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CONSENSUS

2025 International Consensus Meeting on Musculoskeletal Infection: Summary From Biofilm Workgroup on Treatment of Biofilm-Related Infection and Preclinical Models

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ABSTRACT

Despite advancements in surgical techniques, musculoskeletal infections (MSKI) remain severe complications following orthopedic surgery, imposing a substantial financial and personal burden on patients and healthcare systems globally. To establish the current state of knowledge in this field, International Consensus Meetings (ICM) were held in 2013, 2018, and 2025, including a Biofilm Section focused on establishing state-of-the-art basic science and translational research. The latest ICM utilized a 2-year-long Delphi process that commenced on May 31, 2023, and culminated in an in-person meeting involving voting on 30 questions by 47 delegates on May 8–10, 2025, in Istanbul, Turkey. Following the voting process, the Biofilm Section formed three workgroups (Biofilm Basic Science, Biofilm Treatment, and Research Priorities) to interpret the results and disseminate the findings in Consensus Articles that highlight priorities. The following is the summation of the Biofilm Treatment Workgroup, which aims to shape future pre-clinical MSKI research directions and grant funding with respect to: (1) elevating scientific rigor to ensure reproducibility and high-quality data in preclinical research; (2) transitioning mature therapeutic concepts into rigorous in vivo models to definitively prove their clinical feasibility; and (3) accelerating the development of novel molecular targets and advanced drug-delivery systems. Finally, the workgroup acknowledged a critical shift in the funding landscape. As government support faces future challenges, there is an urgent need for increased investment from industry and philanthropic partners. Such support is essential to develop effective treatments for serious orthopedic infections and to improve outcomes for patients facing life-altering illnesses.

Musculoskeletal infections (MSKI) can occur after injury or surgical procedures, leading to serious complications such as fracture non-unions, failed joint replacements, amputations, and death, and standards of care are largely unchanged from surgical and antibiotic treatments developed in the 1980s [1]. While the rates of infection for elective orthopedic surgical procedures are low at 0.5%–1.4%, in part due to stringent protocols for sterility and the use of antibiotic prophylaxis, the consequences of MSKI can be devastating [2]. Notably, the 5-year survival rate for periprosthetic joint infection (PJI) is only ~75%, which is similar to that of the most lethal cancers [3]. Thus, pre-clinical research to develop "game-changing" cost-effective therapies to prevent and treat MSKI with intractable biofilm has increased in recent years, with over 17,000 related journal articles published in the past 4 years. Several physical and pharmaceutical treatments have emerged as potential biofilm-targeted solutions, including novel antimicrobials, local delivery systems, and electrotherapy [4–6]. However, studies demonstrating the efficacy of a treatment modality in vitro or in animal models often fail to translate to clinical practice [7]. These issues were the focus of the Biofilm Workgroup, comprised of 47 out of 1205 total delegates, that contributed to the 2025 International Consensus Meeting (ICM) on MSKI https://www.icmortho.org. The Biofilm Workgroup generated 30 high-priority questions that were systematically reviewed by delegate teams, who wrote a Response with Rationale based on the systematic reviews and expert opinions, which were voted on at the in-person ICM on May 8–10, 2025, in Istanbul (Turkey), and the results are available online https://www.ors.org/2025-icm-on-mski/.

As these Biofilm Questions spanned a broad range of pre-clinical research topics, the authors of these Consensus Articles were tasked with summarizing the results and future directions of the questions related to therapeutic strategies for infection. The following describes our methodology to identify the greatest priorities based on the state-of-the-art, research needs, and impact potential of research that could produce cost-effective treatments for patients with serious MSKI.

1 | Methodology

The main objective of ICM 2025 was to assemble expert recommendations for guiding new research directions and best practices for the incorporation of these therapies into clinical practice. Infection experts from various fields, including biomedical engineering, immunology, infectious diseases, microbiology, pathology, veterinary medicine, and orthopedic surgery, made up the group. An international panel of MSKI experts was asked to select questions for which they could offer expertise to identify the relevant literature and common challenges. Discussion of summarized results sought to achieve consensus on recommendations using the Delphi methodology, as was done for the 2018 ICM for MSKI [8, 9].

Over 10 months, each question was assigned to a liaison who led a group of two or more delegates to review the literature, summarize the data, and construct a narrative summary of the rationale behind the response. Covidence (Veritas Health Innovation, Australia) was used to guide systematic reviews, importing studies with relevant MESH terms. Reviewers then screened titles and abstracts to identify relevant studies, screened full-text articles, and extracted data for analysis. The compiled responses were then published at https://www.icmortho.org/documents for review and comment by all 1205 delegates. The authoring delegates refined their responses based on the comments in preparation for discussion and voting at the in-person consensus meeting held on May 8–10, 2025, in Istanbul (Turkey).

Controversial or unclear recommendations were edited during the discussion to promote broader agreement on questions. Delegates voted to (1) agree; (2) disagree; or (3) abstain, on each response, following Delphi methodology, with results rated as Unanimous Consensus (100%), Strong/High Consensus (≥ 80%), Moderate Consensus (65%–79.9%), Weak Consensus/Low Agreement (50.1%–64.9%), and No Consensus/Excluded (< 50%).

Consensus was reached without compulsion, undue influential power, inability to comprehend another course of action, or impatience with the process of discussion and voting. Full recommendations for each question, with HTML links to

downloadable PDFs for each question, response, consensus, and post-meeting rationale, are available at https://www.ors.org/2025-icm-on-mski/. To ensure a comprehensive review of topics within the Biofilm Workgroup, pre-numbered questions were categorized into thematic areas, including treatment strategies and basic mechanisms, to facilitate a structured analysis. Primary liaisons and delegates for each question subsequently summarized the results, which are supported by the full primary research references available in the source documentation. The following sections detail the 22 prioritized questions related to the evaluation and clinical application of therapeutic strategies against biofilms (organized into topics of models, standard of care antimicrobials, and experimental therapeutics/physical methods, keeping the original numbering so that documents can be accessed for full responses). Figure 1 summarizes the major themes that received high and low agreement in the categories of diagnostics, therapeutics, prevention, and methodology.

