B11: How should antimicrobial properties of an orthopaedic titanium implant be evaluated in animal and clinical studies?

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Response/Recommendation: The antimicrobial properties of orthopaedic titanium implants should be evaluated using a combination of *in vitro*, animal, and clinical studies to ensure comprehensive assessment of safety and efficacy. Animal models provide crucial insights into the biological interactions of implants with host tissues and pathogens, while human studies validate clinical applicability. To effectively evaluate the antimicrobial properties of new orthopaedic titanium implants, it is recommended to use a combination of well-established animal models, appropriate bacterial species, standardized inoculation doses, and comprehensive analytical techniques.

Level of Evidence: Moderate. While there is substantial evidence supporting the evaluation methods, variations in study design, bacterial strains, and outcome measures necessitate further standardization to strengthen clinical translation.

Delegate Vote: Agree: [% vote], Disagree: [%], Abstain: [%]

Rationale: Eligibility criteria: A systematic literature review was conducted of the PubMed and Web of Science databases. Due to the immense number of scientific articles on this topic (2747 studies screened), this review only included studies published from 2015 onwards, focusing on animal models and human studies that evaluate the effectiveness and safety of antimicrobial implants made of titanium (Ti) and its alloys. Review articles, studies restricted to purely *in vitro* models, or focusing on non-orthopaedic devices or biomaterials other than Ti were excluded. Included interventions were antibacterial coatings, nanomaterials, surface modifications, and localized drug delivery systems applied to the implants to prevent bacterial colonisation. Excluded interventions were antimicrobial treatments not part of the implant itself, such as systemic or local antibiotics, debridement treatments, *in situ* sonication, and electrical stimulation. Included outcomes were reduction in biofilm formation, bacterial adhesion, infection rates, antibiotic resistance, bacterial viability, inflammatory response, histological analysis, clinical outcomes, and functional outcomes. Studies that only assessed biocompatibility or osseointegration were excluded.

Introduction: Ti implants are widely used in orthopaedics due to their excellent biocompatibility, mechanical strength, and corrosion resistance. However, implant-associated infections remain a significant challenge, necessitating the development and evaluation of antimicrobial strategies. While *in vitro* models provide initial insights into antimicrobial efficacy, their relevance is limited due to the absence of complex host factors such as immune responses and tissue integration. This review provides an overview of the recent animal and human models used to evaluate the antimicrobial properties of orthopaedic Ti implants. Of the 51 studies analysed, 42 utilized animal models and 9 were human clinical studies (2 randomized controlled trials, 6 cohort studies, and 1 case-control study).

Methodological considerations:

***In vitro* models** are essential for preliminary screening of antimicrobial properties before progressing to *in vivo* studies. *In vitro* models allow for controlled experimentation on bacterial adhesion, biofilm formation, and antimicrobial efficacy under standardized conditions. Common *in vitro* techniques include colony forming unit (CFU) counting, live/dead staining, crystal violet staining, and imaging using confocal laser-scanning microscopy (CLSM) and scanning electron microscopy (SEM). *In vitro* models provide valuable insights into the mechanisms of action of

antimicrobial strategies and help refine experimental conditions for subsequent *in vivo* studies. Animal models and human studies both play crucial roles in evaluating the antimicrobial properties of orthopaedic Ti implants.

