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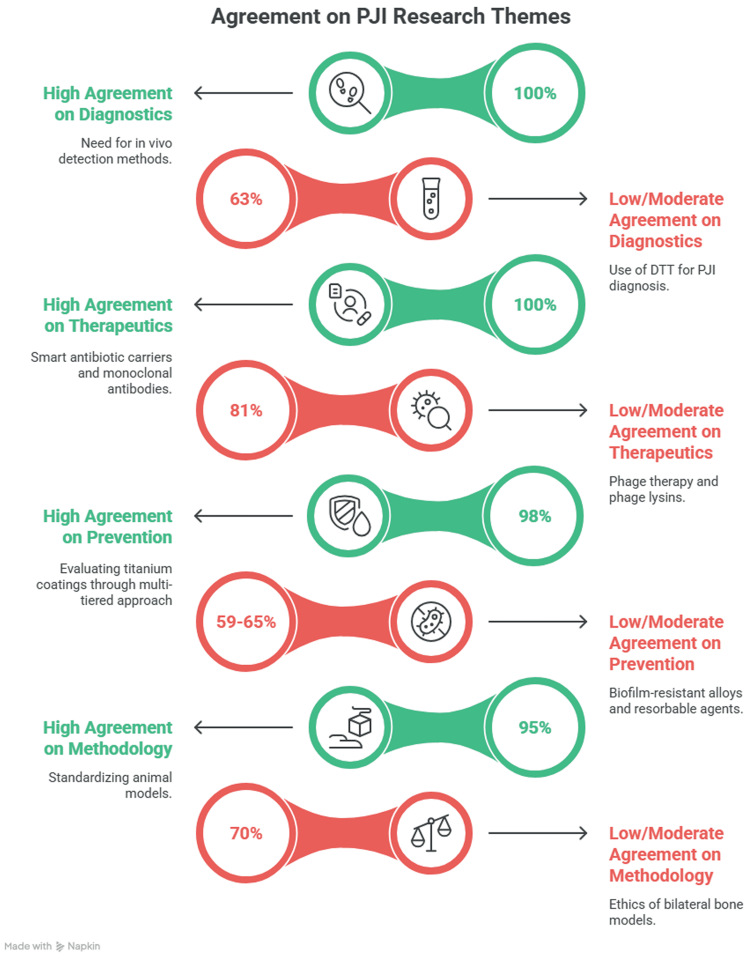

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CONSENSUS

2025 International Consensus Meeting on Musculoskeletal Infection: Summary From the Biofilm Workgroup on Biofilm Formation, Persistence, and Host-Environment Interactions

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ABSTRACT

Musculoskeletal infection (MSKI) remains a major problem after trauma and elective orthopedic surgery. Chronic MSKI is related to the formation of biofilm, which impairs diagnosis and effective treatments. Therefore, to understand and communicate global standards and best practices, the 2025 International Consensus Meeting (ICM) on MSKI created a Biofilm Section to address crucial aspects of

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biofilm biology pertaining to its mechanisms of drug resistance and immune evasion, and potential approaches to overcome them. This featured a 2-year process, with final voting and discussion on May 8–10, 2025, in Istanbul, Turkey. This Consensus Article is the effort of the Biofilm Basic Mechanisms Workgroup, which interpreted the results on ICM questions related to (1) the infectious microenvironment; (2) appropriate inocula in preclinical research; (3) biofilm behavior in infected tissues; and (4) synergy within biofilms and with other comorbidities. Collectively, we find that this field has the necessary research tools to discover the pathophysiology of orthopedic implant-associated biofilm development and maturation, perform clinically relevant studies in animal models, and elucidate mechanisms that allow opportunistic infections in compromised tissues and patients with other health issues.

1 | Introduction

Musculoskeletal infection (MSKI) remains a major source of morbidity following trauma and orthopedic procedures, with consequences that include fracture nonunion, implant failure, amputation, and premature mortality [1–4]. Despite improvements in asepsis and perioperative antibiotic prophylaxis, the absolute burden of MSKI is rising in parallel with the global volume of orthopedic surgical interventions and use in an aging, comorbid population [5–7]. While biofilm is widely regarded as the dominant barrier to eradication in implant-associated infections, fundamental gaps remain in our understanding of how biofilms form, persist, and interface with host tissues [7–10]. Clarifying these basic mechanisms is the prerequisite for designing preclinical models that accurately predict clinical performance and prioritizing suitable translational targets.

The 2025 International Consensus Meeting (ICM) on MSKI convened a Biofilm Workgroup to address high-priority knowledge gaps spanning preclinical science through clinical translation (https://www.icmortho.org; https://www.ors.org/2025-icm-on-mski/). This Consensus Article synthesizes findings and future directions for the "Basic Mechanisms" domain. The questions interrogate: whether an "immune proteome" signature exists in periprosthetic joint infection (PJI) (B3); what models of orthopedic infection are best to evaluate diagnostic technologies (B4); which pathogens should be evaluated in preclinical models (B8); intracellular survival of bacteria in osteoblasts and its role in disease propagation (B9–B10); minimum data elements to standardize orthopedic infection studies (B12); the centrality of biofilm itself as the main treatment challenge (B15); biofilm behavior in synovial fluid (B17); viable-but-non-culturable (VBNC) states in orthopedic contexts (B18); material-specific affinities (B20); regulation of extracellular DNA (eDNA) via autolysis in *Staphylococcus aureus* (B21); potential links between biofilms and bone tumors (B24); and polymicrobial synergy versus antagonism in biofilm formation (B29). Together, these questions aim to align preclinical inquiry with the pathophysiology that drives persistent MSKI and clinical failure [10–12].

2 | Methodology

The objective of the ICM 2025 Biofilm Section was to generate expert, actionable recommendations that sharpen preclinical research questions and harmonize best practices for study design and reporting. The Biofilm Section included experts in microbiology, immunology, biomedical engineering, infectious diseases, and orthopedic surgery. An international panel selected questions aligned with their expertise to (i) scope the relevant literature, (ii) identify recurrent methodological pitfalls, and (iii) propose consensus guidance. As in prior ICMs, recommendations were developed and voted on using Delphi methodology [11, 12].

Over 10 months, each question was assigned to a liaison who coordinated further delegates to perform structured reviews. Covidence was used to manage systematic reviews; searches incorporated appropriate MeSH terms and synonyms. Teams screened titles/abstracts, performed full-text review, and extracted key data elements (model characteristics, organisms, inocula, materials, endpoints, and analytic methods). Draft "Response with Rationale" documents were posted for all delegates at https://www.icmortho.org/documents for open comment. Author teams revised their responses based on feedback in preparation for in-person discussions and voting at the ICM (May 8–10 2025, Istanbul, Turkey).

During the meeting, statements with ambiguity or contention were edited in session to promote clarity and achieve broader agreement. With regard to the proposed response, delegates voted to (1) agree, (2) disagree, or (3) abstain. Outcomes followed predefined thresholds: (a) Simple majority (50.1%–59%): No Consensus; (b) Majority (60%–65%): Weak Consensus; (c) Super Majority (66%–99%): Strong Consensus; (d) 100%: Unanimous Consensus. The 13 questions summarized here specifically address the evaluation of basic mechanisms underpinning biofilm formation, persistence, and interaction with the host, as well as preclinical model standardization. Consensus was reached without compulsion, undue influence, or process constraints. Full question-level recommendations, including downloadable PDFs with responses, consensus votes, and post-meeting rationales, are available at https://www.ors.org/2025-icm-on-mski/. Following voting at ICM 2025, the authors of this manuscript were assigned to the Biofilm Basic Mechanisms Workgroup and tasked with highlighting the most important questions on this topic, interpreting the recommendations and voting results, and providing insights on the way forward. The following represents this work product, and the results are summarized in Table 1.

3 | Results and Discussion

Question B3 asked "Is there an immune proteome in PJI?" The delegates unanimously (100% agreement) answered that there is an immune proteome in PJI, and our understanding of this proteome continues to grow and evolve. Novel high-throughput approaches paired with more targeted protein investigations have indicated that there is an immune proteome in PJI compared to aseptic revisions. This proteome involves the synovial fluid, local tissues, implant, and peripheral blood. While discrepancies exist between compartments, interleukin-6

TABLE 1 | Summary table of the questions for ICM2025 and the consensus agreement.

Question	Consensus
B3: Is there an immune proteome in PJI?	Unanimous Consensus
B4: What is (are) the best preclinical model(s) of orthopedic infection for the evaluation of diagnostic technologies?	Strong Consensus
B8: What crtical pathogens should be routinely evaluated in preclinical models to study important questions in orthopedic infection research?	Strong Consensus
B9: Can bacteria survive in the intracellular space of osteoblasts?	Strong Consensus
B10: Do intracellular bacteria play a role in propagating MSKI?	Strong Consensus
B12: What is (are) the minimum data set for studying orthopedic infections?	Strong Consensus
B15: Are we certain biofilms are the main challenge of treating implant-associated infection?	Strong Consensus
B17: Does biofilm form in the synovial fluid?	Strong Consensus
B18: Does the concept of viable but non-culturable (VBNC) apply to orthopedic infections?	Unanimous Consensus
B20: Does biofilm have different affiniites for different surfaces?	Strong Consensus
B21: What key regulatory mechanisms control the release of extracellular DNA (eDNA) from *S. aureus* during autolysis, which significantly contributes to the structural integrity of biofilm?	Unanimous Consensus
B24: Are biofilms carcinogenic or associated with bone tumors?	Strong Consensus
B29: In the case of polymicrobial infection, do all pathogens participate synergistically in biofilm formation or is there any antagonistic influence by some organisms?	Strong Consensus

(IL-6) was the most commonly elevated cytokine in PJI compared to aseptic revisions. The composition of the proteome also varied based on the detection method used for each study, primarily either LC-MS or bead-based/ELISA assays. As more advanced and unbiased techniques evolve and become more readily available, so too will the understanding of this proteome and how it can be used for diagnosis, treatment, and ultimately prevention of PJI in the future.