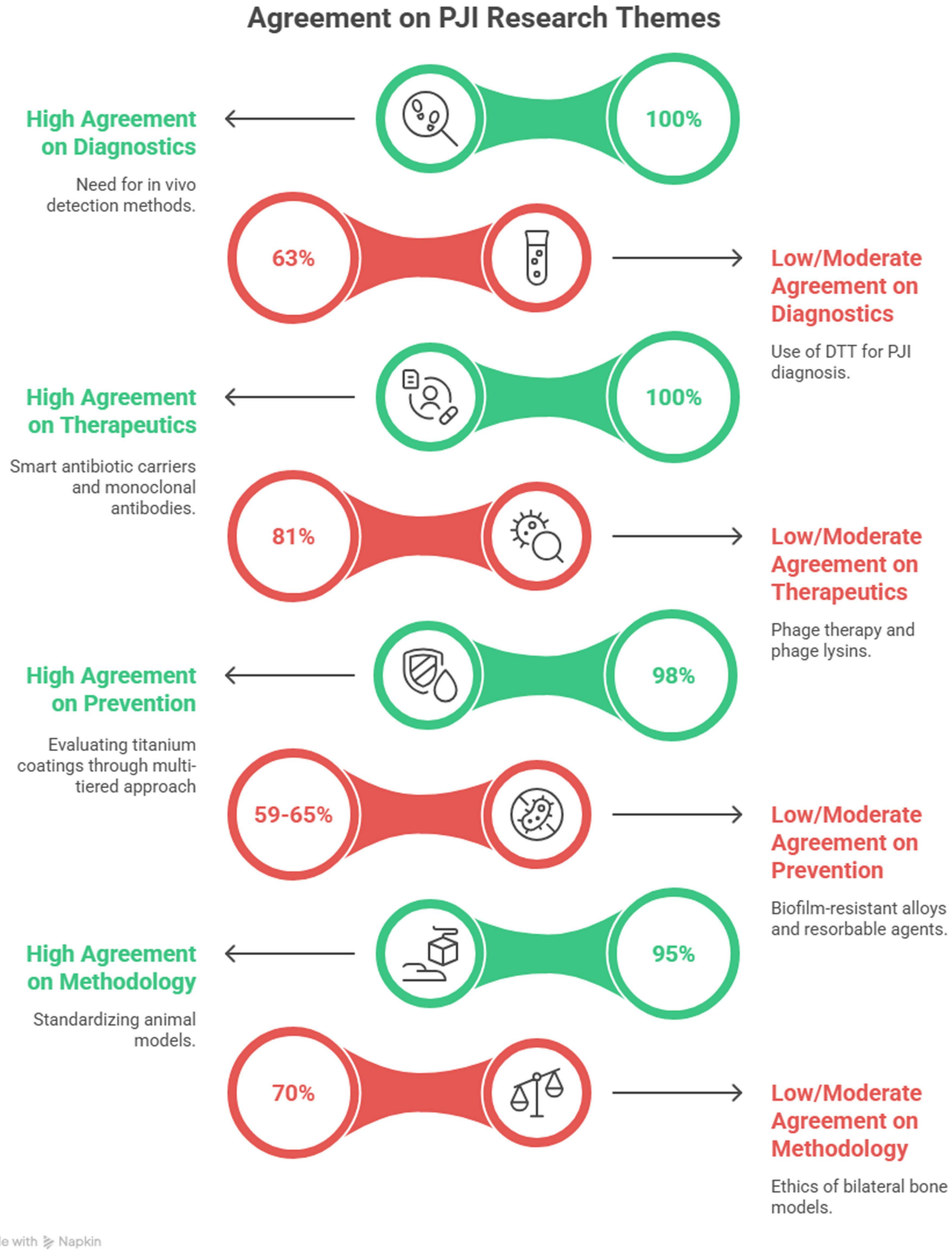

FIGURE 1 | Research themes with high and low/moderate agreement from the biofilm workgroup involving treatment. Made with Napkin.ai.

2 | Major Advances in Preclinical Models of MSKI Demand Rigor and Reproducibility

A central theme of the 2025 ICM was the evolution of experimental technologies and discovery tools for in vivo and ex vivo MSKI research. These advancements have fundamentally changed the landscape since the inaugural ICM in 2013, which largely relied on non-quantitative outcomes and colony-forming assays. Recent editorials and systematic reviews have highlighted a need for greater consistency and scientific rigor in peer-reviewed MSKI research [10, 11]. Further complicating this landscape, government-regulated animal research has undergone significant changes, with substantial variation in guidelines across nations and individual Institutional Animal Care and Use Committees (IACUCs). These discrepancies create challenges for the international dissemination of research, particularly concerning large animal models and trauma-related studies (e.g., war wounds).

To navigate these complexities, the 2025 ICM addressed four questions regarding scientific rigor and the transparent reporting of animal research. The goal is to ensure that clinical findings are not only scientifically sound but also ethically robust enough to support effective translation to clinical practice.

Question B5: What is(are) the best preclinical model(s) of orthopedic infection for the evaluation of therapeutic efficacy?

Response: The "best" model depends on the specific hypothesis; however, studies must include adequate standard-of-care controls, quantification of the pathogenic inoculum, evidence of infection prior to treatment, and quantification of burden at the prospective endpoint.

Voting: 95% Agree, 0% Disagree, 5% Abstain (Strong Consensus).

Taken literally, this question calls for a roadmap for the most rigorous, robust, and cost-effective approach for investigators conducting preclinical evaluations of experimental antimicrobial strategies for MSKI. Because regulatory bodies such as the FDA have not provided specific guidance on this topic, the 2025 ICM focused on establishing minimum requirements for in vivo efficacy studies. This focus was influenced by a vast body of peer-reviewed literature claiming antimicrobial efficacy despite failing to adhere to published recommendations [12]. The ICM found these studies to be scientifically insufficient because their experimental designs lacked essential prospective controls and baseline infection data required to validate their conclusions [10]. To rectify this, the ICM outlines both "must-have" criteria and "rigor-enhancing" evaluations for common animal models (Table 1). Specifically, the ICM recommends that preclinical MSKI studies evaluating antimicrobial efficacy in mice, rats, rabbits, pigs, and sheep must include controls that receive adequate standard of care treatment where possible, quantification of the pathogenic inoculum, evidence of infection prior to treatment, and quantification of the pathogenic burden at the prospective endpoint. The ICM emphasizes that while the "best" model remains hypothesis-dependent, superior studies should go beyond minimal requirements to include statistically powered longitudinal outcomes (such as radiology or bioluminescent imaging) and multi-modal ex vivo analyses (including molecular biology and biochemistry) to ensure high-fidelity clinical translation (Table 1).

Question B6: Are bilateral animal models of infected bone defects ethical?

Response: A thoughtful 5-point recommendation was developed to balance the 3Rs (reducing animal numbers) with the scientific rigor required to evaluate pain and suffering.

Voting: 70% Agree, 0% Disagree, 30% Abstain (Strong Consensus).

This question was largely provoked by diverging opinions on: (i) the 3Rs of animal research, as bilateral models cut the number of animals and costs in half, (ii) the scientific rigor of evaluating pain and suffering and primary outcomes in animals with bilateral infected bone defects, and (iii) the omnipotence of local IACUCs for animal research results that are to be acceptable world-wide. The ICM made a thoughtful five-point Recommendation that addresses these points, summarized visually in Figure 2. The five points are as follows: (1) Scientific assessments of mobility and functionality that likely go beyond established standards for single limb bone defect infection models as they pertain to animal welfare (need for euthanasia) and pain management (need for analgesic administration) should be included. (2) The cumulative effect of all the defects on animal welfare with correctly applied analgesic treatment and animal management should not exceed the effect of a single infected defect. (3) The overall scientific rigor of the research should provide formal proof-of-concept. (4) Assurances that there is a scientific justification for the use of a bilateral model and that it is not being applied primarily for cost-effectiveness reasons. And (5) the research needs to comply with national and international regulations governing animal research, such as the Animal Welfare Act in the United States, Directive 2010/

TABLE 1 | Considerations for animal models of musculoskeletal infections (MSKI).