Animal models of orthopaedic-device related infection (ODRI) offer controlled environments and the ability to perform invasive procedures, providing valuable insights into the implant's effectiveness in preventing infections. The most frequently used animal species in the studies are rats, rabbits, and mice, selected for their cost-effectiveness and translational relevance to human bone physiology. For instance, Sprague-Dawley rats, New Zealand White rabbits, and C57BL/6 mice are commonly employed due to their manageable size and well-characterized immune responses. In addition, sheep and minipigs were also used. The most common sample size per experimental group was n=6 for rabbits,[1-5] n=5-10 for rats and mice,[6-8] and n=7 for sheep and minipigs.[9; 10] The inoculum (CFU and volume) used to induce infection must be appropriate for the animal species and body region, and determined through preliminary pilot studies. Too large an inoculum can cause sepsis and death, while too small a dose may be cleared rapidly. The time required for infection establishment varies by species and differs from clinical cases. Factors such as bacterial strain virulence and growth phase influence the inoculum concentration needed. The choice of bacterial species depends on whether the model targets acute (for example, *Staphylococcus aureus*) or chronic infections (such as, coagulase-negative staphylococci). Reflecting its clinical relevance in implant-associated infections, *S. aureus* was the predominant bacterial species used to induce infections (32 studies), with strains such as ATCC 25923 and Xen29 most common. The inoculation doses to induce infections mimicking clinical conditions vary, with typical doses being 10^6-10^8 CFU/ml for rabbits and 10^6 CFU/ml for rats. Most animal models have so far utilized an initial inoculum of planktonic bacterial cultures. Introducing mature biofilms grown on an implant as the initial inoculum in animal models could further optimise antimicrobial treatment strategies.[11] In addition, bone remodelling kinetics differ between animals and humans, affecting study duration. To enhance clinical relevance, implant placement should allow weight-bearing movement. The types of implants used include rods, screws, nails, pins, and plates, often placed in the femur or tibia to simulate clinical scenarios.[12-21] Typical outcomes measured include bacterial viability, infection rates, clinical outcomes, inflammatory response, and reduction in biofilm formation. Evaluation time-points range from 1 week to several months, depending on the study design. For instance, Zhou *et al*. (2017) evaluated infection reduction and clinical outcomes over 8 weeks in a rabbit model.[5]

Clinical Studies often employ cohort or randomized controlled trial (RCT) designs. The populations studied include patients undergoing orthopaedic surgeries with increased infection risk, such as those with fractures or joint replacements. Similar to the animal studies, clinical studies evaluated antibiotic-loaded coatings, silver coatings, and other antimicrobial strategies. For instance, gentamicin-coated intramedullary nails and silver-coated mega prostheses have been investigated for their efficacy in preventing infections. Among the 9 human studies evaluated, all included clinical outcome measurements, such as patient recovery rates and implant success rates, 6 measured infection rates, and 4 assessed functional outcomes, such as mobility and pain levels.[22-30] Evaluation time-points range from several months to years, 1 year being a common follow-up timepoint. For example, DeMeo *et al*. (2023) reported infection rates and clinical outcomes over a follow-up period of 34.41 ± 9.46 months,[22] and Kawano *et al*. (2023) reported clinical and functional outcomes for 5 years.[24]

Outcome evaluation and analytical techniques: Commonly measured **outcomes** for evaluating the antimicrobial properties of Ti orthopaedic implants in animal and clinical studies include: (1) ***Bacterial viability***, which assesses the proportion of live versus dead bacteria to determine the implant's antimicrobial effectiveness (killing or inhibition). Common methods include live/dead staining, metabolic assays, ATP quantification, and qPCR targeting viable bacteria. (2) ***Bacterial adhesion*** quantifies the initial bacterial attachment to the implant surface, indicating how well the