Question B4 queried "What is (are) the best preclinical model (s) of orthopedic infection for the evaluation of diagnostic technologies?" The absence of a definitive diagnostic is highlighted by the fact that a perennial outcome of MSKI ICMs has been an updated definition of PJI, which remains a high priority with robust research activity. Delegates agreed that the "best" model depends on the specific hypothesis tested and animal welfare considerations. In animal models, translational potential, ethical approval, model design, and statistical success metrics should be predetermined and declared. To evaluate new diagnostics, current industry standards are receiver operating characteristic (ROC) curve analysis of the new technology compared to clinical cultures, clinical signs and symptoms, or other FDA-approved diagnostics of orthopedic infection. Additionally, endpoints should be valid, and diagnosis should involve quantitative microbiological, radiological, serological, histological, and clinical observations. Ultimately, less invasive mechanisms for infection surveillance to reduce animal morbidity and numbers are important goals, and although validated In Silico and In Vitro models to assess diagnostics of orthopedic infection do not currently exist, these technologies are rapidly emerging and may need to be considered in the near future. This recommendation received overwhelming ICM agreement (93%) with three abstaining votes.

Question B8 queried "What critical pathogens should be routinely evaluated in preclinical models to study important questions in orthopedic infection research?" The delegates overwhelmingly agreed (98%) with the recommendation that, in addition to prevalent pathogens like *S. aureus* and *E. coli*, other microorganisms, particularly Gram-negative bacilli, *Enterobacterales*, and *C. acnes*, should also be assessed. This rationale is based on a systematic review of six studies, which identified a range of pathogens as causative agents of disease, including less commonly studied species, such as *S. pseudointermedius* and various fungi. Notably, while most preclinical models have focused on common pathogens, further research into less common organisms is necessary to ensure a comprehensive understanding of the full spectrum of pathogens involved in orthopedic infections and therapeutic development. Until then, a multidisciplinary approach involving orthopedic surgeons and MSKI specialists is crucial in managing these infections.

Question B9 asked "Can bacteria survive in the intracellular space of osteoblasts?" The question considered the evidence of stable intracellular infections of osteoblast-lineage cells, from osteoprogenitors to mature osteocytes. Stable infection was defined as evidence of viable pathogens inside viable host cells for at least 24 h post-infection. The majority of delegates (98% in favor, 2% opposed) agreed that this occurs. Several case reports of chronic osteomyelitis have identified intracellular pathogens, including obligate intracellular organisms, in osteocytes and osteoblasts as being the likely cause of infection chronicity. Additionally, a multitude of In Vitro studies have demonstrated the intracellular persistence of numerous facultative and obligate intracellular pathogenic species in both well-characterized osteoblast and osteocyte cell lines and primary osteoblasts and osteocytes. Pathogens, including *S. aureus*, coagulase-negative staphylococci, *Brucella* species, *Porphyromonas gingivalis*, Mycobacterium species and *Chlamydia pneumoniae*, were all found to be capable of infecting and persisting in viable osteoblast lineage cells for experimental periods ranging from 24 h to 28 days.

Question B10 asked "Do intracellular bacteria play a role in propagating MSKI?" This question extended the findings of

Question B9. The majority of delegates (98% in favor, 2% opposed) agreed that this occurred. Strong evidence supports the notion that both facultative and obligate intracellular pathogens can invade host cells, survive within them, and contribute to the development of chronic or recurrent infections. *S. aureus* is the most extensively studied species in this context, demonstrating persistence in various musculoskeletal cell types, including osteoblasts, osteocytes, macrophages, and osteoclasts, which can last for 21 days or longer. Clinical and preclinical studies further establishing connections between intracellular bacteria and chronic/recurrent infections and antimicrobial resistance are much needed. Mechanisms of bacterial escape and the triggers for pathogenic reactivation remain under investigation. These unresolved issues indicate a need to improve the diagnosis of intracellular infections and for ongoing research to better inform therapeutic strategies against persistent MSKI.

Question B12 asked "What is (are) the minimum data set for studying orthopedic infections?" This question highlighted the variability in reporting registry data, which leads to challenges in comparing studies. The expert recommendation was that a minimum data set should include a sample size estimation, patient characteristics, index operative factors, diagnostic criteria, operative and medical treatment factors, and postoperative outcomes to maximize generalizability and comparability across orthopedic literature (Table 2). To increase the accuracy of clinical data across different studies, it is reasonable to propose a minimum sample size of 300 patients per comparison group. This will increase statistical power and reduce the likelihood of type II errors. Another consideration is host optimization, as it is an important preoperative factor to prevent socioeconomic disparities in healthcare utilization and enhance recovery from orthopedic postoperative complications and infections [13]. At a minimum, patient age, sex, body mass index, surgical history, and comorbidities should be recorded. Optimally, inclusion of socioeconomic factors through a comprehensive approach, such as the Area Deprivation Index, is also recommended. Surgical factors, including indication, operative time, blood loss, transfusion, and hospital stay influence the development of orthopedic infection and should be reported. Diagnostic criteria for infection are also valuable to report, and the recommendation of experts is to use the 2018 ICM Criteria or the 2021 European Bone and Joint Infection Society criteria for hip and knee PJI [14, 15]. Standardizing and reporting outcome measures for treatment success or failure remains challenging. A minimum of 1-year follow-up is recommended, but up to 5-year follow-up would improve outcome tracking. Patient-reported outcome measures (PROMs) are now considered important for assessing treatment success, but do not always give a complete picture of infection treatment success. The "clinical relevance ratio" is a new way to analyze PROMs and can help show how many patients had meaningful

TABLE 2 | Criteria for the minimum data set required to study orthopedic infections.

Sample size	Need for a power analysis to determine the appropriate sample size
Patient characteristics	Age, sex, body mass index
	Relevant surgical history
	Host comprising factors (e.g., diabetes, autoimmune disease, immunosuppressive medication, renal disease, malnutrition)
	Extremity comprising factors (e.g., soft tissue loss, need for plastic surgery, vascular insufficiency)
	Socioeconomic factors (e.g., area deprivation index)
Index operative factors	Surgical indication (e.g., elective *vs.* traumatic)
	Operative time, length of stay
	Estimated blood loss, need for transfusion
	Type of implant used (e.g., primary *vs.* revision components)
	Delayed wound healing, extended antibiotic prescription
Diagnostic criteria	Time of infection onset
	Serological laboratory studies (ESR, CRP)
	Synovial fluid analysis (if applicable), microbiological data
Operative and medical treatment factors	Implant retained, removed, or exchanged (i.e., molecular components, temporary spacer, or real implants)
	Local antimicrobial delivery method
	Systemic antimicrobial therapy (agent and duration)
Postoperative outcomes	Minimum 1-year clinical follow-up
	General health and condition-specific PROMs
	Definition of treatment success: infection control with no continued antimicrobial therapy, infection control with suppressive antimicrobial therapy, retained spacer, need for reoperation, and death

Abbreviations: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PROMs, patient-reported outcome measures.

improvements after treatment [16]. This question received votes from both the general session in the hip and knee category and in the biofilm section. The biofilm section delegates voted 80% in agreement, 7% in disagreement, and 13% abstained. The hip & knee section voted 84.6% in agreement, 7.7% in disagreement, and 7.7% abstained.

Question B15 inquired "Are we certain that biofilms are the main challenge of treating implant-associated infection?" Delegates reached a strong consensus, with 92% voting "agree," 3% "disagree," and 5% "abstain" that biofilms, particularly those formed by *S. aureus* and *S. epidermidis*, drive antibiotic resistance, immune evasion, and chronicity. These findings were supported by both clinical and preclinical evidence. Key implications of this question include the need for improved diagnostics (e.g., sonication, molecular assays), development of biofilm-targeted therapies, and innovations in implant materials. Debate remains over exceptions, such as non-biofilm pathogens, small-colony variants, and strain-specific differences, highlighting that biofilm is not the sole pathogenic mechanism. Overall, biofilms represent the central barrier to effective treatment, but ongoing research must address variability and explore personalized and adjunctive strategies.

Question B17 asked "Does biofilm form in the synovial fluid?" Based on a strong level of evidence, 97% of delegates agreed that suspended biofilm aggregates, particularly of *Staphylococcus* species, can form in synovial fluid both In Vitro and In Vivo. This finding may have significant clinical implications, as these aggregates exhibit reduced antimicrobial susceptibility, potentially contributing to the persistence and treatment resistance of PJIs. While most studies on this topic have focused on *S. aureus*, other PJI pathogens, including other Gram-positive bacteria (e.g., coagulase-negative staphylococci like *S. epidermidis*, and *C. acnes*), Gram-negative bacteria (e.g., *Enterobacterales* and *P. aeruginosa*), and fungi (e.g., *Candida* spp.), have also demonstrated the capability of forming biofilm-like aggregates in synovial fluid. However, the evidence for these additional species remains largely confined to In Vitro studies, and in general, In Vivo evidence for biofilm formation in synovial fluid remains limited. *S. aureus* biofilm aggregates form via binding to fibrinogen, fibronectin, and hyaluronic acid in synovial fluid; the ability to bind to these key synovial fluid components may allow stronger association of bacteria with tissues. Variability in experimental models complicates direct comparisons between results obtained in different studies, and further research is needed to standardize methodologies and better understand the clinical impact of these non-surface-attached biofilms in the management of PJI.

Question B18 asked "Does the concept of viable but non-culturable (VBNC) apply to orthopedic infections?" Delegates unanimously agreed (100% agreement) that the concept of VBNC is relevant in orthopedic infections. Evidence from In Vitro studies, including those using clinical isolates from PJIs, has demonstrated that pathogens such as *S. aureus*, *S. epidermidis*, *S. lugdunensis*, and *P. aeruginosa* can enter the VBNC state under stress conditions such as antibiotic pressure, nutrient depletion, and anoxia when in a biofilm [17–22]. These cells can persist for prolonged periods, evade detection, survive treatment-like conditions, and resuscitate under favorable conditions, contributing to treatment failure and the recurrence of orthopedic infections. While evidence supports a link to culture-negative infections, key challenges remain in clinically validating these findings and developing accurate diagnostics and therapies to target dormant bacterial populations.

Question B20 asked "Does biofilm have different affinities for different surfaces?" Delegates strongly supported the assertion that biofilm exhibits different affinities for distinct surfaces (97% yes, 3% abstain). Bacterial adhesion and biofilm development on the implant surface are influenced by a combination of host factors, microorganism-specific characteristics, exposure time, and implant-related surface properties such as wettability, topography, micro- and nanoscale roughness, surface free energy, material, and charge. Substantial evidence suggests that increased implant surface roughness at the micro- and macrometer scale promotes bacterial adhesion, while surface peaks and valleys provide shelter from shear stresses. Nanoscale roughness, on the other hand, seems to reduce adhesion points. Furthermore, peaks with diameters close to bacterial cells exert mechanical stress on the bacterial membrane, resulting in its rupture. Surface chemistry also directly affects biofilm formation. There are differing opinions among authors regarding the effectiveness of various surface types in preventing bacterial colonization. Some argue that hydrophilic, highly hydrated, and uncharged surfaces are effective barriers, while others contend that hydrophobic surfaces with low surface free energy, such as cobalt chrome, inhibit bacterial growth.

