J* 2020;39:24–36.

78. Mikolai C, Kommerein N, Ingendoh-Tsakmakidis A, Winkel A, Falk CS, Stiesch M. Early host-microbe interaction in a peri-implant oral mucosa-biofilm model. *Cell Microbiol* 2020;22:e13209.

79. Renvert S, Quirynen M. Risk indicators for peri-implantitis. A narrative review. *Clin Oral Implants Res* 2015;26 Suppl 11:15–44.

80. de Avila ED, Avila-Campos MJ, Vergani CE, Spolidorio DM, Mollo Fde A, Jr. Structural and quantitative analysis of a mature anaerobic biofilm on different implant abutment surfaces. *J Prosthet Dent* 2016;115:428–436.

81. Nascimento CD, Pita MS, Fernandes F, Pedrazzi V, de Albuquerque Junior RF, Ribeiro RF. Bacterial adhesion on the titanium and zirconia abutment surfaces. *Clin Oral Implants Res* 2014;25:337–343.

82. Roehling S, Astasov-Frauenhoffer M, Hauser-Gerspach I, et al. In Vitro Biofilm Formation on Titanium and Zirconia Implant Surfaces. *J Periodontol* 2017;88:298–307.

83. de Avila ED, de Molon RS, Vergani CE, de Assis Mollo F, Jr., Salih V. The Relationship between Biofilm and Physical-Chemical Properties of Implant Abutment Materials for Successful Dental Implants. *Materials (Basel)* 2014;7:3651–3662.

84. de Avila ED, de Molon RS, Palomari Spolidorio DM, de Assis Mollo F, Jr. Implications of Surface and Bulk Properties of Abutment Implants and Their Degradation in the Health of Periodontal Tissue. *Materials (Basel)* 2013;6:5951–5966.

85. Serichetaphongse P, Chengprapakorn W, Thongmeearkom S, Pimkhaokham A. Immunohistochemical assessment of the peri-implant soft tissue around different abutment materials: A human study. *Clin Implant Dent Relat Res* 2020;22:638–646.

86. Pandoleon P, Bakopoulou A, Papadopoulou L, Koidis P. Evaluation of the biological behaviour of various dental implant abutment materials on attachment and viability of human gingival fibroblasts. *Dent Mater* 2019;35:1053–1063.

87. Fu JH, Wang HL. Breaking the wave of peri-implantitis. *Periodontol 2000* 2020;84:145–160.

88. Pabst AM, Walter C, Bell A, et al. Influence of CAD/CAM zirconia for implant-abutment manufacturing on gingival fibroblasts and oral keratinocytes. *Clin Oral Investig* 2016;20:1101–1108.

89. Atsuta I, Ayukawa Y, Furuhashi A, et al. Epithelial sealing effectiveness against titanium or zirconia implants surface. *J Biomed Mater Res A* 2019;107:1379–1385.

90. Hu J, Atsuta I, Ayukawa Y, Zhou T, Narimatsu I, Koyano K. Effect of titanium or zirconia implant abutments on epithelial attachments after ultrasonic cleaning. *J Oral Sci* 2020;62:331–334.

91. Rigolin MSM, Barbugli PA, Jorge JH, et al. Effect of the aging of titanium and zirconia abutment surfaces on the viability, adhesion, and proliferation of cells and the adhesion of microorganisms. *J Prosthet Dent* 2019;122:564 e561–564 e510.

92. Tang K, Luo ML, Zhou W, Niu LN, Chen JH, Wang F. The integration of peri-implant soft tissues around zirconia abutments: Challenges and strategies. *Bioact Mater* 2023;27:348–361.

93. Shan Z, Xie L, Liu H, et al. "Gingival Soft Tissue Integrative" Lithium Disilicate Glass-Ceramics with High Mechanical Properties and Sustained-Release Lithium Ions. *ACS Appl Mater Interfaces* 2022;14:54572–54586.

94. Brunot-Gohin C, Duval JL, Verbeke S, et al. Biocompatibility study of lithium disilicate and zirconium oxide ceramics for esthetic dental abutments. *J Periodontal Implant Sci* 2016;46:362–371.

95. Zarone F, Di Mauro MI, Ausiello P, Ruggiero G, Sorrentino R. Current status on lithium disilicate and zirconia: a narrative review. *BMC Oral Health* 2019;19:134.