Animal	Minimal requirements (must have)	Evaluations to enhance rigor
Mouse	Microbiological assessments; Standardized inoculation; Histology	Radiography; Longitudinal bioluminescent imaging; Electron microscopy; Molecular analyses
Rat	Microbiological assessments; Radiography; Histology	Tissue antibiotic concentrations; Cytokine levels (IL-6, TNF-α); Micro-CT
Rabbit	Microbiological assessments; Radiography; Histology	Micro-CT; Weight monitoring; Mortality rates; Systemic antibiotic concentrations
Pig	Microbiological assessments; Routine clinical exam; Histology	Advanced imaging techniques; Serum biomarkers
Sheep	Microbiological assessment; Radiography; Routine clinical exam; Histology	Hematology; Serum biomarkers

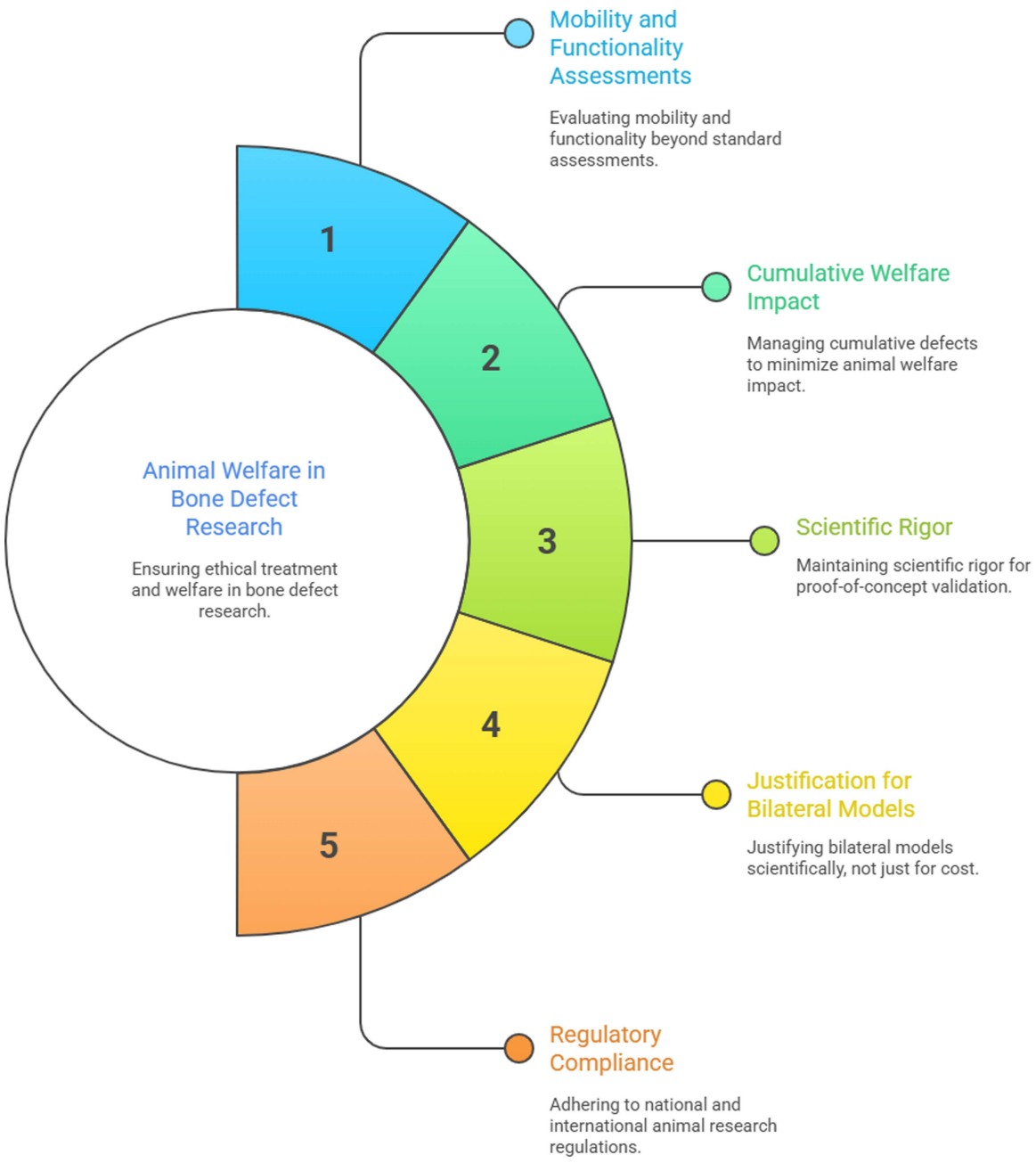

FIGURE 2 | Five-point recommendations for enhancing animal welfare when considering bilateral models. Made with Napkin.ai.

63/EU in the European Union, and guidelines from organizations like the National Institutes of Health and the World Organization for Animal Health. However, the 30% abstention is also noteworthy and likely reflects ongoing controversies that will need to be addressed going forward.

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

Response: Evaluation should involve a multi-tiered approach (in vitro, animal, and clinical) using well-established models, appropriate bacterial species, and standardized inoculation doses.

Voting: 98% Agree, 0% Disagree, 2% Abstain (Strong Consensus).

The ICM recommendation establishes a comprehensive framework for assessing the antimicrobial capabilities of titanium implants. Use of standardized inoculation doses and well-established animal models was proposed as the best method for achieving a high level of evidence for safety and efficacy prior to clinical application. The workgroup identified several "mature" technologies currently under review, including active agent coatings (hydrogels, nanoparticles, nanofibers, or nanotubes impregnated with antibiotics, antiseptics, antimicrobial peptides, or ions) and surface modifications (classic silver-coated surfaces and other physical-chemical alterations to the titanium substrate) [13]. The workgroup also supported a multi-tiered valuation approach to include progression from in vitro to animal to clinical studies. While lacking biological complexity, in vitro studies are critical for refining technology parameters and establishing initial dosing conditions. In vivo evaluations are primarily conducted in small rodents, though sheep and swine are utilized for larger load-bearing models [12]. *Staphylococcus*

aureus remains the primary pathogen, with outcomes focusing on biofilm reduction, histology, and infection rates. For clinical studies, the workgroup recommends a focus on high-risk patient populations, prioritizing functional outcomes and re-infection rates.

The workgroup highlighted several deficiencies in current experimental designs that should be addressed to ensure long-term clinical utility, including antimicrobial resistance (AMR) testing, evaluation of a diverse set of strains, and inclusion of informative biomarkers. Current studies often fail to assess whether a coating induces bacterial resistance over time, and it will be important to evaluate long-term exposure to sub-lethal concentrations of antimicrobial ions or peptides for the selection of resistant strains. The group also felt that there is a critical need to expand testing beyond *S. aureus* to include coagulase-negative staphylococci and other common clinical isolates. Finally, to enhance translational value, the incorporation of physiological outcome preclinical measures and biomarkers (e.g., serum inflammatory markers) would allow direct alignment with clinical monitoring tools. By incorporating these rigorous scientific standards, investigators can better predict the real-world success of antimicrobial titanium implants in preventing life-altering MSKI.