Affinity arises from the complex interaction between surface properties and bacterial physicochemical properties. The same biomaterial can interact differently with various bacteria since each bacterial strain has different affinities for distinct surfaces. In light of these factors, establishing a universally valid correlation or identifying a surface that consistently prevents biofilm formation remains challenging. Despite this, multiple studies suggest that ceramic, cobalt-chromium, and titanium alloys outperform polyethylene, stainless steel, and Polyether-ether-ketone (PEEK) in preventing biofilm formation. The majority of evidence is derived from In Vitro or preclinical models, raising concerns regarding the influence of host immunity, tissue response, and the dynamic biological environment. Enhanced translational research on bacterial surface affinities is essential for developing biofilm-resistant implant designs.

Question B21 asked "What key regulatory mechanisms control the release of extracellular DNA (eDNA) from *S. aureus* during autolysis, which significantly contributes to the structural integrity of biofilm?" The release of eDNA contributes significantly to the structural integrity, growth, and immune-evasive properties of *S. aureus* biofilms in orthopedic infections. This process is primarily mediated by autolysis via murein hydrolase, encoded by the *atl* gene, and modulated by the *cidABC* and *lrgAB* operons, which regulate cell death and lysis. Additional mechanisms, such as those involving phosphodiesterase and thermonuclease, also contribute to eDNA release, albeit independent of autolysis. The delegates voted unanimously (100% agreement) with these proposed mechanisms. The scientific context underscores the crucial role of eDNA in biofilm stability, offering potential therapeutic targets for bacterial eradication. Although there is a strong consensus regarding the underlying mechanisms, unresolved issues remain regarding the role of environmental factors like oxygen and glucose levels

in modulating eDNA release. Furthermore, the influence of subinhibitory antibiotic concentrations on eDNA production within biofilms warrants further investigation for clinical implications in treating MSKI.

Question B24 asked "Are biofilms carcinogenic or associated with bone tumors?" Delegates voted 81% in agreement, 2% in disagreement, and 17% abstaining that biofilms have a role in carcinogenesis. The oncogenic potential of biofilm has been studied extensively in colorectal cancers, where In Vitro and animal studies have shown both pathogen-specific and biofilm-related effects on cancer progression. Microbiome analyses have revealed changes in the local microbiome associated with a wide range of tumor types. Specific pathogens have been implicated in altering the tumor microenvironment, including *F. nucleatum, S. typhi*, and *S. epidermidis*. Proposed oncogenic mechanisms include the induction of an inflammatory environment and specific products of bacterial metabolism. While several cancers show strong associations with biofilm activity, there is a lack of data specific to bone tumors. Questions such as the microbiome of primary bone cancers and the oncogenic potential of pathogens specific to orthopedic infections remain unresolved.

Question B29 asked "In case of polymicrobial infection, do all pathogens participate synergistically in biofilm formation or is there any antagonistic influence by some organisms?" Delegates voted 97% in agreement and 3% in disagreement that both synergistic and antagonistic effects occur depending on the species involved, with a limited level of evidence supporting this recommendation. Evidence, primarily from In Vitro studies, shows that while some pathogen pairs, such as C. albicans with S. aureus, enhance biofilm formation, antimicrobial tolerance, and virulence [23], others, such as S. aureus and P. aeruginosa, may initially compete before later developing synergistic colonization strategies [24] or exhibit antagonistic interactions [25]. Gram-positive organisms, particularly S. aureus, often outcompete Gram-negatives in bone or implant niches, but synergistic relationships can arise depending on the environment and infection stage [24, 25]. These findings have important implications for MSKI management, as polymicrobial interactions can alter pathogenicity, immune evasion, and, in some cases, antimicrobial susceptibility [23–25]. However, data specific to MSKI remain limited, and further In Vivo research is needed to inform targeted treatment strategies for mixed-species biofilms. Most current investigations are constrained by reliance on In Vitro models, limited pathogen diversity, and insufficient representation of clinically relevant infection environments [23–25].

4 | Conclusion

These recommendations from the ICM 2025 illustrate the current state of knowledge regarding biofilms and their role in MSKI. Specifically, this review highlighted the complexity of pathogen behavior, the need for robust preclinical models, and the importance of multidisciplinary approaches to improve patient outcomes. Multiple recommendations underscored the need for In Vivo and clinical studies for promising In Vitro findings. Filling in these knowledge gaps regarding biofilm is crucial to the development of better diagnostics and therapeutics.

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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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 ($\geq$ 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.

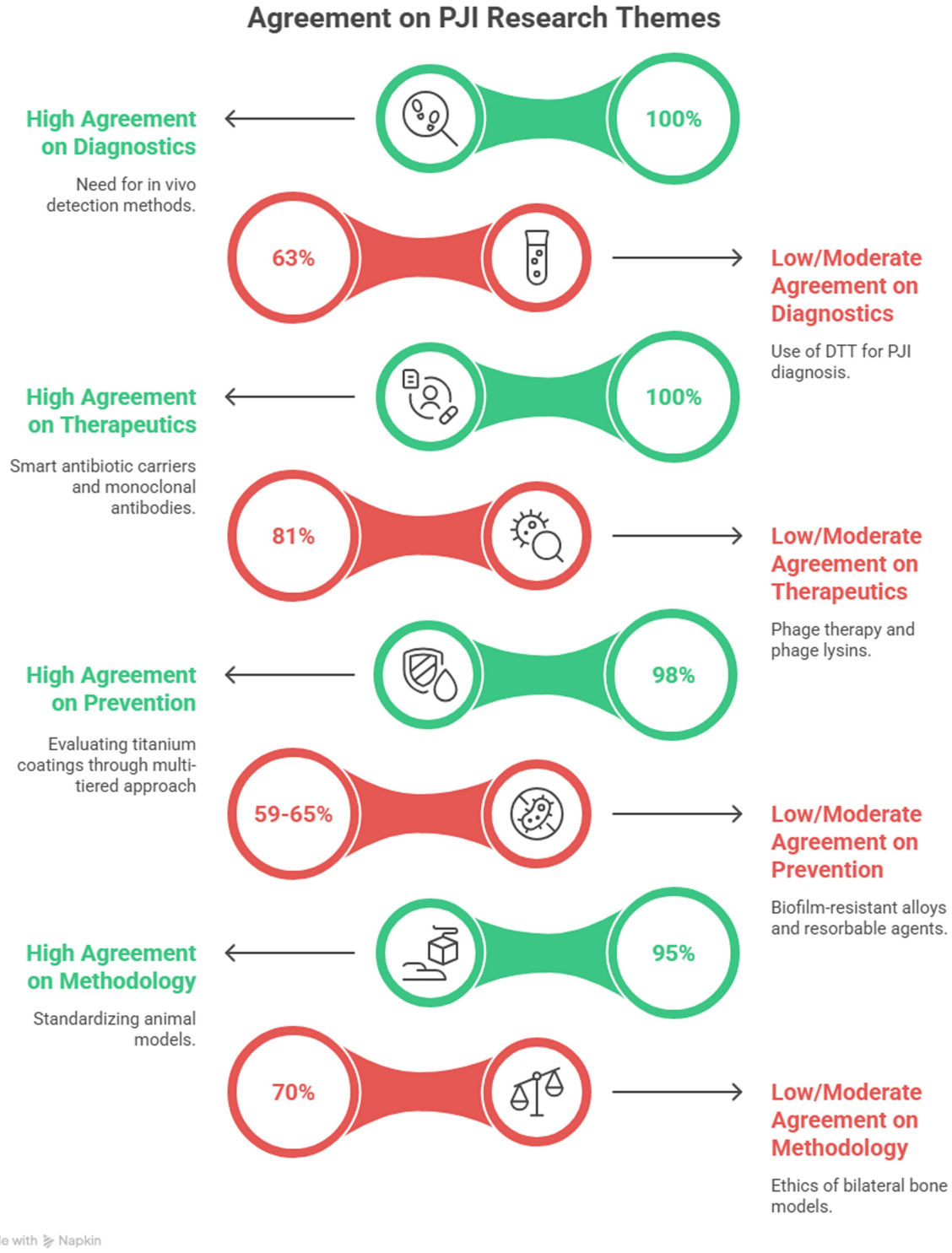

FIGURE 1 | Research themes with high and low/moderate agreement from the biofilm workgroup involving treatment. Made with Napkin.ai.

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.

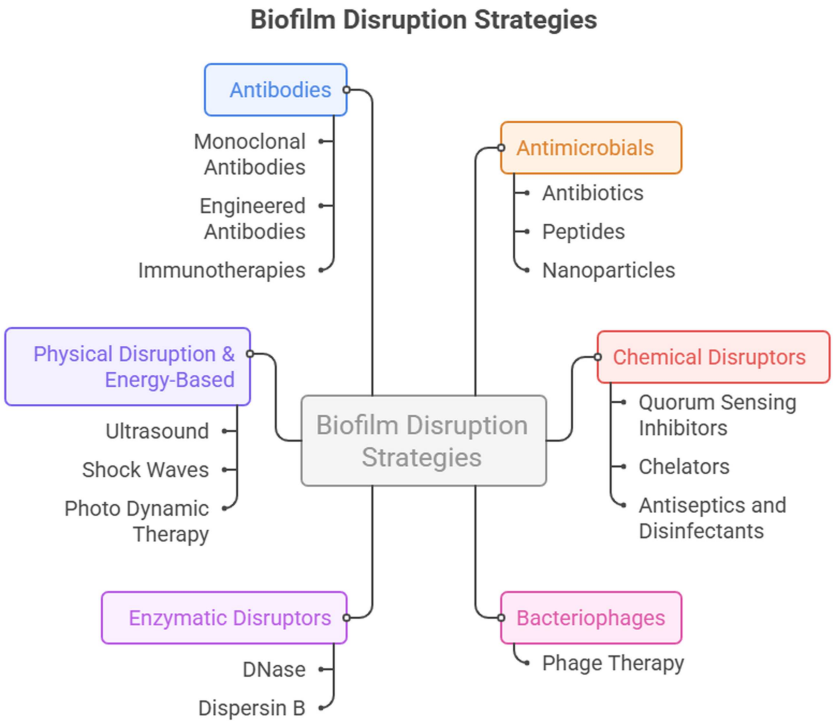

FIGURE 3 | Six categories of biofilm disruption strategies. Made with Napkin.ai.