implant prevents colonization. Measurement methods include CFU counting after surface detachment by sonication and vortexing, fluorescence microscopy, crystal violet staining, and SEM imaging. (3) ***Reduction in biofilm formation*** measures the implant's ability to prevent or reduce mature biofilms, which are hard to treat. Methods for assessing biofilm formation include crystal violet staining for biofilm biomass quantification, CLSM, SEM imaging, and CFU counting from biofilm dispersal. (4) ***Infection rates*** track the incidence and severity of infections post-implantation to assess the clinical effectiveness of antimicrobial strategies. Methods for measuring infection rates include clinical observation, microbiological cultures, imaging (X-ray, MRI), and infection scoring systems. (5) ***Clinical outcomes*** assess patient health and implant success, including recovery rates and complication rates, implant integration, and overall health outcomes following implantation. Measurement methods include clinical examination, patient-reported outcomes (including pain, quality of life), imaging, and complication tracking. (6) ***Inflammatory response*** monitors local and systemic inflammatory markers to detect infections or adverse reactions caused by the implant. Methods for assessing inflammatory response include blood tests, histological analysis, and immunohistochemistry. Fewer studies include (7) ***functional outcomes*** to evaluate the implant's impact on physical function, ensuring it does not impair performance.[5; 26] Measurement methods include mobility tests, range of motion assessments, pain scales, and functional scoring systems. (8) ***Antibiotic resistance*** evaluates whether bacteria exposed to the implant develop resistance to antibiotics over time, which could compromise long-term antimicrobial effectiveness. Measurement methods include antibiotic susceptibility testing (MIC), detection of resistance genes, and whole-genome sequencing. Interestingly, only one of the studies evaluated antibiotic resistance after exposure to the implant.[22] Recent technological advances have significantly enhanced the evaluation of antimicrobial properties in orthopaedic implants. Technologies such as 3D-printing, bacterial genomic sequencing, and real-time *in vivo* imaging have improved preclinical models, making them better at mimicking clinical infections and evaluating antimicrobial materials. These advances enable more accurate and detailed assessments of how implants interact with bacteria and the host immune system. Among the evaluated animal studies, the most frequently employed analytical techniques were: (1) ***histology*** (90%) to examine tissue samples for signs of infection, inflammation, and bone integration (H&E, Gram, Giemsa, and ALP/TRAP staining were commonly used); (2) ***CFU counting*** (67%) to quantify the number of viable bacteria on the implant surface and surrounding tissues; (3) ***radiographic imaging (X-ray)*** (36%) to evaluate bone healing, implant integration, and signs of infection; (4) ***haematological analyses*** (29%) of inflammatory markers (white blood cell counts (WBC), C-reactive protein (CRP), TNF-α, and IL-6) to assess the animal's inflammatory response to the implant; (5) ***micro-computed tomography (micro-CT)*** (19%) to provide detailed 3D images of the bone and implant interface; (6) ***microscopy*** for the visualisation of biofilms on implants or tissues (***SEM*** (17%), ***CLSM*** combined with Live/Dead® BacLight™ staining (7%)); and (7) ***bacterial bioluminescence*** imaging using IVIS (*in vivo* imaging system) for bacterial tracking (17%).[31; 32] These methods provide quantitative and qualitative assessments of ODRI and host response. Among the 9 human studies evaluated, outcomes were typically evaluated by: (1) ***Bacterial culturing*** of tissue biopsies,[22; 29] (2) ***radiographic imaging*** for detection of osseointegration, implant stability, and signs of implant loosening and failure;[22; 24-26] (3) ***haematological analyses*** including complete blood count (CBC) paired with serum analysis,[23] and (4) the use of various ***scoring/classification systems*** (such as Harris Hip Score (HHS),[23] Gustilo–Anderson classification for open fractures,[22] Japanese Orthopaedic Association (JOA) score for spine,[24] ASEPSIS wound healing score,[25] Lower Extremity Functional Scale (LEFS),[27] Short Assessment of Patient Satisfaction (SAPS),[27] and Implant failure modified classification by Henderson *et al*).[30]

Antimicrobial strategies: The reviewed studies employed various antimicrobial strategies, most often including antibiotic-loaded coatings (such as gentamicin, vancomycin),[33; 34] antiseptic-loaded coatings (including silver,[23; 24; 28] iodine,[30] chlorhexidine[35-37]), or antimicrobial peptides

(AMPs).[6; 38; 39] These antimicrobial agents were often loaded in hydrogels, nanoparticles, or nanotubes bound to the Ti implant surface. Most of the strategies demonstrated efficacy in reducing bacterial colonization and preventing osteomyelitis. For example, gentamicin-coated implants demonstrated significant reductions in infection rates and improved clinical outcomes in both animal and human studies.[27; 40] Similarly, silver-coated implants demonstrated effective antimicrobial properties and reduced bacterial colonization without significant adverse effects.[41; 42] Localized drug delivery systems, using biodegradable polymers and nanomaterials,[43-45] provided sustained release of antimicrobial agents, effectively reducing biofilm formation and infection rates.