Question B23: Are there any methods to detect biofilms in vivo?

Response: No. There are currently no in vivo biofilm detection methods available for clinical practice, though several experimental methods are in development.

Voting: 100% Agree (Unanimous Consensus).

While preclinical studies have clarified biofilm structure on both organic and inorganic surfaces, current clinical diagnostic tools remain inadequate for in situ detection. In orthopedic implant-associated infections, clinicians currently rely on non-specific X-rays or radiolabeled white blood cell imaging. These methods are often coupled with invasive peri-prosthetic tissue or fluid samples that must be cultured [14]. Culturing patient-derived samples is the standard for identifying microorganisms, yet it frequently fails to detect biofilm-encased bacteria for several reasons, including mechanical adhesion and the metabolic state of microorganisms within biofilm. Biofilms are difficult to remove from surfaces or disaggregate without significant mechanical disruption. The presence of dormant "persister" cells or small colony variants means bacteria may not grow under standard laboratory conditions [15]. These limitations result in low sensitivity and specificity, often leading clinicians to miss the underlying infection or misidentify it as aseptic failure.

Misinterpretation of diagnostic results can lead to inappropriate antimicrobial treatment, unnecessary surgery, or "culture-negative" infections [16]. To address this, research is exploring various chemical agents (e.g., crystal violet, methylene blue, biosurfactants) and nanotechnology-based methods that have shown promise in laboratory settings. Because molecular tools like PCR and Next-Generation Sequencing cannot distinguish between a biofilm lifestyle and free-floating (planktonic) bacteria, microscopy remains the primary tool for visualizing biofilm structure. Despite advancement efforts, these technologies have not yet transitioned into validated clinical tools.

3 | Standard of Care Antimicrobial Therapies

Five questions in the Biofilm Workgroup and two questions in the General Session were identified as related to traditional therapies used in the clinic and whether they have been proven to be effective based on current evidence from the literature.

Question B2: Has the in vitro antimicrobial efficacy of antiseptic irrigation solutions translated to a reduction in SSI/PJI in clinical practice?

Response: Yes. There is encouraging evidence, particularly for Povidone-iodine (PI), although meta-analyses are mixed and lack consistent protocols.

Voting: 100% Agree (Unanimous Consensus).

This question recommends that there is some evidence from in vitro studies that supports the use of antiseptic irrigation solutions, though at a low to moderate level of evidence. The most widely studied antiseptic for this purpose is PI [17]. While some national and international guidelines offer weak or conflicting recommendations on its use, the evidence for PI is mixed but promising. Some meta-analyses show that PI reduces postoperative infection rates, while others find no significant difference. Recently, high-quality studies have shown a decrease in PJI when PI is used for irrigation during revision total joint arthroplasty and, in combination with other agents, during primary TJA [18]. Similarly, for spine surgery, some randomized controlled trials (RCTs) have demonstrated a significant reduction in SSIs with PI irrigation, though other studies show no difference [19]. Overall, the evidence is encouraging, although a lack of consistent protocols and small sample sizes in many studies prevent a definitive recommendation. More large-scale, prospective RCTs are needed to confirm the effectiveness of PI and other antiseptic solutions in preventing SSIs and PJIs.

Question B7: Are there any specific agents that have been shown to be efficacious against biofilm in pre-clinical models?

Response: Yes. Pre-clinical evidence exists for antimicrobials, chemical disruptors, physical disruptors, bacteriophages, enzymatic disruptors, and antibodies.

Voting: 100% Agree (Unanimous Consensus).

Biofilm is widely recognized as the primary obstacle to the successful eradication of MSKI. While historical research focused on creating agents to physically disrupt the biofilm matrix to improve antibiotic penetration, modern evidence suggests that antibiotic failure is often driven by the altered physiology and metabolic dormancy of bacteria within the biofilm, rather than simple penetration barriers [20]. The 2025 ICM scrutinized the literature for pre-clinical studies evaluating experimental strategies to overcome these challenges. The delegates unanimously agreed ($n = 40$) that six distinct therapeutic modalities have demonstrated pre-clinical efficacy against biofilms (Figure 3):

- **Antimicrobials**: Use of specialized or high-dose agents designed to target the unique metabolic states of biofilm-associated bacteria.
- **Chemical Disruptors**: Agents such as biosurfactants or chelators that destabilize the biofilm's protective exopolymer matrix.
- **Physical and Energy-Based Disruptors**: Use of ultrasound, shockwaves, or thermal energy to mechanically weaken biofilm structures.

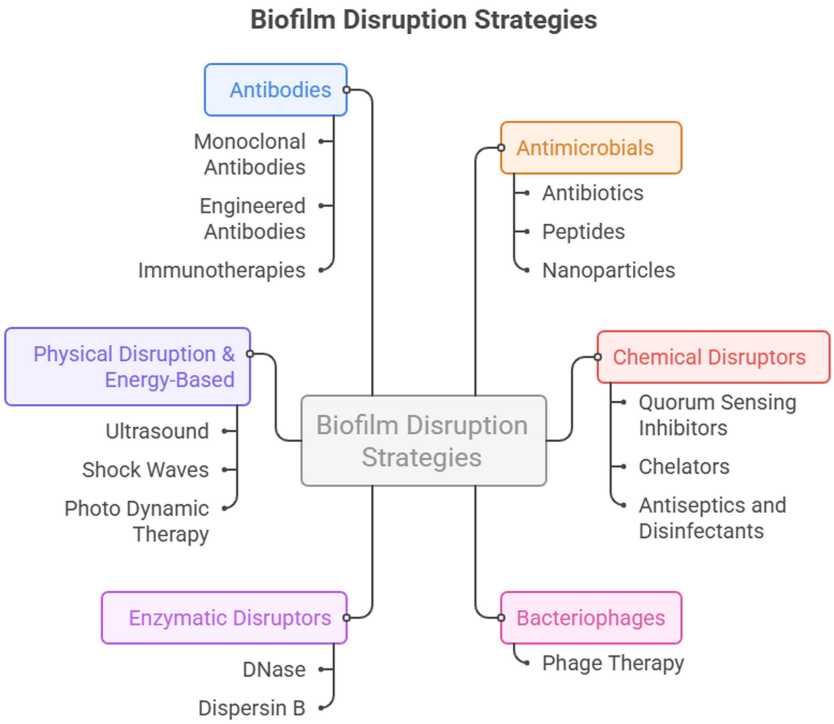

FIGURE 3 | Six categories of biofilm disruption strategies. Made with Napkin.ai.

- **Bacteriophages**: Viruses that specifically infect and lyse specific pathogenic bacteria, often containing biofilm matrix-degrading enzymes.
- **Enzymatic Disruptors**: Specific enzymes (e.g., DNase or glycoside hydrolases) that degrade the structural components of the biofilm matrix.
- **Antibodies**: Immunotherapies designed to target biofilm-specific surface proteins, neutralizing their protective capabilities or marking them for immune clearance.