- **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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CONSENSUS

The 2025 International Consensus Meeting on Musculoskeletal Infection: Research Priorities and Future Directions

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ABSTRACT

Musculoskeletal infection (MSKI) is a leading cause of implant failure following orthopedic surgery for trauma or elective procedures and it is associated with catastrophic outcomes for patients and healthcare systems worldwide. International Consensus Meetings (ICM) aim to define state-of-the-art, influencing clinical standards of care and accelerating discoveries by setting research priorities. The 3rd ICM was held on May 8–10, 2025 in Istanbul (Turkey) and included a 2-year-long Delphi process that culminated with in-person voting by 1205 delegates on 102 General and 30 Biofilm-specific MSKI questions. Consistent with prior ICMs, a Research Priorities Workgroup was established after the voting to interpret the results and summarize the most important future directions. Here, the group reports on several critical research priorities that emerged, which should be addressed to advance the field. These include: (1) improving diagnostics through standardized patient sampling, advanced non-invasive imaging technologies, and biofilm-specific biomarkers; (2) developing clinically relevant in-vitro and in-vivo models to rigorously and reproducibly test antibiofilm strategies; (3) identifying high priority immunological research areas, including deciphering the role of T-cell immunity in biofilm persistence, and if T cell targeting therapies can be harnessed to disrupt chronic biofilm-associated infection; (4) clinically evaluating novel anti-biofilm technologies on larger cohorts of patients; and (5) addressing translational barriers through the use of multi-center data collection and large-scale data

ICM 2025 Biofilm Research Priorities Workgroup

tools to accelerate clinical application. These research priorities aim to enhance the prevention, diagnosis, and treatment of biofilm-associated MSKI.

1 | Introduction and Background

Musculoskeletal infection (MSKI) remains a major source of morbidity after trauma care and arthroplasty, contributing to nonunion, implant failure, reoperation, and increased mortality. Procedure volumes continue to expand in aging populations; in the United States, revision hip and knee arthroplasty are projected to rise by ~280% and ~400% from 2014 to 2040, respectively, with substantial growth in estimated costs and revision burden [1]. Registry data reveal that aseptic loosening is no longer the major cause of failure, while septic loosening is increasing as a complication [2]. Concurrently, fracture-related infection (FRI) imposes a sustained global burden [3]. Across implant-associated infections, biofilm is the dominant barrier to eradication, driving antimicrobial tolerance and immune evasion. These clinical and mechanistic challenges keep translational thresholds high and create the demand for better models and outcomes that predict clinical performance [4, 5].

Previous clinical International Consensus Meetings (ICM) on orthopaedic infection have standardized key definitions, diagnostic criteria, and management pathways, and they employed predefined Delphi thresholds for grading agreement (No Consensus 50.1%–59%, Weak 60%–65%, Strong 66%–99%, Unanimous 100%) [6, 7]. This framework improves cross-study comparability and clinical decision-making while delineating domains where evidence remains insufficient and where preclinical work must better align with clinical questions.

Specifically regarding biofilm, a workgroup consisting of 28 experts convened at the 2018 clinical consensus meeting in Philadelphia discussed 13 questions regarding the following topics: (1) surface modifications to prevent/inhibit biofilm formation; (2) therapies to prevent and treat biofilm infections; (3) polymicrobial biofilms; (4) diagnostics to detect active and dormant biofilm in patients; (5) methods to establish minimal biofilm eradication concentration for biofilm bacteria; and (6) novel anti-infectives that are effective against biofilm bacteria. The summary stated that despite the intention to discuss these questions from the perspective of clinical intervention, the data were largely based on preclinical studies and there were minimal to low levels of clinical data on these topics [8], which resulted in narrative expert opinions. A major topic of interest was the characterization of the timeline of biofilm infections (acute vs. chronic) to potentially enable better diagnostics and treatment. It was not clear to what extent such a definition could influence clinical management of infections. There was consensus that all involved implant surfaces were susceptible to biofilm formation despite evidence that physicochemical properties, such as surface charge and topography, interfered with the timeline and extent of biofilm formation in vitro. The limitations of using the minimum inhibitory concentration (MIC) of antibiotics for guiding treatments were defined with the recommendation of focusing on the minimum biofilm eradication concentration (MBEC) for biofilm relevance. Moreover, obtaining more clinical information on bacteriophage therapy was considered a priority.

Encouraged by the progress made in guiding clinical consensus by previous efforts, a research consensus effort was organized by the Musculoskeletal Infection Research Interest Group (RIG) of the Orthopaedic Research Society (ORS) [9] and considered 65 questions regarding in-vitro testing [10], host immunity [11], treatments, and animal models [12]. The main motivation was to outline a path for increasing the standardization and rigor of musculoskeletal infection-related research.

The Istanbul ICM2025 and the ORS research track delineated a research agenda with five pillars: (1) Extending pathogen scope and ecology beyond staphylococci to Gram-negative rods, Cutibacterium/anaerobes, fungi, and defined polymicrobial consortia studied in host-relevant microenvironments; (2) Understanding synovial-fluid aggregates by resolving formation kinetics, phenotype, antimicrobial tolerance, and dispersal, and standardization of models that recapitulate joint fluid; (3) Understanding persistence biology including bacterial populations in intracellular reservoirs, small-colony variants, extracellular DNA (eDNA) regulation, and host immune signatures linked to treatment failure; (4) Investigating methods of improving study quality including defining minimum datasets, appropriate controls, and harmonized reporting to permit cross-study comparison; and (5) Demonstrating the breadth of therapeutic innovation by optimizing systemic and local antibiotic exposures and carriers with pharmacokinetic/pharmacodynamic (PK/PD) principles, and evaluate non-antibiotic strategies using prespecified, clinically anchored endpoints. Collectively, these priorities specify focus areas and the methodological stringency required for results to be robust, reproducible, and clinically meaningful.

The goal of this document is to present a prioritized research agenda in the pursuit of understanding, preventing, and treating MSKI.

2 | Methodology

At the conclusion of the voting at ICM2025 in Istanbul, a quorum of the biofilm section was assigned this consensus article. The work commenced with the review of all the questions (https://www.icmortho.org/documents) and identifying those that were deemed the most important research priorities going forward.

Based on this review, the questions whose scope was considered in this article were B1-B32 (except B1, B6, and B31), which pertained to the in-vitro and preclinical evaluation of biofilm and G5, G7-8, G11-12, G25, G36, G37, G50-56, G69-73, G85-87, G92, G94-95 from the general session, which were deemed relevant. Here, we provide research priorities and describe the consensus discussion on these questions with expert opinions on future directions.

This document outlines key themes for prioritization of research efforts and funding. The identified themes from the consensus questions were diagnostics and infection characterization, testing

strategies against biofilm-associated infections, advances in the understanding of immunity against infections and clinical treatment strategies. Additionally, it was also deemed important to include potentially significant areas that should include questions for consensus building in the next ICM; namely, those that can aid in overcoming translational barriers: the enhancement of reproducibility and rigor, the integration of artificial intelligence and machine learning, and the improvement of in-vitro organoid/organ-on-chip models.

3 | Results and Discussion

3.1 | Improving Diagnostics and Infection Characterization (Figure 1)

Biofilms are now recognized as the most important etiologic agent in implant-associated infections and the primary driver of chronicity, antimicrobial tolerance, and immune evasion (B15). Clinical data show that biofilm-forming organisms (B8)—particularly *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa*—are responsible for persistent infections that resist conventional treatment. Despite the centrality of biofilm-associated challenges in PJI, the detection workflows often ignore the presence of these biofilms. Thus, robust biofilm detection and the incorporation of an efficient "biofilm-aware" diagnostic framework in the clinical setting is a priority.

Bacterial biofilms exist in distinct reservoirs: on the implant surface, in local soft and osseous tissue, and in the osteo-lacuno canalicular network of cortical and trabecular bone within the joint environment (B9, B10 [13]). There is also strong evidence suggesting the existence of biofilm-like aggregates in the synovial fluid (B17). Sampling methodologies are crucial for precise downstream diagnosis. Current protocols include pre- and post-operative swabs of the surgical site and implant materials, as well as testing of debrided tissue and synovial fluid. There is a significant gap in our characterization of biofilm as these methodologies selectively detect viable and culturable bacteria in suspension and often fail to capture microbial communities embedded in a matrix with various physiological traits unique to the biofilm life cycle. The biofilm retrieval and recovery methodologies must be optimized and standardized to reduce pre- and post-operative diagnostic variability. There is currently no validated method for detecting biofilms in vivo in routine clinical practice (B23). The detection of dormant bacterial phenotypes, including viable but non-culturable (VBNC) cells (B18), small-colony variants (SCVs), and intracellular pathogens, represents another challenge in biofilm diagnostics. These phenotypes often evade conventional detection methods, contributing to treatment failure. Furthermore, in polymicrobial infections, antagonistic interactions among the organisms could interfere with accurate pathogen detection and treatment [14].

Identifying biomarkers that reflect biofilm burden and activity is a crucial and necessary area in facilitating the shift towards biofilm diagnostics from the current diagnostic methods for bacteria in suspension. Extracellular DNA, neutrophil extracellular traps (NETs), and nuclease activity (B21) are emerging as potential indicators of biofilm presence and characteristics. These markers could enable earlier detection and stratification of infection severity. To support these efforts, physiologically relevant in-vitro models must be developed and standardized. Models incorporating human, animal, or synthetic synovial fluid and host factors, such as fibrinogen and hyaluronic acid, can better simulate the in-vivo biofilm environment, allowing for more accurate testing of diagnostic and therapeutic interventions. Emerging technologies, including fluorescence imaging, optical coherence tomography (OCT), confocal laser-scanning microscopy (CLSM), and peptide nucleic acid fluorescence in situ hybridization (PNA-FISH) probes, have shown promise in preclinical models (B23). Biofilm-specific detection technologies, next-generation "omics"-based approaches, sensitive viability dyes, and advanced imaging techniques are needed to clarify microbial physiological status and capture elusive phenotypes within biofilms. Robust preclinical testing is necessary to adapt standardized sampling and biofilm detection tools for human use and integrate these technologies into surgical and diagnostic workflows.