96. Liao C, Li Y, Tjong SC. Polyetheretherketone and Its Composites for Bone Replacement and Regeneration. *Polymers (Basel)* 2020;12.

97. Bathala L, Majeti V, Rachuri N, Singh N, Gedela S. The Role of Polyether Ether Ketone (Peek) in Dentistry - A Review. *J Med Life* 2019;12:5–9.

98. Ramenzoni LL, Attin T, Schmidlin PR. In Vitro Effect of Modified Polyetheretherketone (PEEK) Implant Abutments on Human Gingival Epithelial Keratinocytes Migration and Proliferation. *Materials (Basel)* 2019;12.

99. Milinkovic I, Krasavcevic AD, Jankovic S, Sopta J, Aleksic Z. Immunohistochemical analysis of soft tissue response to polyetheretherketone (PEEK) and titanium healing abutments on dental implants: a randomized pilot clinical study. *BMC Oral Health* 2022;22:484.

100. Rea M, Ricci S, Ghensi P, Lang NP, Botticelli D, Soldini C. Marginal healing using Polyetheretherketone as healing abutments: an experimental study in dogs. *Clin Oral Implants Res* 2017;28:e46–e50.

101. Borie M, Bosshardt D, Liegeois L, et al. Peri-Implant Supracrestal Tissue Characteristics Related to Abutment Materials: A Comparative Histomorphometry Study. *Clin Implant Dent Relat Res* 2025;27:e70083.

102. Laskar P, Kumar KH, Kakkar T, et al. In Vitro Comparison of Different Implant Abutment Materials on Gingival Fibroblast Attachment. *J Pharm Bioallied Sci* 2025;17:S1472–S1474.

103. Glauser R, Schupbach P. Early bone formation around immediately placed two-piece tissue-level zirconia implants with a modified surface: an experimental study in the miniature pig mandible. *Int J Implant Dent* 2022;8:37.

104. Brunello G, Rauch N, Becker K, Hakimi AR, Schwarz F, Becker J. Two-piece zirconia implants in the posterior mandible and maxilla: A cohort study with a follow-up period of 9 years. *Clin Oral Implants Res* 2022;33:1233–1244.

105. Simons HL, Severs NJ. Plasmalemmal vesicles and the effects of sterol-binding agents in rabbit aortic endothelium. *J Cell Sci* 1986;83:141–153.

106. Ibanez JC, Nicolini M, Galdoporpora JM, et al. Biological Response of Soft Tissues to Three Abutment Materials Titanium, Zirconia, and Lithium Disilicate. In Vitro Comparative Study. *Journal of Osseointegration* 2024;16:71–80.

107. Paul SJ, Nesic D. Influence of the Transmucosal Surface of Dental Implants on the Soft Tissue Attachment Level and Marginal Bone Loss in Preclinical Studies: A Systematic Review. *Int J Oral Maxillofac Implants* 2024;39:173–183.

108. Doll PW, Husari A, Ahrens R, Spindler B, Guber AE, Steinberg T. Enhancing the soft-tissue integration of dental implant abutments-in vitro study to reveal an optimized

microgroove surface design to maximize spreading and alignment of human gingival fibroblasts. *J Biomed Mater Res B Appl Biomater* 2021;109:1768–1776.

109. Kim JC, Lee M, Yeo IL. Three interfaces of the dental implant system and their clinical effects on hard and soft tissues. *Mater Horiz* 2022;9:1387–1411.

110. Belloni A, Argentieri G, Orilisi G, et al. New insights on collagen structural organization and spatial distribution around dental implants: a comparison between machined and laser-treated surfaces. *J Transl Med* 2024;22:120.

111. Takebe J, Miyata K, Miura S, Ito S. Effects of the nanotopographic surface structure of commercially pure titanium following anodization-hydrothermal treatment on gene expression and adhesion in gingival epithelial cells. *Mater Sci Eng C Mater Biol Appl* 2014;42:273–279.

112. Wang C, Wang X, Lu R, Cao X, Yuan D, Chen S. Influence of surface nanotopography and wettability on early phases of peri-implant soft tissue healing: an in-vivo study in dogs. *BMC Oral Health* 2023;23:651.

113. Baraka S, Sam A, Krysmann M, Cook N, Ahmed W, Barrak F. Soft tissue attachment of human gingival fibroblasts to titanium dioxide nanotubes compared to commercially pure titanium and its alloys: a systematic review. *BDJ Open* 2025;11:58.