While these six categories show significant promise, the ICM noted a critical lack of standardization between studies. Without uniform models and comparative metrics, it remains difficult to objectively determine which agents are most effective for clinical translation. The Workgroup emphasized that future research must move beyond mere disruption and address the physiological resilience of biofilm-resident microbes.

Question B19: Does local delivery of antimicrobials have any influence on the removal of biofilm in orthopedic infections?

Response: Unknown. Due to a lack of high-level evidence, a definitive supportive or oppositional recommendation cannot be provided.

Voting: 94% Agree, 3% Disagree, 3% Abstain (Strong Consensus).

The authors of the response concluded that while the potential role of local antibiotic delivery has been explored in vitro, animal, and human case studies, there is a substantial lack of Level 1 evidence to demonstrate its efficacy in removing biofilm associated with MSKI [21]. Specific gaps in knowledge include optimal dosing, delivery methods, antimicrobial selection, and standardization. There is no consensus on the concentration required to eradicate biofilm in vivo. Evidence is insufficient to recommend specific carriers (e.g., bone cement, beads, or spacers). It remains unclear which specific agents are most effective against established biofilms in a clinical setting. The absence of standardized formulation protocols prevents objective comparisons between different treatment regimens.

A significant development in this field is the SOLARIO (Short or Long-term Antibiotics for Orthopedic Infection) trial (NCT03815578) [22]. This study investigates whether a shorter course of systemic antibiotics can be just as effective as the standard longer course when supplemented with local antibiotic delivery. Inclusion criteria were patients with confirmed MSKI (including prosthetic joint infections, fracture-related infections, and osteomyelitis) where the surgeon felt a "short course" of systemic antibiotics was potentially appropriate. All infected metalwork must have been removed, or the patient must have had no retained infected hardware at the time of the intervention. Exclusion criteria included patients requiring long-term suppressive therapy or those with infections where local antibiotic delivery was not feasible. The total number of patients was 473 participants, who were randomized into (i) short group ($n = 237$) receiving 7 days or less of postoperative systemic antibiotics and (ii) long group ($n = 236$) receiving the standard of care (typically 4 weeks or more). Both groups received implanted local antibiotics as part of their surgical treatment. Participants in the short-duration arm received a mean of 27.5 antibiotic days over 12 months, compared to 74.9 days in the standard care arm. While the trial suggests that local antibiotics may allow for a significant reduction in systemic antibiotic exposure, the full peer-reviewed publication—including specific details on pathogen types (e.g., *S. aureus* vs. others) and specific implant locations—is currently pending.

Question G52: Is there a role for the use of resorbable agents that deliver antibiotics locally for the prevention of infection?

Response: Yes. Resorbable agents like calcium sulfate and hydrogels may reduce infection rates in high-risk patients, though study quality is generally low.

Voting: 59.2% Agree, 27.3% Disagree, 13.5% Abstain (No Consensus).

A total of nine studies were identified. The rationale is supported by multiple studies, primarily focusing on high-risk patients, such as those undergoing arthroplasty or revision surgeries, showing that these agents significantly reduce infection rates compared to standard care [23]. Key clinical implications include their potential for reducing infections in high-risk patients, but caution is warranted due to the relatively small number and low quality of available studies. The high percentage of "Disagree" and "Abstain" votes reflects significant reservations among the delegates. The lack of consensus was driven by the following factors:

- **Low Quality of Evidence:** While the results are promising, the workgroup noted that the overall quality of available studies is low and the total number of studies (nine) is relatively small.
- **Safety and Adverse Events:** There are notable concerns regarding complications, specifically with calcium sulfate, including prolonged serous wound drainage and potential hypercalcemia.
- **Unclear Superiority:** There is currently no definitive evidence demonstrating the superiority of one resorbable agent (e.g., calcium sulfate vs. hydrogels) over another.
- **Unresolved Efficacy:** The delegates determined that further research is required to validate these findings and address ongoing questions regarding long-term safety and efficacy.

While resorbable agents show potential for reducing infections in high-risk patients, the 2025 ICM emphasizes that caution is warranted. The transition of these "mature conceptual therapies" into clinical practice requires more rigorous in vivo validation to establish standardized safety and delivery protocols.

Question B27: Is there sufficient data demonstrating that any antibiotics possess antibiofilm properties?

Response: Yes. Rifampicin shows consistent activity against bacteria, while echinocandins show activity against *Candida* species.

Voting: 97% Agree (Strong Consensus).

The consensus vote for this question supports the antibiofilm properties of some antibiotics, though clinical evidence for antibiofilm activity is scarce, with most data available being derived from in vitro studies and studies in animals. Interpretation of antibiofilm efficacy is further complicated by variability in experimental models, lack of standardized metrics, strain-specific differences in antibiofilm activity, and the predominance of studies on monospecies biofilms [24–26]. The recommendation also concluded that rifampicin has demonstrated the most consistent antibiofilm activity against bacteria, especially in combination therapies, and effective combinations include rifampicin with vancomycin, daptomycin, fluoroquinolones (e.g., levofloxacin, ciprofloxacin, moxifloxacin), doxycycline, minocycline, and fusidic acid [27]. Among antifungals, echinocandins (micafungin, caspofungin, anidulafungin) show the strongest antibiofilm activity towards *Candida* spp. Amphotericin B lipid formulations are also effective, while fluconazole and other azoles generally lack activity [28, 29].

Question B28: Can Dithiothreitol (DTT) be considered a useful tool for breaking down biofilms present in synovial fluid?

Response: Yes. DTT is a reducing agent that facilitates the identification of bacteria in synovial fluid by breaking down the biofilm matrix.

Voting: 63% Agree, 1% Disagree, 33% Abstain (Majority/Weak Consensus).

Biofilms are bacterial aggregates protected by an extracellular matrix that often hinder accurate pathogen identification, a critical step for effective treatment of PJI. DTT serves as a reducing agent that specifically breaks disulfide bonds within this matrix, releasing encased bacteria and making them accessible for culture. DTT fluid cultures demonstrate higher sensitivity than standard saline-based methods or sonication, particularly when infection is not initially suspected [30]. It remains effective in cases where patients have received preoperative antibiotics—a common scenario where traditional culture methods frequently fail [31]. DTT is a cost-effective and user-friendly alternative to sonication, as it does not require specialized equipment and features a faster protocol with a lower risk of contamination. Emerging data suggest DTT may disrupt fungal biofilms (e.g., *Candida albicans* and *Aspergillus* spp.) by the same disulfide-reduction mechanism, potentially aiding in the detection of pathogens resistant to conventional diagnostics [31]. Despite the generally favorable recommendation based on moderate-level evidence, the 33% abstention rate highlights significant concerns within the expert community. The Biofilm Treatment Workgroup identified several areas requiring further development:

- **Diagnostic Protocols:** There is a pressing need for better standardization of PJI diagnostic protocols across clinical settings.
- **Parameter Consistency:** The research community must establish standardized DTT concentrations and pretreatment timing to ensure reproducible results.
- **Compositional Variability:** DTT's effectiveness may vary depending on the specific composition of the biofilm, and its role in diagnosing fungal infections requires further rigorous research.