Antibiotics remain our most potent tool against MSKI despite their reduced activity against biofilms without prior debridement. Future research should also focus on improving antibiotic

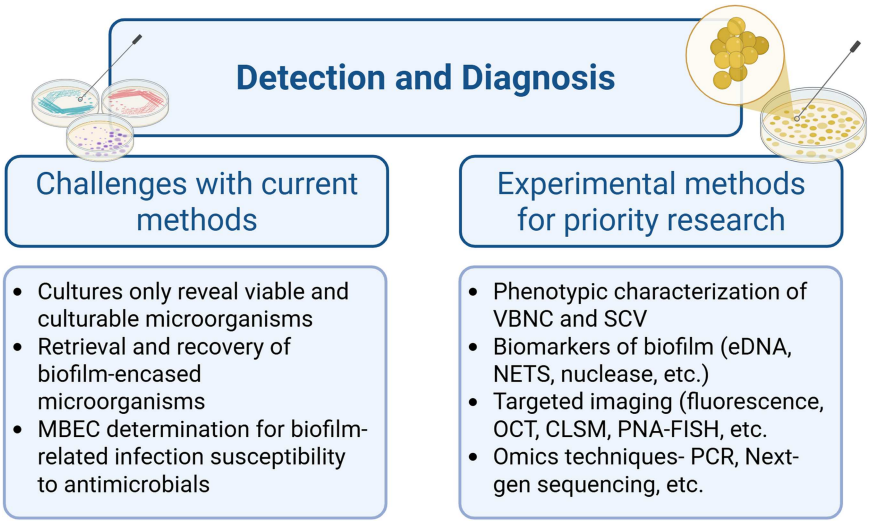

FIGURE 1 | Detection and diagnosis. Overview of challenges in current diagnostic approaches and emerging experimental methods for detecting biofilm-related infections.

selection and regimens for biofilm infections. While MIC testing is standardized and clinically validated, it assesses the susceptibility of planktonic cells and is a poor predictor of biofilm susceptibility (B18, B23 [15]). The minimum biofilm eradication concentration (MBEC) offers more realistic insights, but testing remains experimental, without standardized parameters or proven clinical value (G86). Evidence shows that although antibiotics may achieve MIC levels in bone and joint tissues, they often fall short of MBEC thresholds, exposing a major gap in our understanding of how to optimize current treatment strategies. The understanding of factors that contribute to the differences between the MBEC values of experimental biofilms in vitro and clinical biofilms is also an important issue (G86) [16]. Research priorities include developing standardized MBEC protocols, incorporating the relevant pharmacokinetics of active agents in the study of biofilm, and evaluating whether MBEC-guided clinical therapy improves outcomes compared with MIC-based approaches. Tolerability at MBEC-level dosing must also be assessed (G87), as effective concentrations in vitro often exceed safe levels in vivo. Integration of MBEC testing into diagnostic workflows, correlation with outcomes, and investigation of synergistic antibiotic combinations are priorities to refine treatment of implant-associated biofilm infections. Recommended follow-on questions are listed in Table 1.

TABLE 1 | Improving diagnostics and infection characterization: Recommended follow-on questions.

- Can standardized sampling methodologies to retrieve bacteria/biofilm from all relevant locations be developed?
- Are there methods to identify SCVs and VBNCs that can be integrated into the clinical PJI diagnosis framework?
- Is there a set of methods to decisively determine biofilm presence in vivo with an integrated imaging and biomarker detection workflow?
- Should antibiotic PK/PD be determined in biofilm environments for antibiotic dosing guidance?
- Is there a minimum set of data to determine whether biofilm-based susceptibility (e.g., determination of the MBEC) has an added clinical value?
- Can biofilm-based susceptibility testing identify synergistic antibiotic combinations effective against biofilm-associated infections?

3.2 | How to Test Strategies Against Biofilm-Associated Infections (Figure 2)

The preclinical testing strategies for anti-infective and antibacterial prevention and treatment remain extremely varied in study design as well as reported outcomes, both in vitro and in animal models of MSKI. There was strong consensus on the need to have a minimum set of reported outcomes (B4, B5, B11, B12), especially to improve the level of scientific evidence and the reproducibility across different studies. The need for improved rigor and reproducibility was a recurring theme of discussion and agreement, and is undoubtedly a priority in MSKI research. This goal can be achieved by higher scrutiny of the power of statistical study design, as well as randomization and blinding. Ethical considerations, especially under the framework of the 3Rs (Replacement, Reduction, Refinement), must also guide model design and reporting, with ongoing debate about whether ethical concerns themselves should constitute a special level of evidence.

In-vitro/ex-vivo testing conditions are currently not sufficiently representative of clinical conditions and therefore, study outcomes cannot be applied to the clinical management of infections. Thus, developing more clinically relevant testing methods in vitro and in preclinical environments is a priority. The current consensus effort identified clear and complementary outcome measures in preclinical models (B11) towards this priority. In addition, current research confirms that biofilm formation in synovial fluid is not only possible but also highly relevant to prosthetic joint infections (B17). In-vitro studies consistently demonstrate that suspended biofilm aggregates of *S. aureus* (including both methicillin-sensitive and resistant strains) and coagulase-negative staphylococci (CoNS) readily

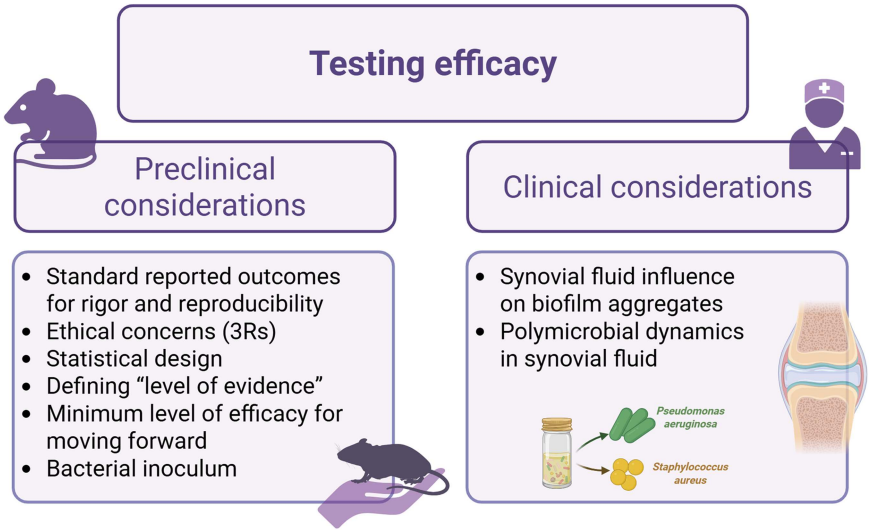

FIGURE 2 | Testing efficacy. Comparison of preclinical and clinical considerations in testing antimicrobial efficacy against biofilm infections.

form in human, bovine, porcine, and equine synovial fluid as well as in various synthetic synovial formulations. More recently, a broader range of pathogens, including Gram-negative bacteria (such as *Pseudomonas aeruginosa, E. coli, Klebsiella pneumoniae*, and *Proteus mirabilis*), Gram-positive bacteria (*including Streptococcus agalactiae*, *Enterococcus faecalis*, and *Cutibacterium acnes*), as well as *Candida* species, have also been shown to aggregate in synovial fluid, further demonstrating its importance in testing environments [17].

A limitation of the current evidence is its reliance on a limited range of microorganisms. However, systematic reviews indicate that this narrow focus overlooks the broader microbial diversity present in both clinical and animal infections (B8, G80-G85). To address this knowledge gap between in-vitro and in-vivo conditions, there was strong consensus that preclinical testing needs to include more clinically relevant microorganisms and especially polymicrobial communities, which constitute a large fraction of infections. Concurrently, better profiling of the clinical infection environment is desirable with the pathogen characteristics, the composition of the synovial fluid (e.g., fibrinogen, fibronectin, hyaluronic acid) and antibiotic tolerance. Additionally, clarification is needed on how polymicrobial communities interact with one another, and whether these interactions substantially impact clinical outcomes. In-vitro/ex-vivo testing with higher clinical relevance can only be designed with this knowledge. The recommended follow-on questions are listed in Table 2.

TABLE 2 | How to test strategies against biofilm-associated infections: Recommended follow-on questions.

- Is there a minimum level of efficacy that justifies moving forward to clinical testing?
- Can combination therapies be tested preclinically in a clinically relevant manner?
- Are in-vitro testing conditions for antibacterial efficacy comparable to *in vivo* conditions?
- Does synovial fluid influence biofilm formation in vivo and how?
- Should the antibiotic susceptibility and physiological status of biofilms be tested in the presence of synovial fluid?
- Are polymicrobial biofilm dynamics known in the presence of synovial fluid?
- Is there a preferred statistical design for "conclusively" predicting clinical efficacy based on in-vitro or preclinical results?
- Is there a definition of the level of evidence in preclinical studies (low, moderate, strong), and should ethical concerns be indicated as a special "level"?
- Is there a minimum level of bacterial inoculum to establish a periprosthetic infection in each animal model?

3.3 | What is the Role of Host Immunity in the Fight Against Biofilm? (Figure 3)

Recently, there have been developments in further understanding host-pathogen interactions in MSKI. Neutrophils and macrophages, which are the primary defensive cells against bacteria, remain ineffective against bacterial cells encased within biofilm. In addition to the physical barrier of the biofilm extracellular matrix, biofilms create local microenvironmental conditions that can impair leukocyte activity [18]. Recent work visualizing real-time interactions of these immune cells with implant surfaces revealed that immune cells reached and colonized these avascular surfaces effectively; however, they were unable to prevent biofilm maturation [19]. This work may redefine our understanding of the "race to the surface" [20], which has been the foundation for the development of surfaces that delay bacterial attachment and biofilm formation with the hypothesis that this delay could prevent infections. This new finding suggests the limited utility of this strategy alone, also supporting the earlier consensus discussion on the lack of impact on clinical management.