114. Deng Z, Yu L, Kuang Y, Zhou Z, Li X. Highly Ordered Nanotube-Like Microstructure on Titanium Dental Implant Surface Fabricated via Anodization Enhanced Cell Adhesion and Migration of Human Gingival Fibroblasts. *Int J Nanomedicine* 2024;19:2469–2485.

115. Alali AQ, Abdal-Hay A, Gulati K, Ivanovski S, Fournier BPJ, Lee RSB. Influence of Bioinspired Lithium-Doped Titanium Implants on Gingival Fibroblast Bioactivity and Biofilm Adhesion. *Nanomaterials (Basel)* 2021;11.

116. Chen Z, Zhang Y, Li J, Wang HL, Yu H. Influence of Laser-Microtextured Surface Collar on Marginal Bone Loss and Peri-Implant Soft Tissue Response: A Systematic Review and Meta-Analysis. *J Periodontol* 2017;88:651–662.

117. Romero-Gavilan F, Arias-Mainer C, Cerqueira A, et al. Roughness affects the response of human fibroblasts and macrophages to sandblasted abutments. *Biomed Eng Online* 2024;23:68.

118. Roth LA, Bastos MF, Melo MA, et al. The Potential Role of a Surface-Modified Additive-Manufactured Healing Abutment on the Expression of Integrins alpha2, beta1, alphav, and beta6 in the Peri-Implant Mucosa: A Preliminary Human Study. *Life (Basel)* 2022;12.

119. Leong A, De Kok I, Mendonca D, Cooper LF. Molecular Assessment of Human Peri-implant Mucosal Healing at Laser-Modified and Machined Titanium Abutments. *Int J Oral Maxillofac Implants* 2018;33:895–904.

120. Anitua E, Murias-Freijo A, Tierno R, Tejero R, Hamdan Alkhraisat M. Effect of implant abutment surface treatments on bacterial biofilm composition and structure. *J Oral Microbiol* 2025;17:2459922.

121. Sinjab K, Sawant S, Ou A, Fenno JC, Wang HL, Kumar P. Impact of surface characteristics on the peri-implant microbiome in health and disease. *J Periodontol* 2024;95:244–255.

122. Brull F, van Winkelhoff AJ, Cune MS. Zirconia dental implants: a clinical, radiographic, and microbiologic evaluation up to 3 years. *Int J Oral Maxillofac Implants* 2014;29:914–920.

123. Karapataki S, Vegh D, Payer M, Fahrenholz H, Antonoglou GN. Clinical Performance of Two-Piece Zirconia Dental Implants After 5 and Up to 12 Years. *Int J Oral Maxillofac Implants* 2023;38:1105–1114.

124. Harada A, Sasaki H, Asami Y, et al. Effects of the application of low-temperature atmospheric plasma on titanium implants on wound healing in peri-implant connective tissue in rats. *Int J Implant Dent* 2024;10:15.

125. Hall J, Neilands J, Davies JR, Ekestubbe A, Friberg B. A randomized, controlled, clinical study on a new titanium oxide abutment surface for improved healing and soft tissue health. *Clin Implant Dent Relat Res* 2019;21 Suppl 1:55–68.

126. Milleret V, Lienemann PS, Gasser A, Bauer S, Ehrbar M, Wennerberg A. Rational design and in vitro characterization of novel dental implant and abutment surfaces for balancing clinical and biological needs. *Clin Implant Dent Relat Res* 2019;21 Suppl 1:15–24.

127. Gittens RA, Scheideler L, Rupp F, et al. A review on the wettability of dental implant surfaces II: Biological and clinical aspects. *Acta Biomater* 2014;10:2907–2918.

128. Ma T, Ge X, Zhang Y, Lin Y. Effect of Titanium Surface Modifications of Dental Implants on Rapid Osseointegration. *In: Sasaki, K, Suzuki, O, Takahashi, N (eds) Interface Oral Health Science 2016 Springer, Singapore* 2017.

129. Miyauchi T, Yamada M, Yamamoto A, et al. The enhanced characteristics of osteoblast adhesion to photofunctionalized nanoscale TiO2 layers on biomaterials surfaces. *Biomaterials* 2010;31:3827–3839.

130. Att W, Hori N, Iwasa F, Yamada M, Ueno T, Ogawa T. The effect of UV-photofunctionalization on the time-related bioactivity of titanium and chromium-cobalt alloys. *Biomaterials* 2009;30:4268–4276.