In ***animal studies***, various antimicrobial strategies have been evaluated for their effectiveness in preventing infections associated with Ti orthopaedic implants. For instance, the use of hydroxypropyltrimethyl ammonium chloride chitosan coatings on Ti exhibited significant infection control and reduced bone destruction.[46] Similarly, polymeric nanofiber coatings loaded with antibiotics significantly reduced infection rates and biofilm formation while enhancing implant integration.[33] The combination of gentamicin and vancomycin in fluorine- and phosphorus-doped nanotubular oxide layers effectively decreased biofilm density without compromising implant integration.[47] Other studies highlighted the benefits of surface modifications, such as the incorporation of AMPs and angiogenic sequences, which reduced infection rates and inflammatory markers while improving vascularization and osseointegration.[48] Additionally, the use of oligo-ampicillin hydrogels prevented bacterial colonization and osteomyelitis,[49] and an AMP-based coating (OP-145) significantly reduced bacterial colonization and infection signs.[50] Various other strategies, including silver coatings, hierarchical TiO_2 nanotubes loaded with cinnamaldehyde, and antibiotic-loaded hydrogels, also demonstrated promising results in reducing infection rates, biofilm formation, and inflammatory responses.[1; 51; 52]

Human studies have also explored the efficacy of different antimicrobial coatings on orthopaedic implants. For example, gentamicin-coated nails were effective in curing infections, promoting bone healing, and yielding good patient-reported outcomes.[27] Similarly, antibiotic-loaded hydrogel coatings significantly reduced fracture-related infections, although some complications were noted.[22] Silver-coated implants demonstrated lower infection rates and improved clinical outcomes in various studies.[24; 29] The use of iodine-coated Ti implants revealed a lower infection incidence in both prevention and treatment cases, with no significant difference between one-stage and two-stage replacements.[30] In addition, antibiotic-loaded resorbable hydrogels significantly reduced surgical site infections without adverse events.[25]

Conclusion: Most studies reviewed demonstrate a statistically and clinically significant reduction in infection rates and improvement in clinical outcomes with antimicrobial coatings on orthopaedic Ti implants. A multi-tiered approach incorporating *in vitro*, animal, and clinical studies is essential for evaluating the antimicrobial properties of orthopaedic Ti implants, ensuring safety and efficacy before clinical introduction. Recent technological advances have further improved the evaluation process, enabling more accurate and detailed assessments. Most animal models of ODRI use *S. aureus*, but future studies should also include other clinically relevant bacteria including coagulase-negative staphylococci, a common cause of chronic infections. Future research should also focus on antibiotic resistance development, which can significantly compromise the long-term effectiveness of antimicrobial strategies in orthopaedic implants. This will enhance the overall success and sustainability of antimicrobial treatments in orthopaedic applications.

References:

1. Fabritius M, Al-Munajjed AA, Freytag C, et al. 2020. Antimicrobial Silver Multilayer Coating for Prevention of Bacterial Colonization of Orthopedic Implants. MATERIALS 13.