Beyond innate immunity, the role of adaptive and humoral responses in biofilm infections is still poorly understood. Biofilm protects bacteria from host defenses, making immune clearance difficult even when neutrophils and macrophages reach the surface. Preclinical studies have provided evidence that vaccines may protect against biofilm-related infections [21]. However, no effective vaccine has been developed so far,

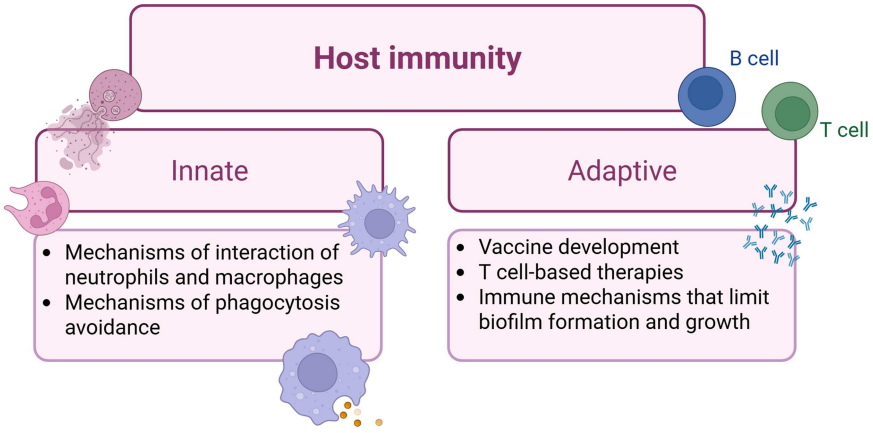

FIGURE 3 | Host immunity. Summary of innate and adaptive immune mechanisms relevant to biofilm infections.

underscoring a significant gap in our understanding of protective adaptive immunity. Clarifying how host immunity interacts with biofilm, and identifying which immune mechanisms can limit biofilm growth, will be essential to guide rational vaccine development. A better understanding of these processes could also support broader immunological approaches to prevent or control biofilms and is an important priority for MSKI research (G94). The recommended follow-on questions are listed in Table 3.

3.4 | Clarification of Efficacious Clinical Strategies and Evaluation of the Feasibility of Novel Treatments (Figure 4)

Among the discussed questions and answers, there was a clear distinction between the optimization of conventional treatments, such as irrigation solutions, and demonstrating the feasibility of novel treatments. Thus, we present the research priorities in two subsections.

TABLE 3 | Host immunity: recommended follow-on questions.

- Are the mechanisms by which neutrophils and macrophages interact with biofilm structures on implant or tissue surfaces known?
- Are the molecular mechanisms that prevent phagocytosing immune cells from effectively clearing biofilm bacteria known?
- Are the specific in-vivo adaptive and humoral immune mechanisms that contribute to either limiting or failing to limit biofilm growth known?
- Can T cell–based therapies or vaccines enhance protective immunity against biofilm-associated infections?
- Are there specific immune pathways or targets for immunomodulation in preventing or disrupting biofilm formation and maturation? (High-level evidence)

3.5 | Approaches Aiming to Optimize the Delivery of Antibacterials (Figure 4)

Application of antibiotics in free forms topically or in delivery vehicles as well as antimicrobial coatings for implanted devices was considered to optimize existing antimicrobial strategies (G34, G36, G37, G52, G56). Although there is some evidence that different antimicrobials and other therapies exhibit antibiofilm activity preclinically (B7, B27), mechanical debridement of established biofilms is currently the clinical method with the most consistent results. Antibiotics then support the prevention of re-infection and there is a plethora of new technologies designed to enable or enhance antibiotic action at the site of an orthopaedic infection. The consensus for most of these technologies representing some antibacterial efficacy was positive (B19), however delegates' detailed responses showed that there is a lack of robust, standardized preclinical (as mentioned in 3.2) and clinical data to support their use. Currently there isn't sufficient evidence to change established biofilm management without mechanical debridement and antibiotic treatment. Randomized clinical studies with clearly stated hypotheses that can serve specific indications and capture the diversity of the patient population and treatment types are a priority for conclusively evaluating new strategies [22].

The technologies that have progressed furthest along the pipeline are antibiotic and silver-coated implants (B22, B27, G53, G56). These implants are not approved for use in the US but have been on the European market for over a decade; despite this, there are no large-scale prospective clinical studies that aim to evaluate their efficacy. Silver-coated megaprostheses are indicated for limb-salvage surgeries; thus, their outcomes are limited to a small number of patients and are generally heterogeneous. Similarly, antibiotic and silver-coated fracture fixation devices have been applied in trauma care, with generally positive results, but a relatively limited number of patients were included in these studies. Nevertheless, these technologies may result in cost-effectiveness in patients at high risk of infection [23]. Another technology for providing sustained release of antibiotic agents is a degradable hydrogel based on hyaluronic acid. These technologies have shown promise in small studies and may be more widely applicable as the carrier can be used with any medical device similar to antibiotic-loaded cement

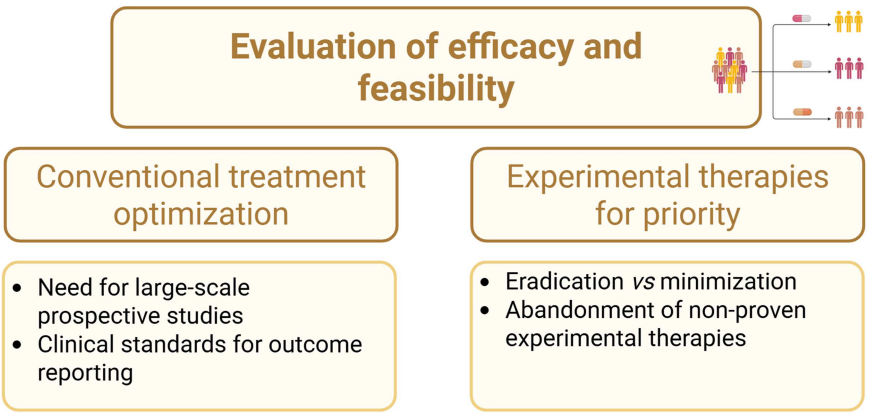

FIGURE 4 | Evaluation of efficacy and feasibility. Framework for optimizing conventional treatments and prioritizing experimental therapies. Emphasis is placed on the need for large-scale, standardized clinical studies and the differentiation between eradication vs. minimization strategies. Non-evidence-based experimental approaches should be discontinued.

coatings. The planned randomized controlled trial using this antibiotic-loaded hydrogel coating (SINBIOSE-H [24]), if successful, may set a new standard for evaluating these technologies. The ongoing lack of randomized controlled trials for conclusively evaluating the efficacy of these technologies with the potential to decrease the infection burden on a larger group of patients is creating a bottleneck for their widespread use.

Likewise, capturing the outcomes of diverse groups of patients retrospectively in a coherent and rigorous manner, for example by detailed reporting of MSKI cases available in registries (G101), can also be valuable in guiding future RCTs. However, there is an urgent need to set a clinical standard for what constitutes effective local antibacterial technology. Studies should include careful definition of the patient population, randomization, inclusion of appropriate controls, robust outcome measures, such as implant survival and reoperation rate over a sufficiently long follow-up period, in addition to outcomes such as wound healing complications, wound drainage/leakage, surgical site infection and PJI (G97, G100). Standardizing clinical study protocols to evaluate not only direct outcomes but also to generate feedback for future clinical studies and enhance preclinical study design with greater predictive power is essential.

3.6 | Novel Technologies Designed to Combat Biofilm (Figure 5)

As biofilms are the main challenge of treating implant-associated infection (B15), the development of a treatment that can "eradicate" established biofilm on an implant in a patient (e.g., PJI) would be transformative for MSKI. Many new technologies aimed at detaching or destroying biofilm in situ are in development. The ICM considered questions on potential treatments using physical technologies such as ultrasound (B16), electric fields (B26) and photodynamic therapy (B32), active agents targeting bacteria and biofilm such as monoclonal antibodies (B13), proteolytic enzymes (B14), antimicrobial peptides (G58, G59, G95), and bacteriophage therapy (G57, G95). The consensus was that while these technologies demonstrate promising antibiofilm effects in vitro, the current level of evidence for their use in clinical infections is low and there is a need to increase rigor, reproducibility and standardization of preclinical evaluation methods. Based on the information available, ICM2025 concluded that there is no direct evidence for these discussed technologies that they: (1) are feasible as defined by a rational dosing regimen that can be given to a broad patient population based on biofilm characteristics and location; and/or (2) can "eradicate" biofilm in vivo defined as disintegration of the extracellular polymeric substance and killing all associated microbes. The strategies with the highest potential antimicrobial effect remain those that provide an active agent as their main mechanism of action and several are worth mentioning due to ongoing clinical efforts to demonstrate their impact.

Anti-DNABII antibodies (B13) target DNABII proteins that stabilize extracellular DNA crosslinks within the biofilm matrix, leading to collapse of the biofilm and restoration of bacterial susceptibility to antibiotics and host immunity [25]. They are species-agnostic and exhibit broad-spectrum antibiofilm activity against more than 20 tested Gram-positive and Gram-negative species, encompassing all ESKAPEE pathogens. Intravenous TRL1068—a fully human anti-DNABII monoclonal antibody (mAb)—was safe across escalating doses in a phase 1 hip and knee PJI trial and demonstrated preliminary reductions in bacterial burden and increased culture negativity at the explanted prosthesis during two-stage revision (ClinicalTrials.gov: NCT04763759) [26]. A phase 2 trial is now recruiting to evaluate TRL1068 in conjunction with debridement, antibiotics, and implant retention (DAIR) for hip and knee PJI (ClinicalTrials.gov: NCT06621251). CMTX-101, a humanized anti-DNABII mAb, completed phase 1 intravenous dose-escalation safety testing in healthy volunteers and is in an ongoing phase 1b/2a trial in individuals with cystic fibrosis chronically infected with *Pseudomonas aeruginosa* (ClinicalTrials.gov: NCT06159725).