131. Han A, Ding H, Tsoi JKH, Imazato S, Matinlinna JP, Chen Z. Prolonged UV-C Irradiation is a Double-Edged Sword on the Zirconia Surface. *ACS Omega* 2020;5:5126–5133.

132. Guo L, Smeets R, Kluwe L, et al. Cytocompatibility of Titanium, Zirconia and Modified PEEK after Surface Treatment Using UV Light or Non-Thermal Plasma. *Int J Mol Sci* 2019;20.

133. Izard T, Brown DT. Mechanisms and Functions of Vinculin Interactions with Phospholipids at Cell Adhesion Sites. *J Biol Chem* 2016;291:2548–2555.

134. Yamada M, Miyauchi T, Yamamoto A, et al. Enhancement of adhesion strength and cellular stiffness of osteoblasts on mirror-polished titanium surface by UV-photofunctionalization. *Acta Biomater* 2010;6:4578–4588.

135. Hori N, Ueno T, Minamikawa H, et al. Electrostatic control of protein adsorption on UV-photofunctionalized titanium. *Acta Biomater* 2010;6:4175–4180.

136. Yang Y, Zheng M, Yang Y, et al. Inhibition of bacterial growth on zirconia abutment with a helium cold atmospheric plasma jet treatment. *Clin Oral Investig* 2020;24:1465–1477.

137. Blazquez-Hinarejos M, Ayuso-Montero R, Jane-Salas E, Lopez-Lopez J. Influence of surface modified dental implant abutments on connective tissue attachment: A systematic review. *Arch Oral Biol* 2017;80:185–192.

138. Susin C, Finger Stadler A, Musskopf ML, de Sousa Rabelo M, Ramos UD, Fiorini T. Safety and efficacy of a novel, gradually anodized dental implant surface: A study in Yucatan mini pigs. *Clin Implant Dent Relat Res* 2019;21 Suppl 1:44–54.

139. Cao X, Wu K, Wang C, et al. Graphene Oxide Loaded on TiO(2)-Nanotube-Modified Ti Regulates the Behavior of Human Gingival Fibroblasts. *Int J Mol Sci* 2022;23.

140. Hormia M, Sahlberg C, Thesleff I, Airenne T. The epithelium-tooth interface--a basal lamina rich in laminin-5 and lacking other known laminin isoforms. *J Dent Res* 1998;77:1479–1485.

141. Huang G, Li F, Zhao X, et al. Functional and Biomimetic Materials for Engineering of the Three-Dimensional Cell Microenvironment. *Chem Rev* 2017;117:12764–12850.

142. Perdomo SJ, Fajardo CE, Cardona-Mendoza A. Laminin 332 functionalized surface improve implant roughness and oral keratinocyte bioactivity. *Heliyon* 2024;10:e34507.

143. Hersel U, Dahmen C, Kessler H. RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. *Biomaterials* 2003;24:4385–4415.

144. Truskey GA, Proulx TL. Relationship between 3T3 cell spreading and the strength of adhesion on glass and silane surfaces. *Biomaterials* 1993;14:243–254.

145. Khalili AA, Ahmad MR. A Review of Cell Adhesion Studies for Biomedical and Biological Applications. *Int J Mol Sci* 2015;16:18149–18184.

146. Yang M, Jiang P, Ge Y, et al. Dopamine self-polymerized along with hydroxyapatite onto the preactivated titanium percutaneous implants surface to promote human gingival fibroblast behavior and antimicrobial activity for biological sealing. *J Biomater Appl* 2018;32:1071–1082.

147. Wang Z, Wu G, Yang Z, Li X, Feng Z, Zhao Y. Chitosan/Hyaluronic Acid/MicroRNA-21 Nanoparticle-Coated Smooth Titanium Surfaces Promote the Functionality of Human Gingival Fibroblasts. *Int J Nanomedicine* 2022;17:3793–3807.

148. Han J, Andree L, Deng D, et al. Biofunctionalization of dental abutments by a zinc/chitosan/gelatin coating to optimize fibroblast behavior and antibacterial properties. *J Biomed Mater Res A* 2024;112:1873–1892.

149. Li W, Yang Y, Zhang H, et al. Improvements on biological and antimicrobial properties of titanium modified by AgNPs-loaded chitosan-heparin polyelectrolyte multilayers. *J Mater Sci Mater Med* 2019;30:52.