2. Hazer DB, Sakar M, Dere Y, et al. 2016. Antimicrobial Effect of Polymer-Based Silver Nanoparticle Coated Pedicle Screws: Experimental Research on Biofilm Inhibition in Rabbits. Spine 41:E323-E329.
3. Lee JW, Cho JA, Roh YJ, et al. 2024. Antibacterial Immunonegative Coating with Biocompatible Materials on a Nanostructured Titanium Plate for Orthopedic Bone Fracture Surgery. BIOMATERIALS RESEARCH 28.
4. Miller RJ, Thompson JM, Zheng J, et al. 2019. In Vivo Bioluminescence Imaging in a Rabbit Model of Orthopaedic Implant-Associated Infection to Monitor Efficacy of an Antibiotic-Releasing Coating. JOURNAL OF BONE AND JOINT SURGERY-AMERICAN VOLUME 101.
5. Zhou L, Liu QB, Zhou ZH, et al. 2017. Efficacy of tobramycin-loaded coating K-wire in an open-fracture rabbit model contaminated by staphylococcus aureus. INTERNATIONAL JOURNAL OF CLINICAL AND EXPERIMENTAL MEDICINE 10:6004-6016.
6. Liu DQ, Xi YJ, Yu SZ, et al. 2023. A polypeptide coating for preventing biofilm on implants by inhibiting antibiotic resistance genes. BIOMATERIALS 293.
7. Peng ZX, Ao HY, Wang L, et al. 2015. Quaternised chitosan coating on titanium provides a self-protective surface that prevents bacterial colonisation and implant-associated infections. RSC ADVANCES 5:54304-54311.
8. Zhou W, Peng X, Ma Y, et al. 2020. Two-staged time-dependent materials for the prevention of implant-related infections. ACTA BIOMATERIALIA 101:128-140.
9. Jaekel C, Windolf CD, Sager M, et al. 2022. Plate-associated localized osteitis in mini-pig by biofilm-forming Methicillin-resistant Staphylococcusaureus (MRSA): establishment of a novel experimental model. EUROPEAN JOURNAL OF TRAUMA AND EMERGENCY SURGERY 48:3279-3285.
10. Williams DL, Epperson RT, Ashton NN, et al. 2019. In vivo analysis of a first-in-class tri-alkyl norspermidine-biaryl antibiotic in an active release coating to reduce the risk of implant-related infection. ACTA BIOMATERIALIA 93:36-49.
11. Williams DL, Costerton JW. 2012. Using biofilms as initial inocula in animal models of biofilm-related infections. J Biomed Mater Res B Appl Biomater 100:1163-1169.
12. Zhang B, Braun BM, Skelly JD, et al. 2019. Significant Suppression of Staphylococcus aureus Colonization on Intramedullary Ti6Al4V Implants Surface-Grafted with Vancomycin-Bearing Polymer Brushes. Acs Appl Mater Inter 11:28641-28647.
13. Dong JL, Zhou W, Hu XL, et al. 2024. Honeycomb-inspired ZIF-sealed interface enhances osseointegration via anti-infection and osteoimmunomodulation. BIOMATERIALS 307.
14. Park J, Jeong D, Lee Y, et al. 2024. Bioactivated lubricant-infused surfaces: A dual-action strategy for enhancing osseointegration and preventing implant-associated infections. CHEMICAL ENGINEERING JOURNAL 482.
15. Ren H, Wang P, Huang HW, et al. 2024. N-Halaminated spermidine-containing polymeric coating enables titanium to achieve dual functions of antibacterial and osseointegration. BIOMATERIALS SCIENCE 12:2648-2659.
16. Li ZH, Jin LQ, Yang XJ, et al. 2024. A multifunctional ionic liquid coating on 3D-Printed prostheses: Combating infection, promoting osseointegration. MATERIALS TODAY BIO 26.
17. Zhang LJ, Yang YR, Xiong YH, et al. 2023. Infection-responsive long-term antibacterial bone plates for open fracture therapy. BIOACTIVE MATERIALS 25:1-12.
18. Metsemakers WJ, Emanuel N, Cohen O, et al. 2015. A doxycycline-loaded polymer-lipid encapsulation matrix coating for the prevention of implant-related osteomyelitis due to doxycycline-resistant methicillin-resistant Staphylococcus aureus. JOURNAL OF CONTROLLED RELEASE 209:47-56.