Bacteriophage therapy presents a mechanistically novel treatment against bacteria with high potential for efficacy due to the variety of naturally occurring bacteriophages and their specificity for interacting with bacteria. Unlike conventional

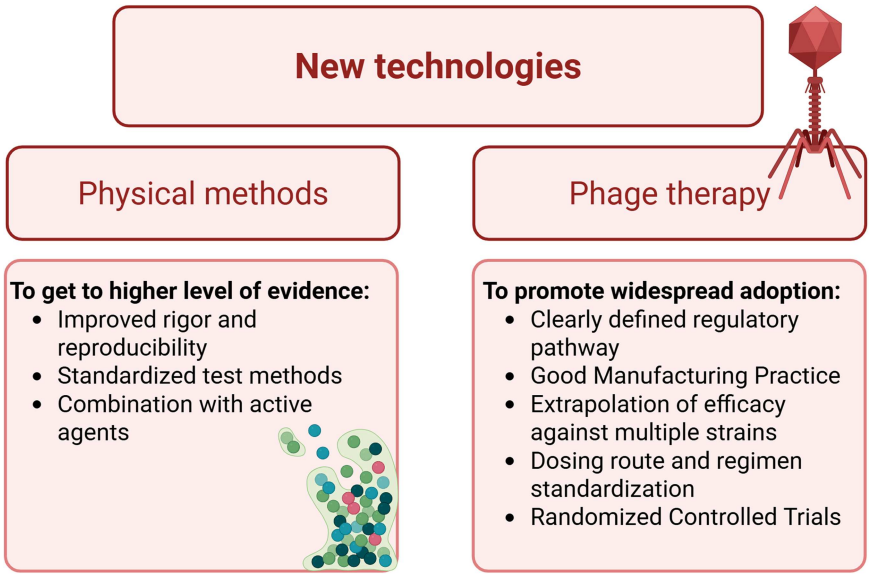

FIGURE 5 | New technologies. Emerging technologies for biofilm management, highlighting physical methods and bacteriophage therapy.

antibiotics, it is not impacted by the global challenge of antimicrobial resistance. There is growing interest in the application of bacteriophage therapy for the treatment of MSKI, as reflected by the establishment of dedicated phage therapy centers worldwide, and targeted funding initiatives such as the **S**trategies for **H**ealing **I**mplant-associated Infections and **E**nhancing **L**ongevity in **D**evices (SHIELD) doctoral network [27, 28] and multiple related questions raised during the ICM 2025 conference (G57, G58, G95). Despite its promise, the approach is accompanied by several uncertainties that must be addressed before widespread clinical adoption is feasible. A challenge is the absence of a clearly defined regulatory pathway to produce bacteriophages under Good Manufacturing Practice (GMP) conditions [29]. Another is the lack of clarity on how efficacy data obtained for a specific bacteriophage against a single pathogen can be extrapolated to the broader spectrum of pathogens and infection types encountered in clinical practice. Furthermore, optimal routes of administration and dosing regimens remain poorly defined, and the potential for host immune responses, particularly the development of neutralizing antibodies during treatment, raises additional concerns. The clinical evidence base remains limited [30, 31]. Although published low-level clinical reports are generally positive, some larger trials outside of musculoskeletal infection conducted in the past have failed to demonstrate a clear therapeutic benefit [32]. Thus, despite the exciting prospect of bacteriophage therapy as a novel and effective tool against biofilm, the prioritization of answering the most clinically relevant safety and efficacy questions is crucial. There is a pressing need for adequately powered randomized controlled trials (RCTs) to rigorously assess efficacy.

The general lack of direct clinical evidence of antibacterial effects of new technologies using active agents is partly due to the absence of rigorous and reproducible assays to evaluate the dosing, pharmacokinetics, pharmacodynamics of the proposed agent(s) and their efficacy against biofilm. However, now that appropriate experimental systems are discussed and minimal datasets for diagnostic (B4), therapeutic (B5), and clinical (B12) claims have been agreed upon by ICM2025, a more rigorous and critical evaluation of mature technologies can be performed. The recommended follow-on questions are listed in Table 4.

TABLE 4 | Clarification of efficacious clinical strategies and evaluation of the feasibility of novel treatments: Recommended follow-on questions.

- Is there standardized guidance for RCT outcomes for evaluating antimicrobial efficacy?
- Is there a harmonized set of outcomes in clinical and preclinical studies for antimicrobial efficacy?
- Is there a minimum set of outcomes that registries should collect/report for MSKI?
- Are there clinically relevant endpoints/outcome measures in preclinical (in-vivo) studies regarding antimicrobial safety?
- Are there clinically relevant endpoints/outcome measures in preclinical (in-vivo) studies regarding antimicrobial efficacy?
- Are there clinically relevant endpoints/outcome measures in in-vitro studies regarding antimicrobial safety?
- Are there clinically relevant endpoint measures in in-vitro studies regarding antimicrobial efficacy?

3.7 | Translational Barriers

While it was apparent that the efficacy of many device strategies was strongly supported against infections in vitro and in preclinical studies, for example, regarding new technologies such as smart antibiotic carriers (B30), there is a significant gap in the translation of these strategies to the clinic. This gap is caused by both the lack of predictive algorithms for the clinical impact of preclinical results and regulatory barriers to translation. Of the more than 1000 orthopaedic devices approved for clinical use in the US by the Food and Drug Administration (FDA) through the 510 K and premarket approval (PMA) mechanisms in 2024 and 2025, there are only a handful addressing infection and 16 combination products. Those that were approved for use via the 510 K pathway are largely antibiotic-eluting cement and those that were approved for use via PMA are bone grafts. Only 2 devices were approved by the DeNovo designation; ELEOS Limb salvage system with NanoCept technology (Onkos Medical, NJ), which is a "Limb and joint salvage device with coating for bacteria reduction" and Orthobond Mariner pedicle screw system (Orthobond, NJ), which is a "spinal fusion system with 12-methacryloyloxydodecyl pyridinium bromide coating" designed to reduce bacterial contamination. There is also one investigational drug; VT-X7 (Osteal Therapeutics, TX), which is a combination of the antibiotics tobramycin and vancomycin delivered into the joint via an irrigation pump. All these devices were approved for use in infected revisions and limb salvage while no anti-infective devices for prevention of infection in primary surgeries were approved in the same period. The current portfolio of devices indicates that there is a preference towards incremental advances rather than novel therapeutics and devices due to the challenges of the regulatory process and the large investments needed to develop these products, whose efficacy can take many years, many sites, and tens of thousands of patients to demonstrate, especially for infection prevention.

For this reason, the most effective use of resources is in prioritizing "high-risk" patients first for novel strategies, as this approach provides sufficient patient volume to power such studies and significantly reduces cost requirements. Identifying "high-risk" patients and standardizing study protocols remains a challenge and there is a gap in the conceptual understanding and methods of the evaluation of the risk of treatment failure for proposed technologies. More standardized, clinically relevant, accurate and cost-effective methods of evaluating the benefit-to-risk ratio of anti-infective devices/treatments and incorporating realistic regulatory considerations into research activities at the study design stage [33] are important research and clinical priorities.

The testing guidance and methods that regulatory agencies rely on are often developed by standards organization, such as the International Standards Organization (ISO) or the American Society for Testing and Materials (ASTM). These organizations develop standard guidance and methods based on the input and

consensus of the stakeholders in the area of interest, including industry, academic research, and regulatory agencies. Although there are specific tests for determining the antimicrobial efficacy of surfaces that are commonly used such as ASTM E-2149 and ISO 22196, there is an increasing need for test methodology specific to implantable medical devices in the context of biofilm and guidance on how to use existing methods for a comprehensive evaluation. Only by establishing methods of strong preclinical support can the widespread use of MSKI treatment strategies be successfully enabled. Increasing the clinical relevance of in-vitro testing strategies with better clinical performance predictions can reduce long-term follow-up requirements for clinical studies and can make a considerable impact in reducing the investments for drug and device development without increasing the risk for patients. This shift is imperative to also guide research efforts in the most efficient direction in treating infections and away from the production of strategies and products that cannot be cleared for safe and effective clinical use. Recommended follow-on questions are listed in Table 5. In the next sections, we present the biggest opportunities in overcoming translational barriers and providing successful ways of minimizing MSKI.

TABLE 5 | Translational barriers: recommended follow-on questions.

- Is there a minimum cohort of patients to confirm
  - antibacterial efficacy in prophylaxis?
  - antibacterial efficacy in treatment?
- Is there a known set of risks associated with
  - antimicrobial MSKI prevention?
  - antimicrobial MSKI treatment?
- Is there a preclinical and clinical method of quantifying resistance risk for novel antibacterial treatments?
- Is there guidance for the evaluation of
  - The safety risk of new antimicrobial technologies in MSKI treatment?
  - treatment failure of new antimicrobial technologies in MSKI treatment?
- Should there be a defined clinical indication while reporting the in-vitro testing of new technologies?

3.8 | Opportunities: Enhancement of Reproducibility and Rigor (Figure 6)

The previous consensus meeting in 2018 identified the need for clear definitions related to clinical PJI and emphasized the need for these definitions to be based on and supported by what is known about the biology of biofilms. While the previous ICM efforts established a clear definition of PJI based on clinical diagnostic plasma and synovial fluid markers supported by tissue culture, there are still gaps in our knowledge of bacterial diagnosis and characterization in the clinic in a timely and accurate manner. Addressing these gaps can enable the more accurate determination of antibiotic selection, dosing, and duration of treatment as well as more accurate evaluation of efficacy for existing and novel treatments and technologies.

Improvement is needed both in the quality of clinical outcome studies to be more informative for research studies and in the predictive value of in-vitro methods of biofilm characterization and treatment. This gap is due largely to the lack of standardization/harmonization of outcome measures and the lack of high level of evidence, both in preclinical and in clinical studies. For example, clinical studies often rely on clinical measures such as ranked pain scoring or probabilistic outcomes such as infection rate, which do not provide direct feedback to in-vitro or preclinical work. Addressing the gap requires a

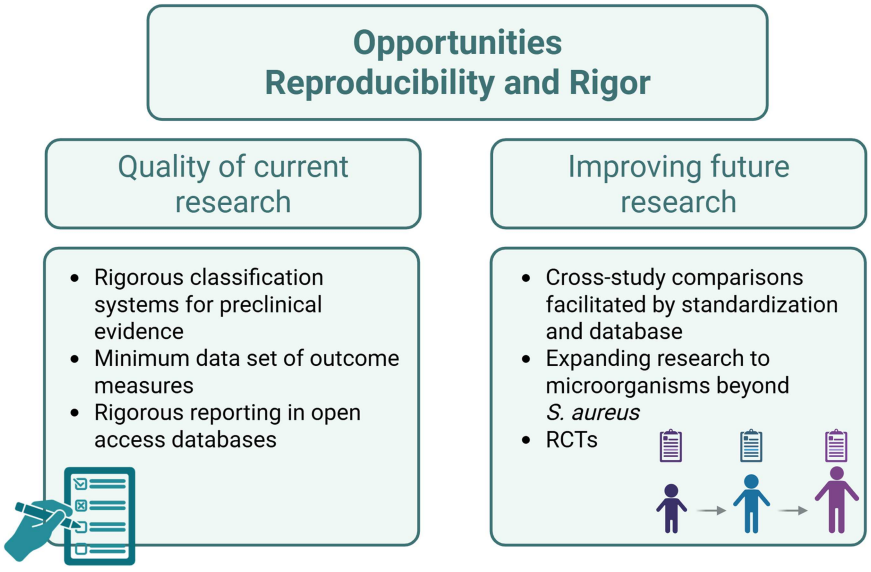

FIGURE 6 | Opportunities for reproducibility and rigor: Strategies to improve the quality and consistency of biofilm research. Enhancing current studies involves standardized data reporting and classification systems, while future directions include expanding beyond *Staphylococcus aureus* and promoting cross-study comparisons and randomized controlled trials (RCTs).

multi-pronged effort. First, the field must adopt rigorous classification systems for preclinical evidence, analogous to clinical hierarchies, and enforce standardized definitions of efficacy. Second, journals and funders should require a minimum data set of outcome measures, including inoculum quantification, infection verification, appropriate controls, burden measurement, imaging, and histology, to ensure reproducibility (B11). Third, implant studies must move toward protocol standardization in animal models (species, inoculum size, bacterial strains, outcomes). Finally, the creation of a shared, open-access database of infection model outcomes (similar to GEO for OMICS) will allow benchmarking, cross-study comparisons, and more robust meta-analyses. Only by embedding rigor, harmonizing reporting, and ensuring transparency can the gap between preclinical findings and clinical outcomes be bridged.

