

QUESTION 4: What are some of the emerging pre-clinical methods for evaluating novel antimicrobial technologies?

RECOMMENDATION: At present, most in vitro testing provides limited insight into the potential of novel antimicrobial technologies. More recently, in vitro models that incorporate animal or human tissue are emerging to test adherence and colonization to devices in contact with human tissues. Further development and validation of these models is needed, as well as approaches to include the element of human immune response.

LEVEL OF EVIDENCE: Limited

DELEGATE VOTE: Agree: 81%, Disagree: 2%, Abstain: 17% (Super Majority, Strong Consensus)

RATIONALE

The Food and Drug Administration (FDA) held a workshop in 2014 on antimicrobial/antibiofilm technologies and has published a white paper on the workshop outcomes [1] as well as a book chapter in 2016 [2]. The FDA recognizes the public health impact of medical device associated infections including prosthetic joint infections. There are two types of pre-clinical antimicrobial effectiveness testing: in vitro and in vivo. In this response, in vitro testing is addressed.

In Vitro Testing

At present, most in vitro testing provides limited insight into the potential of novel antimicrobial technologies. Most Clinical and Laboratory Standards Institute (CLSI) and United States Pharmacopeia (USP) tests (e.g., CLSI M02-A11, CLSI M07-A9 and USP 51) are for planktonic bacteria and/or are not ideal for medical device technologies. Some of the newer American Society for Testing and Materials (ASTM) methods are focused on creation of reproducible microbial biofilms for testing, but are not specifically developed with methods and endpoints that are appropriate for medical devices. Medical devices have a range of patient contact types (e.g., indwelling, transcutaneous and implanted) and duration (e.g., prolonged vs. permanent contact). A notable consideration for permanent contact implants is how to identify an effective dose that can prevent biofilm formation where multiple applications of the antimicrobial are not feasible). Therefore, modification and careful development of protocols to demonstrate in vitro effectiveness is necessary for specific medical device applications.

Differences based on material properties are more easily detected in adhesion studies since they are typically conducted using short times while in saline, where bacterial growth is minimal. Thus, adhesion testing is better suited for comparing early stage bacterial interactions with different antimicrobial technologies or libraries of materials. The ASTM E2647 drip flow reactor or similar type flow systems have been used to study early stage bacterial adhesion and biofilm formation [3,4]. An alternative approach to adhesion testing is to put samples in microtiter plates with an orbital incubator and to extract colonies after testing the antimicrobial strategy [5]. While this approach is simpler to set up and does not require sophisticated and costly confocal microscopy equipment to visualize cells, it is an endpoint method rather than a real-time approach. There may also be limitations due to the extraction technique employed and the presence of viable but non-culturable (VBNC) bacteria. When testing adhesion, one should keep in mind that surfaces which initially repel bacteria may fail after some period of time due to buildup on the surface, fouling by dead bacteria and interactions with bodily fluid and tissues.

For longer-term biofilm testing, the ASTM E2562 CDC flow reactor is a lab-scale model suitable for testing coupons from medical devices or entire small devices [6]. It has been used extensively in the literature for testing antimicrobial device technologies. A limitation of this approach is that bacteria are typically provided continuous nutrients so that a mature and fully-saturated biofilm is achieved. This can reduce the sensitivity for comparing between similar materials with slight differences, such as different types of patterned/textured surfaces. The ASTM E2799 minimum biofilm eradication concentration (MBEC) assay is a higher throughput format than the CDC reactor, but requires modification to be used with medical devices [7]. It is challenging to perform successfully due to the number of steps and requires significant work to optimize for each material and strain.

Two promising in vitro approaches that have the potential to increase realism in testing are human cell-based co-culture and ex vivo tissue models. Bacterial co-culture with human cells is challenging and its use for testing is still in experimental development. It can include human tissue cells [8] and/or human immune cells [9]. A more achievable approach at this time is ex vivo tissue-based models. The use of ex vivo porcine skin explants has shown great promise as a tool to study the development of more mature biofilms with greater resistance to antimicrobials [9–11]. The next logical step is the use of human tissue models such as a recent article showing how the use of human epithelial tissues has yielded valuable information on the fitness of bacteria to adhere to and colonize human cells [12]. Such models could potentially allow for simulation of the tissues in contact with an orthopaedic implant for evaluation of antibiofilm strategies.

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