Question G87: Do currently available products safely achieve local antibiotic concentrations above Minimum Biofilm Eradication Concentration (MBEC) for a sufficient time?

Response: No. There is no clear evidence to confirm this in clinical practice due to concerns over toxicity and species-specific variability.

Voting: 89.7% Agree, 3.4% Disagree, 6.8% Abstain (Strong Consensus).

This question examined whether current products can safely maintain local antibiotic concentrations above the MBEC long enough to eradicate orthopedic biofilm-associated infections. Strong consensus supported the view that there is no clear evidence to confirm this in clinical practice. While in vitro and

some clinical studies demonstrate that direct antibiotic infusion systems, high-dose antibiotic-loaded carriers, and novel biodegradable delivery methods can achieve high local concentrations, variability in MBEC by bacterial species, implant status, and antibiotic type, as well as concerns over toxicity, make consistent clinical achievement uncertain [32]. Sustaining concentrations above MBEC without causing harm remains unproven, and further well-designed trials are needed to establish safe and effective protocols.

4 | Physical Methods and Experimental Therapeutics

Eleven questions related to experimental therapeutics that are not in widespread clinical use or physical removal methods for biofilm that may complement traditional antimicrobial therapies.

Question B13: Are monoclonal antibodies (mAbs) capable of eradicating biofilms in orthopedic infections?

Response: Unknown. While preclinical data are promising, current clinical evidence remains limited.

Voting: 100% Agree (Unanimous Consensus).

Preclinical studies strongly support the potential of mAbs to target and disrupt biofilms through a variety of mechanisms [33, 34]. These include disruption of biofilm structural components (e.g., DNA binding proteins in the family DNABII, Poly-N-acetylglucosamine, amyloid fibers) [35–37], interference with biofilm-associated enzymes and adhesins (e.g., *S. aureus* autolysin Atl, clumping factor A) [38], and, in the case of conjugated mAbs, targeted delivery of antibiotics [39] or radiopharmaceuticals [40, 41] to biofilm sites. Collectively, these strategies promote biofilm matrix degradation, improve bacterial clearance through immune or localized antimicrobial mechanisms, and increase susceptibility to conventional antibiotics. Among these mAbs, only anti-DNABII therapies have progressed to early-phase clinical trials, with Phase I data supporting safety and feasibility in patients with PJI [42]. Further translational and clinical research is needed to evaluate the full potential and therapeutic efficacy of mAb-based strategies for eradicating biofilms in the orthopedic setting.

Question G94: Is there a role for immunotherapy in patients with orthopedic implant-associated infections?

Response: Potentially. Immunotherapy could be promising, but clinical validation in humans is necessary.

Voting: 91% Agree (Strong Consensus).

The Workgroup defines immunotherapy in the context of MSKI as the manipulation of host-microbe interactions to enhance bacterial clearance or modulate the inflammatory environment. This differs from oncological definitions and focuses on strategies such as modulating topography by embedding metal ions into porous surfaces, utilizing host defense peptides, and releasing agents like hydrogen sulfide (H_2S) to stimulate anti-inflammatory cytokines that promote wound healing [43]. Delegates voted for the agreement that immunotherapy could be promising in some ways to control biofilm, but human clinical studies are necessary to validate it. There are some in vitro and preclinical studies of manipulating the topography of the implant, along with embedding metal ions/nanoparticles to combat implant-associated biofilm. Liu et al. fabricated a porous surface that effectively trapped bacteria, allowing the embedded copper to exert antibacterial effects and promote pro-inflammatory macrophage in vitro and in animal models [44]. Su et al. utilized an acidity-activated metal organic framework to release H_2S that stimulated cytokine production by anti-inflammatory macrophages to promote wound healing and had direct antibacterial effects [44]. Host defense peptides can induce antibacterial and immunomodulatory effects. In the context of implant-associated infection, Innate Defense Regulator peptide 1 (IDR-1), an innate defensin, modulated genetic transcription leading to a controlled inflammatory environment that resulted in enhanced bacterial clearance in vitro and in mice [45]. Similarly, Wang et al. utilized DJK-5 to promote direct antimicrobial killing, promote enhanced macrophage phagocytosis, and inhibit potentially harmful inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α).

Question B14: Is there a role for the use of proteolytic enzymes to treat orthopedic infections?

Response: No. While enzymatic therapies show promise in preclinical settings, their safety and efficacy remain unproven in clinical orthopedic scenarios.

Voting: 87% Agree (Strong Consensus).

The current level of evidence supporting the clinical application of proteolytic enzymes in MSKI, specifically PJI, remains insufficient. The primary therapeutic rationale for these agents is their ability to degrade the EPS matrix of biofilms [46]. This disruption is intended to expose sequestered bacteria to host immune surveillance and increase the penetration of systemic antimicrobial agents. Numerous in vitro and in vivo studies have identified enzymes such as dispersin B, DNase I, proteinase K, and serratiopeptidase (SPEP) as promising agents for inhibiting biofilm formation and promoting the detachment of mature biofilms. In animal models, these enzymes—particularly SPEP—have synergistic effects when combined with antibiotics, resulting in enhanced bacterial clearance and reduced intracellular invasion. Phage-derived lysins, including LysECD7, lysostaphin, and exebacase (CF-301), have also shown significant anti-biofilm activity in preclinical settings. However, the existing data are highly heterogeneous and largely restricted to *S. aureus* and *Staphylococcus epidermidis* isolates, and clinical translation has proven challenging. Notably, exebacase recently failed to demonstrate clinical superiority in a Phase III trial for *S. aureus* bacteremia and endocarditis, a significant setback that underscores the gap between experimental models and clinical outcomes [47].

Several barriers prevent the immediate adoption of these therapies:

- **Systemic Dissemination:** Enzymatic degradation of the biofilm matrix may inadvertently release planktonic bacteria into the systemic circulation, increasing the risk of distal seeding or sepsis.
- **Lytic Risks:** There is a theoretical risk that rapid bacterial lysis could release potent intracellular toxins (e.g., alpha-hemolysin), potentially exacerbating the local inflammatory response.

- **Biofilm Heterogeneity**: The biochemical composition of biofilms varies significantly across clinical isolates, complicating the development of standardized, broad-spectrum enzymatic protocols.

In conclusion, while proteolytic and phage-derived enzymes represent a promising adjunctive strategy for biofilm-associated MSKI, they remain strictly experimental. Clinical translation will require high-quality RCTs to establish safety profiles, optimal dosing, and standardized delivery methods—such as local elution from spacers or carriers—relative to current standard-of-care antibiotic regimens.

Question G57: Is there a role for bacteriophage therapy in the treatment of orthopedic infections?

Response: Yes. It is a reasonable option in select cases based on its safety profile and clinical improvement in multidrug-resistant cases.

Voting: 77% Agree (Strong Consensus).