150. Li SD, Zhao W, Huang YJ, et al. A Sericin@Cu Coating on Zirconia to Orchestrate a Sequential Antibacterial-to-Regenerative Cascade for Enhancing Peri-Implant Soft Tissue Seal. *Adv Healthc Mater* 2026:e05161.

151. Zhang CN, Zhou LY, Qian SJ, Gu YX, Shi JY, Lai HC. Improved response of human gingival fibroblasts to titanium coated with micro-/nano-structured tantalum. *Int J Implant Dent* 2021;7:36.

152. Riivari S, Narva E, Kangasniemi I, Willberg J, Narhi T. Focal adhesion formation of primary human gingival fibroblast on hydrothermally and in-sol-made TiO(2) -coated titanium. *Clin Implant Dent Relat Res* 2023;25:583–591.

153. Abdallah MN, Badran Z, Ciobanu O, Hamdan N, Tamimi F. Strategies for Optimizing the Soft Tissue Seal around Osseointegrated Implants. *Adv Healthc Mater* 2017;6.

154. Xiang E, Gomez-Cerezo MN, Ali Y, et al. Surface Modification of Pure Zinc by Acid Etching: Accelerating the Corrosion Rate and Enhancing Biocompatibility and Antibacterial Characteristics. *ACS Appl Mater Interfaces* 2022;14:22554–22569.

155. Pereira MMA, de Molon RS, Barao VAR, et al. Surveying coating strategies for peri-implantitis management: Clinical implications and classificatory approaches. *J Periodontol* 2025;96:1077–1087.

156. Pereira MMA, Piazza RD, Barbugli PA, et al. Targeted Infection Control and Tissue Integration via pH-Sensitive Smart Coatings on Implant Surfaces. *ACS Appl Mater Interfaces* 2026;18:2584–2603.

157. Gessl A, Waldhausl W. Elevated CD69 expression on naive peripheral blood T-cells in hyperthyroid Graves' disease and autoimmune thyroiditis: discordant effect of methimazole on HLA-DR and CD69. *Clin Immunol Immunopathol* 1998;87:168–175.

158. Van den Borre CE, Zigterman BGR, Mommaerts MY, Braem A. How surface coatings on titanium implants affect keratinized tissue: A systematic review. *J Biomed Mater Res B Appl Biomater* 2022;110:1713–1723.

159. Zheng TX, Li W, Gu YY, Zhao D, Qi MC. Classification and research progress of implant surface antimicrobial techniques. *J Dent Sci* 2022;17:1–7.

160. Canullo L, Penarrocha Oltra D, Pesce P, et al. Soft tissue integration of different abutment surfaces: An experimental study with histological analysis. *Clin Oral Implants Res* 2021;32:928–940.

161. Panayotov IV, Vegh AG, Martin M, et al. Improving dental epithelial junction on dental implants with bioengineered peptides. *Front Bioeng Biotechnol* 2023;11:1165853.

162. Cunha DM, Santana AP, Guerreiro MM, et al. Reconceptualizing Peri-Implantitis: Dual-Factor Inflammation and Its Role in Advancing Biomaterial Coatings. *Clin Implant Dent Relat Res* 2026;28:e70108.

163. Alves Pereira MM, Piazza R, Santana AP, et al. Unraveling the Applicability of LbL Coatings for Drug Delivery in Dental Implant-Related Infection Treatment. *ACS Biomater Sci Eng* 2025;11:13–32.

164. He Y, Vasilev K, Zilm P. pH-Responsive Biomaterials for the Treatment of Dental Caries-A Focussed and Critical Review. *Pharmaceutics* 2023;15.

165. Inchingolo AM, Malcangi G, Ferrante L, et al. Surface Coatings of Dental Implants: A Review. *J Funct Biomater* 2023;14.

166. de Avila ED, Nagay BE, Pereira MMA, Barao VAR, Pavarina AC, van den Beucken J. Race for Applicable Antimicrobial Dental Implant Surfaces to Fight Biofilm-Related Disease: Advancing in Laboratorial Studies vs Stagnation in Clinical Application. *ACS Biomater Sci Eng* 2022;8:3187–3198.

167. de Molon RS, Steffens JP, de Avila ED, Teughels W, Van Dyke TE. Adjunctive Therapies in Periodontitis: Current Concepts and the Future. *J Periodontal Res* 2026.