19. Wang K, Gao MZ, Fan JC, et al. 2024. SrTiO₃ Nanotube-Based "Pneumatic Nanocannon" for On-Demand Delivery of Antibacterial and Sustained Osseointegration Enhancement. ACS NANO 18:16011-16026.
20. Wang YK, Teng WSY, Zhang ZJ, et al. 2021. A trilogy antimicrobial strategy for multiple infections of orthopedic implants throughout their life cycle. BIOACTIVE MATERIALS 6:1853-1866.
21. Skelly JD, Chen FY, Chang SY, et al. 2023. Modulating On-Demand Release of Vancomycin from Implant Coatings via Chemical Modification of a Micrococcal Nuclease-Sensitive Oligonucleotide Linker. Acs Appl Mater Inter 15:37174-37183.
22. De Meo D, Cera G, Pica R, et al. 2023. Antibiotic-Loaded Coatings to Reduce Fracture-Related Infections: Retrospective Case Series of Patients with Increased Infectious Risk. ANTIBIOTICS-BASEL 12.
23. Eto S, Kawano S, Someya S, et al. 2016. First Clinical Experience With Thermal-Sprayed Silver Oxide-Containing Hydroxyapatite Coating Implant. JOURNAL OF ARTHROPLASTY 31:1498-1503.
24. Kawano S, Ueno M, Fujii M, et al. 2023. Case Series of Silver Oxide-Containing Hydroxyapatite Coating in Antibacterial Cementless Total Hip Arthroplasty: Clinical Results of 50 Cases at 5-Year Follow-Up. ARTHROPLASTY TODAY 19.
25. Malizos K, Blauth M, Danita A, et al. 2017. Fast-resorbable antibiotic-loaded hydrogel coating to reduce post-surgical infection after internal osteosynthesis: a multicenter randomized controlled trial. JOURNAL OF ORTHOPAEDICS AND TRAUMATOLOGY 18:159-169.
26. Moghaddam A, Weis J, Haubruck P, et al. 2019. Evaluation of the clinical use of the ETN PROtect® in non-union therapy. INJURY-INTERNATIONAL JOURNAL OF THE CARE OF THE INJURED 50:32-39.
27. Pujol O, Vicente M, Castellanos S, et al. 2023. Preliminary Outcomes of a Staged Percutaneous Retrograde Prefabricated Gentamicin-coated Intramedullary Nail to Manage Complications after Ankle Fusion through Tibial Bone Transport. STRATEGIES IN TRAUMA AND LIMB RECONSTRUCTION 18:155-162.
28. Schoder S, Lafuente M, Alt V. 2022. Silver-coated versus uncoated locking plates in subjects with fractures of the distal tibia: a randomized, subject and observer-blinded, multi-center non-inferiority study. Trials 23.
29. Scoccianti G, Frenos F, Beltrami G, et al. 2016. Levels of silver ions in body fluids and clinical results in silver-coated megaprostheses after tumour, trauma or failed arthroplasty. INJURY-INTERNATIONAL JOURNAL OF THE CARE OF THE INJURED 47:S11-S16.
30. Shirai T, Tsuchiya H, Terauchi R, et al. 2023. Iodine-supported implants in prevention and treatment of surgical site infections for compromised hosts: a prospective study. JOURNAL OF ORTHOPAEDIC SURGERY AND RESEARCH 18.
31. Li FP, Huang K, Wang JB, et al. 2023. A dual functional Ti-Ga alloy: inhibiting biofilm formation and osteoclastogenesis differentiation via disturbing iron metabolism. BIOMATERIALS RESEARCH 27.
32. Stavrakis AI, Zhu SW, Hegde V, et al. 2016. In Vivo Efficacy of a "Smart" Antimicrobial Implant Coating. JOURNAL OF BONE AND JOINT SURGERY-AMERICAN VOLUME 98:1183-1189.
33. Ashbaugh AG, Jiang XS, Zheng J, et al. 2016. Polymeric nanofiber coating with tunable combinatorial antibiotic delivery prevents biofilm-associated infection in vivo. PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 113:E6919-E6928.
34. Diefenbeck M, Schrader C, Gras F, et al. 2016. Gentamicin coating of plasma chemical oxidized titanium alloy prevents implant-related osteomyelitis in rats. BIOMATERIALS 101:156-164.