This document explored the potential role of bacteriophage therapy in treating orthopedic infections, specifically those that do not respond to standard treatments, such as bone and joint infections. The consensus recommendation supports the use of phage therapy, assigning it a moderate strength of recommendation. This reflects a general agreement that it can be a reasonable option in select cases. The rationale for this recommendation is backed by a growing body of clinical evidence, including nearly 100 reported cases, which indicate favorable safety profiles, minimal adverse events, and high rates of clinical improvement, especially when phage therapy is used in conjunction with antibiotics and surgical interventions [48]. Clinically, phage therapy shows promise against multidrug-resistant pathogens and biofilm-associated infections, which aligns with the World Health Organization's (WHO) call for the development of non-traditional antibacterial approaches. However, there are notable limitations to consider, such as the variability in phage preparation and administration, the reliance on case reports that may be subject to publication bias, and unresolved challenges like regulatory hurdles, standardization, and the potential for phage resistance. Therefore, while phage therapy is regarded as safe and may be effective, large-scale clinical trials are needed to confirm its efficacy and establish guidelines for routine use.

Question G58: Is there a role for phage lysins in the treatment of patients with orthopedic infections?

Response: Yes, with cautious support. Laboratory studies show promise, but clinical data are sparse.

Voting: 82% Agree (Strong Consensus).

While phage lysins show promise, particularly due to their antibacterial and anti-biofilm activity demonstrated in laboratory studies, clinical data remain sparse. The WHO classifies lysins as priority non-traditional agents in the fight against AMR [49]. Notably, there is one published clinical report that describes the successful use of the recombinant lysin CF-301 (exebacase) in a salvage approach for four elderly patients suffering from multidrug-resistant *Staphylococcus epidermidis* PJI [50]. In this report, two patients experienced favorable outcomes without any adverse events. Phage lysins are scientifically appealing because they may provide broader activity and scalability compared to whole phage therapy, and they could serve as an adjuvant in conservative surgical strategies. However, the evidence base is still minimal. There are ongoing unpublished experiences in this field and a clinical trial that was halted, highlighting both the interest in lysins and the challenges associated with their use. Key unresolved issues include the need for larger clinical trials, standardized protocols for their application, and a clearer understanding of their long-term role in the routine management of refractory orthopedic infections.

Question B16: Are ultrasonic debridement devices useful for the removal of biofilm in orthopedic infections?

Response: Yes. They effectively break up biofilms on metal and bone surfaces, making bacteria more susceptible to antibiotics.

Voting: 95% Agree, 0% Disagree, 5% Abstain (Strong Consensus).

Of the studies reviewed, 5 out of 14 studies were clinical, while the rest were in vitro. Ultrasonic debridement devices have been shown to effectively break up biofilms from common pathogens like *Pseudomonas aeruginosa* and *S. aureus* [51]. High-frequency ultrasound alters the biofilm structure, making bacteria more susceptible to antibiotics [52]. In some studies, this method removed over 90% of biofilms from metal and bone surfaces without significant tissue damage [53]. Low-frequency devices showed improved penetration into irregular surfaces, which is beneficial for orthopedic applications [54]. While limited, clinical studies suggest that ultrasonic debridement, when used alongside traditional surgical methods, can improve infection clearance rates in chronic wounds and implant-related infections. However, there is a notable gap between the promising experimental results and the limited clinical data, especially for orthopedic implants. The evidence for ultrasonic debridement is strong in preclinical evaluations and in dentistry, but clinical data specific to orthopedic implants is limited. The existing evidence, however, points to potential benefits, such as superior biofilm removal, shorter treatment times, and less surgical damage compared to conventional methods.

Question B22: Are there any novel alloys that are resistant to biofilm formation?

Response: No. No currently available alloy has demonstrated complete resistance to bacterial colonization under clinical conditions.

Voting: 65% Agree, 5% Disagree, 30% Abstain (Weak/Majority Consensus).

Implant alloys play a critical role in influencing surgical outcomes, long-term implant success, patient well-being, and morbidity rates. While biocompatibility remains the most important requirement for implant materials, ongoing research and development efforts increasingly focus on addressing the persistent clinical challenge of implant-associated infections through improved control of bacterial adhesion and biofilm formation. This systematic review identified several promising strategies aimed at enhancing the antibacterial properties of implant alloys. Doping alloys with antimicrobial elements, such as silver or copper, has been shown to reduce bacterial load and inhibit biofilm formation on alloy surfaces [55]. However, the incorporation of such elements bears a risk, as ion release may adversely affect human tissues and compromise biocompatibility, raising concerns about long-term safety.

Nanostructured titanium alloys represent another promising direction [56]. The nano-modification of the alloy itself and the included surface modifications have demonstrated reductions in bacterial adhesion and biofilm development while maintaining favorable interactions with host tissues. Similarly, emerging alloys incorporating elements such as niobium and tantalum have exhibited excellent biocompatibility and initial antibacterial effects [57, 58]. These materials are of particular interest for load-bearing applications, given their mechanical performance and compatibility with bone.

Despite these advances, it is important to note that no currently available alloy has demonstrated complete resistance to bacterial colonization, growth, or biofilm formation under clinical or experimental conditions. The development of a fully biofilm-resistant alloy remains an unmet need. A significant challenge in this field is achieving an optimal balance between antimicrobial efficacy and biocompatibility. Overall, while current trends in alloy development and surface engineering have led to materials with improved antibacterial performance, a definitive solution to prevent implant-associated infections entirely has not yet been achieved. Continued research is required to refine these materials and approaches, with the goal of enhancing implant success rates and improving patient outcomes in orthopedic surgery.

Question B26: Can electric fields be used to detach and destroy biofilm?

Response: Yes, though evidence is currently weak. Electric fields can disrupt bacterial membranes and enhance antibiotic activity.

Voting: 97% Agree, 0% Disagree, 3% Abstain (Strong Consensus).

The response offered was "Yes," but based on weak evidence. Electric forces are involved in bacterial adhesion to surfaces and interbacterial communication, two important aspects of biofilm formation [59]. Further, Electric fields are emerging as a promising approach for biofilm control and treatment with their mechanical, chemical, and synergistic effects [60]. There are several mechanisms of its activity against biofilm, including electrostatic repulsive force, electroporesis, and generation of reactive oxygen species (ROS) [61]. All these mechanisms lead to detachment of biofilm, increased permeability of the cell membrane, and enhanced antibiotic activity. Both direct current (DC) and alternating currents have been found to be effective against biofilm at specific intensities, frequencies, and durations [62, 63]. Pulsed currents were found to be more effective in some studies due to enhanced pore formation from the high-intensity electric field, while generating less heat due to short duration. Silver-Zinc redox electroceutical dressings were found effective in vitro, ex vivo, and in vivo, outperforming traditional wound care in biofilm reduction. During the meeting, there was a discussion about the efficacy of electrolytic surface cleaning in dental applications [64]. In electrolytic surface cleaning, hydrogen bubbles are generated by the application of electrical fields. Recent studies suggest that bubble-mediated detachment is highly effective for disrupting dense, mature biofilms that are otherwise resistant to conventional cleaning methods [65]. Hydrogen bubble generation offers a minimally invasive, efficient, and scalable solution for managing biofilms in both clinical and non-clinical environments. Despite its success in cleaning implants in situ in dental applications, applying this technique in an orthopedic context, such as to accompany a debridement, antibiotics, and implant retention, would be challenging. In conclusion, while there are some preclinical studies of electric fields against biofilm, more rigorous research and clinical studies are needed to refine the electric field parameters to develop a safe and effective protocol for biofilm treatment.