As mentioned above, preclinical research needs to systematically expand the range of pathogens to increase clinical relevance. Models should incorporate Gram-negative bacilli, polymicrobial infections, host-specific pathogens, and fungi to reflect the true clinical diversity of orthopaedic infections. It is also essential for clinical strains to be publicly available for sequencing and characterization to facilitate access, reproducibility, and comparability across studies. Additionally, publication standards must evolve to require quantitative documentation of pathology in bone and soft tissue for each tested strain. To enable and facilitate such broad and equitable access to clinical strains and their characterization, it is a priority to establish nation/regionwide repositories (e.g., ATCC (www.atcc.org) or CCUG (www.ccug.se). By broadening the microbial landscape and standardizing strain documentation, the field can generate more clinically relevant data and better anticipate therapeutic challenges. Furthermore, the construction of these resources can minimize translational barriers as FDA guidance suggests an even wider array of microorganisms, such as *Pseudomonas aeruginosa* and *Candida* spp., that should be considered in antimicrobial testing.

While there are clinical reports that generally describe positive outcomes for several technologies, there is a pressing need for adequately powered randomized controlled trials (RCTs) to rigorously assess their efficacy. The economic burden of MSKI is tremendous and increasing and all the stakeholders in this field have the responsibility to decrease the burden of this condition on patients by demonstrating the efficacy/inefficacy of available treatments and by improving the flexibility and accessibility of treatments. There is a need to highlight this field on a "consortium" level with the engagement of multiple research and clinical groups to enable standardization of methods and to improve the rigor and reproducibility of all preclinical and clinical studies. The ongoing lack of consortium efforts to bring together large sets of patients often necessary for sufficiently powered efficacy studies for infection is a major factor preventing the widespread availability of novel technologies for patients undergoing surgery for musculoskeletal conditions.

3.9 | Opportunities: Artificial Intelligence and Machine Learning (Figure 7)

Despite the wide range of topics addressed at the 2025 ICM on Orthopaedic Infections, artificial intelligence (AI) and machine learning (ML) were not emphasized as part of the prioritized research agenda. However, the meeting did include a dedicated question (G98) examining whether AI/ML has a role in the management of orthopaedic infections. The response acknowledged that AI holds potential advantages in data mining, systematic information collection, and the development of predictive, diagnostic, and treatment tools, with promising applications across prediction, prevention, diagnosis, treatment, and prognosis. Nonetheless, the consensus highlighted that current research remains limited, with significant barriers related to external validation, generalizability, and utilizing the "black box" nature of many ML models, ultimately assigning a low level of evidence to its use. Thus, while AI/ML are starting to be discussed within the field, their absence from the core list of research priorities underscores that orthopaedic infection research remains at an early stage of engaging with digital health innovations. Unlike other medical domains where AI applications are rapidly advancing, the field has yet to recognize these tools as immediate priorities. This reflects both the complexity of the clinical problem and the gap between technological development and clinical applicability. There is currently no integration of AI or ML in vitro or in preclinical research with unrealized opportunity for harmonizing between laboratory discovery, translational pathways, and clinical implementation. This limitation for AI or ML utilization for MSKI research is further challenged by low quality data [34, 35] a substantial barrier, as robust, high quality data sets are the foundation on which this technology must be built. However, these fundamental challenges that hinder meaningful translation into clinical practice, including small and imbalanced data sets, a lack of universally accepted diagnostic standards, difficulties in integrating data from multiple centers, and emerging regulatory hurdles, should be addressable going forward based on the ICM's mandated minimal datasets for diagnostic (B4), therapeutic (B5), and clinical (B12) claims. Thus, efforts to create shared, accessible datasets for integration into AI/ML development is a priority.

3.10 | Opportunities: Prioritization of In-Vitro Models (Figure 8)

NIH recently announced the award of contracts for establishing the Standardized Organoid Modeling (SOM) Center, prioritizing

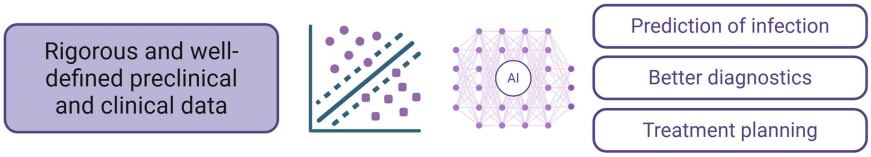

FIGURE 7 | Integration of artificial intelligence. The role of artificial intelligence (AI) in leveraging robust preclinical and clinical datasets for infection prediction, improved diagnostics, and treatment planning, emphasizing data quality and standardization as key prerequisites.

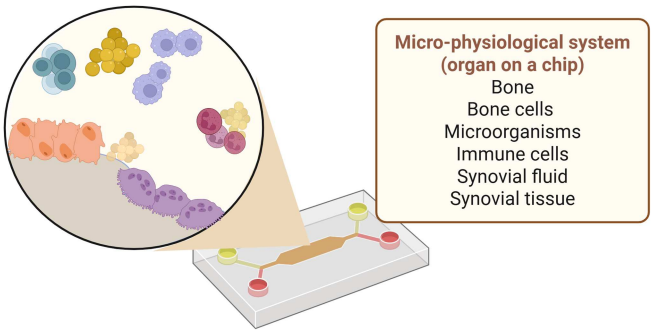

FIGURE 8 | Micro-physiological systems (organ-on-a-chip). Schematic representation of an organ-on-a-chip model integrating bone, synovial tissue, immune cells, and microorganisms. This system aims to mimic complex environments in vivo to study host-pathogen interactions and biofilm-associated infection mechanisms.

research that uses human-based technologies and models and reducing reliance on animal research [36]. This effort aims to utilize animal research when scientifically appropriate, justifiable and with proper animal oversight as well as to guide the direction of biomedical research towards innovation in in-vitro models with increased clinical relevance. The announcement states that funding opportunities will necessitate complementing animal studies with human-focused approaches, such as real-world data. This direction is also in line with our prioritization of approaches to close the gap between in-vitro and preclinical findings and their clinical interpretation and utility.

While the NIH SOM Center will first focus on heart, lung, liver, and intestine, this initiative is a great opportunity for musculoskeletal researchers to work towards developing musculoskeletal organoid/organ-on-a-chip models and those in which medical associated infections can be tested in a more clinically relevant manner. While bone organoids have been proposed for producing healthy and diseased musculoskeletal tissue models [37] and few models for osteomyelitis [38], a full in-vitro model of the infection environment of the joint including the synovium and synovial fluid does not exist. The construction of a synovial joint organoid model is an opportunity to enhance the in-vitro testing environments while aligning with the goal of improving the clinical relevance of preclinical testing. Ultimately, gaining a better understanding of the dynamics of synovial fluid biofilms will be crucial for developing diagnostics and therapies targeting these elusive, non-surface-attached biofilm forms.

The construction of advanced in-vitro micro-physiological systems, such as a synovial joint organoid model, is an important opportunity to enhance the in-vitro testing environments while aligning with the goal of improving the clinical relevance of preclinical testing. These models should ideally incorporate not only host and bacterial cells but also the relevant physical environment including flow conditions and incorporate sensors for real-time monitoring and feedback of micro-physiological stimuli [39]. For example, advanced imaging and molecular techniques can be leveraged to elucidate bacterial aggregate size, structure, and metabolic states, along with host cell response. Importantly, micro-physiological models can enable high- throughput screening of technologies and facilitate reproducibility across laboratories, generating high-quality, robust data sets. In turn, this will accelerate the integration of artificial intelligence as a new tool for improving orthopaedic infection prevention, diagnosis, and treatment, and contribute to the establishment of international standards for clinically relevant testing.

TABLE 6 | Opportunities: recommended follow-on questions.

- Is there an international standard (ASTM or ISO) for clinically relevant testing the antibacterial properties of implantable medical devices?
- Is there a shared, open-access database of infection model outcomes?
- Is there a known set of clinical factors to be included in the design of a synovial joint organoid?
- Is there a known set of clinical factors to be included in the design of an infected bone organoid?
- Is there data from animal models of infection that can be replaced by testing in an organoid?

The recommended follow-on questions are listed in Table 6.

4 | Conclusion and Outlook

Musculoskeletal infections, especially implant-associated infections, continue to present a significant burden to all stakeholders including, first and foremost, the patients and their caregivers, and the healthcare system. By bringing together the expertise of orthopaedic surgeons, infectious disease specialists, biofilm researchers as well as those who work in medical device development, clinical consensus meetings offer an opportunity to define the status quo and identify knowledge gaps. Here, we have presented an expert interpretation of our current knowledge of biofilm-related fields, identified immediate research priorities and recommended potential questions to be asked at the next consensus meeting. The most promising opportunities lie in *organizing, analyzing, and sharing the vast amount of data* that can be collected using advanced technologies such as various 'omics'-approaches and AI/ML, as well as in enabling *multi-center clinical studies with large and comprehensive cohorts* and *standardized techniques* which subsequently *inform preclinical testing to increase clinical relevancy*. Such efforts are essential for the much-needed improvement of rigor and reproducibility, in the identification of effective interventions and treatments (including devices) and in defining a more rigorous framework for introducing new technologies by better assessing risk and benefit.

Acknowledgments

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