35. Perni S, Alotaibi HF, Yergeshov AA, et al. 2020. Long acting anti-infection constructs on titanium. JOURNAL OF CONTROLLED RELEASE 326:91-105.
36. Riool M, Dirks AJ, Jaspers V, et al. 2017. A CHLORHEXIDINE-RELEASING EPOXY-BASED COATING ON TITANIUM IMPLANTS PREVENTS STAPHYLOCOCCUS AUREUS EXPERIMENTAL BIOMATERIAL-ASSOCIATED INFECTION. EUROPEAN CELLS & MATERIALS 33:143-157.
37. Shiels SM, Bouchard M, Wang H, et al. 2018. CHLORHEXIDINE-RELEASING IMPLANT COATING ON INTRAMEDULLARY NAIL REDUCES INFECTION IN A RAT MODEL. EUROPEAN CELLS & MATERIALS 35:178-194.
38. Dong JY, Chen FM, Yao YY, et al. 2024. Bioactive mesoporous silica nanoparticle-functionalized titanium implants with controllable antimicrobial peptide release potentiate the regulation of inflammation and osseointegration. BIOMATERIALS 305.
39. Zhou H, Ren YL, Zou KX, et al. 2024. Efficacy of pH-Responsive Surface Functionalized Titanium Screws in Treating Implant-associated S. aureus Osteomyelitis with Biofilms Formation. ADVANCED HEALTHCARE MATERIALS.
40. Neut D, Dijkstra RJB, Thompson JI, et al. 2015. A BIODEGRADABLE GENTAMICIN-HYDROXYAPATITE-COATING FOR INFECTION PROPHYLAXIS IN CEMENTLESS HIP PROSTHESES. EUROPEAN CELLS & MATERIALS 29:42-56.
41. Kose N, Çaylak R, Peksen C, et al. 2016. Silver ion doped ceramic nano-powder coated nails prevent infection in open fractures: In vivo study. INJURY-INTERNATIONAL JOURNAL OF THE CARE OF THE INJURED 47:320-324.
42. Yang N, Wu T, Li M, et al. 2025. Silver-quercetin-loaded honeycomb-like Ti-based interface combats infection-triggered excessive inflammation via specific bactericidal and macrophage reprogramming. BIOACTIVE MATERIALS 43:48-66.
43. Ghimire A, Skelly JD, Song J. 2019. Micrococcal-Nuclease-Triggered On-Demand Release of Vancomycin from Intramedullary Implant Coating Eradicates Staphylococcus aureus Infection in Mouse Femoral Canals. ACS CENTRAL SCIENCE 5:1929-1936.
44. Woelfle UC, Briggs T, Bhattacharyya S, et al. 2020. DUAL LOCAL DRUG DELIVERY OF VANCOMYCIN AND FARNESOL FOR MITIGATION OF MRSA INFECTION IN VIVO -A PILOT STUDY. EUROPEAN CELLS & MATERIALS 40:38-57.
45. Yang Y, Ao HY, Yang SB, et al. 2016. In vivo evaluation of the anti-infection potential of gentamicin-loaded nanotubes on titania implants. Int J Nanomed 11:2223-2234.
46. Ao HY, Yang SB, Nie BE, et al. 2019. Improved antibacterial properties of collagen I/hyaluronic acid/quaternized chitosan multilayer modified titanium coatings with both contact-killing and release-killing functions. JOURNAL OF MATERIALS CHEMISTRY B 7:1951-1961.
47. Auñón A, Esteban J, Doadrio AL, et al. 2020. Staphylococcus aureus Prosthetic Joint Infection Is Prevented by a Fluorine- and Phosphorus-Doped Nanostructured Ti-6Al-4V Alloy Loaded With Gentamicin and Vancomycin. JOURNAL OF ORTHOPAEDIC RESEARCH 38:588-597.
48. Chen JJ, Hu GS, Li TJ, et al. 2021. Fusion peptide engineered "statically-versatile" titanium implant simultaneously enhancing anti-infection, vascularization and osseointegration. BIOMATERIALS 264.
49. Chen FY, Skelly JD, Chang SY, et al. 2024. Triggered Release of Ampicillin from Metallic Implant Coatings for Combating Periprosthetic Infections. Acs Appl Mater Inter 16:24421-24430.
50. de Breij A, Riool M, Kwakman PHS, et al. 2016. Prevention of Staphylococcus aureus biomaterial-associated infections using a polymer-lipid coating containing the antimicrobial peptide OP-145. JOURNAL OF CONTROLLED RELEASE 222:1-8.

51. Ma ZR, Zhao Y, Xu Z, et al. 2024. 3D-printed porous titanium rods equipped with vancomycin-loaded hydrogels and polycaprolactone membranes for intelligent antibacterial drug release. Scientific reports 14.
52. Mao Y, Xie XR, Sun GX, et al. 2024. Multifunctional Prosthesis Surface: Modification of Titanium with Cinnamaldehyde-Loaded Hierarchical Titanium Dioxide Nanotubes. ADVANCED HEALTHCARE MATERIALS 13.