Question B30: Are there any technological advances in creating smart antibiotic carriers?

Response: Yes. Extensive preclinical evidence supports stimulus-responsive carriers that enhance penetration and targeted release.

Voting: 100% Agree (Unanimous Consensus).

The systematic review discussed several recent advances in the development of biofilm-targeting smart antibiotic carriers and provided a strong level of evidence. The recommendation of the working group was that extensive preclinical evidence supports innovations that enhance antibiotic delivery, biofilm penetration, and overall anti-biofilm efficacy. Smart antibiotic carriers improve antibiotic efficacy by enhancing antibiotic penetration and retention within biofilms, facilitating targeted antibiotic accumulation at infection sites, and/or enabling precise, stimulus-responsive antibiotic release for optimal antimicrobial effect [66]. Stimulus-responsive antibiotic carriers have been engineered to release antibiotics upon exposure to a variety of stimuli, including pH, enzyme, ROS, light, heat, ultrasound, and magnetic field. Smart antibiotic carriers have also been designed to specifically target biofilm by enhancing biofilm penetration [67–69], binding to tissue with biofilm [70], and binding directly to the biofilm itself [71]. Despite preclinical success, smart antibiotic carriers for bacterial biofilm face biocompatibility, regulatory, and scalability challenges, delaying clinical translation [72]. In particular, a gold-standard, minimally invasive biomarker for biofilm burden in clinical settings is needed for longitudinal evaluation of therapeutic efficacy [14, 73].

Question B32: Are there any physical non-cytotoxic methods that can disrupt biofilm?

Response: Potentially. Methods like photodynamic therapy (PDT) and ultrasound show potential in preclinical stages but lack clinical proof of efficacy.

Voting: 83% Agree, 4% Disagree, 13% Abstain (Strong Consensus).

Experts agreed that physical, non-cytotoxic methods, such as PDT, ultrasound, and electromagnetic techniques, show potential to disrupt biofilms in orthopedic infections, though their clinical efficacy and safety have yet to be demonstrated in clinical trials, and the current level of evidence remains weak [74]. PDT employs light-activated photosensitizers to produce ROS, which effectively disrupts biofilm structures [75]. PDT demonstrated effectiveness against both Gram-positive and Gram-negative organisms and fungi, with minimal host tissue damage. However, its clinical application is limited by the need for direct exposure to light and the lack of human trial data. Ultrasound of both high-frequency and low-frequency modalities can enhance antibiotic penetration, promote cavitation-induced mechanical disruption, and suppress biofilm-associated gene expression. Combined therapies with antibiotics or sonosensitizers have shown enhanced effects. Concerns remain about potential tissue or implant damage and variability in

effectiveness. Electrical and electromagnetic approaches, including DC, cathodic voltage-controlled stimulation, and non-contact induction heating (NCIH) that have shown promise in disrupting biofilms and synergizing with antibiotics. Electromagnetic fields can reduce bacterial metabolism and facilitate localized thermal effects. These modalities are under preclinical development, and safety in humans needs further evaluation. Other physical methods include cold plasma (e.g., dielectric barrier discharge), laser therapy, extracorporeal shock wave therapy, and cryoablation. These have shown biofilm eradication potential in experimental models, but standardized clinical protocols are lacking. In summary, while various non-cytotoxic physical methods offer innovative strategies for biofilm disruption, further clinical studies are needed to establish their safety, efficacy, and standardized use in orthopedic infections.

Question G95: Are there any effective anti-biofilm technologies that can be used in clinical practice?

Response: Yes. Approaches such as bacteriophage therapy, mechanical disruption, and novel antibiotics (e.g., PLG0206) are identified as promising.

Voting: 81% Agree (Strong Consensus).

A review of 240 studies on antibiofilm therapies for orthopedic infections, particularly PJIs, highlighted several promising approaches, including bacteriophage therapy, implant coatings, novel antibiotics/antimicrobials, NCIH, bioactive glass, and mechanical disruption methods. Bacteriophage therapy shows potential for personalization against specific pathogens and has demonstrated success when combined with antibiotics [76]. Various implant coatings, such as carbon-infiltrated nanotubes, tantalum-titanium lattice structures, and silver nanoparticles, have exhibited anti-biofilm properties in vitro, though clinical evaluation is still needed. Notably, the novel antibiotic PLG0206 (WLBU2) is the first of its class to show independent biofilm activity, with proven safety and tolerability in early clinical trials, while efficacy studies remain ongoing [77]. NCIH has demonstrated in vitro effectiveness but requires further translational research. Mechanical disruption methods—such as pulsed magnetic fields, extracorporeal shock wave therapy, and ultrasound—have also shown promise in vitro. Similarly, bioactive glass has been shown to be effective in preventing bacterial adherence and biofilm formation in vitro; however, further in vivo studies are required.

5 | Conclusion

The recommendations and voting results from the ICM 2025 can help direct future preclinical research on treating MSKI. While there was consensus on all recommendations, many responses were based on limited or low-quality evidence from existing literature. Additionally, several questions had high abstention rates, indicating a lack of definitive data, relevance, and/or existing controversy. The 2025 ICM delegates and workgroup members pinpointed several key areas where research needs to shift to enhance clinical outcomes for patients with MSKI. Priorities for in vivo diagnostic tool development include creating non-invasive, real-time imaging techniques to detect biofilm load without invasive sampling, establishing minimally invasive biomarkers for ongoing monitoring of treatment effectiveness in clinical environments, and standardizing protocols (including those using DTT) to improve the sensitivity of synovial fluid cultures, especially for culture-negative or fungal infections. To address the lack of clinical translation, the workgroup emphasizes a shift toward more rigorous and reproducible animal research, recommending minimum requirements including standardized pathogenic inoculums, evidence of infection prior to treatment, and quantification of microbiology burden at endpoints. Recommendations also included using longitudinal outcomes, such as bioluminescent imaging and radiology, for multi-tiered approaches (in vitro to large animal models) to evaluate safety and efficacy before initiating clinical trials. To better serve high-risk patient populations, high-quality clinical trials must be prioritized to evaluate the superiority, safety, and cost-effectiveness of resorbable local agents, such as calcium sulfate. While several treatment approaches, such as bacteriophages, lysins, smart delivery systems, and immunotherapy, show promise, they require rigorous clinical validation in animal models to support translation and confirm safety. Future work should investigate synergies between physical methods and systemic antibiotics to enhance biofilm detachment and kill rates.

Author Contributions

All authors participated in data generation (identification of the research questions and voting on their priority), contributed to the writing, and have read and approved the final submitted manuscript.

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