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QUESTION 3: What methods can the Food and Drug Administration (FDA) and other regulatory bodies use to evaluate the efficacy of novel anti-infective technologies?

RECOMMENDATION: The FDA and other regulatory bodies can use in vitro cell culture methods to evaluate the antimicrobial efficacy against pathogens, followed by animal studies to evaluate osseointegration issues and a subsequent osteomyelitis/periprosthetic joint infection (PJI) animal model to evaluate the in vivo efficacy. However, clinical trials may be required for clearance or approval of some novel anti-infective technologies.

LEVEL OF EVIDENCE: Moderate

DELEGATE VOTE: Agree: 80%, Disagree: 3%, Abstain: 17% (Super Majority, Strong Consensus)

RATIONALE

Human clinical trials of anti-infective technologies are inherently difficult to perform according to Lazzarini et al. [1], due to the low incidence of implant-associated infections, the heterogeneous patient population, various treatment options in arthroplasty, the surrounding tissue condition after debridement and the broad range of causative pathogens and associated virulence patterns [2]. A cascade of in vitro cell culture methods and especially meaningful experimental animal models have to serve to fill this inevitable gap [1].

During the development of anti-infective biomaterials and devices and the determination of their anti-microbial properties, reliable in vitro test methods are essential to characterize implant surfaces [1,3]. In any evaluation procedure, cell proliferation has to be included as an important step in the course of infection [3]. For appropriate anti-microbial efficacy testing the independent aspects adhesion, proliferation and detection of bactericidal activity shall be considered in a consistent approach [3,4].

In the almost identical anti-microbial test methods, described with Japanese Industrial Standard (JIS) Z 2801:2010 and the International Organization for Standardization (ISO) 22196:2011 standards, the bacteria are applied onto the sample surface and covered under a sterile film, whereas for the American Society for Testing and Materials (ASTM) E 2180 test method the bacteria are applied as a thin agar slurry film. After 24 hours, by recovering vital bacteria from the samples, both test methods' anti-microbial efficacy is determined as the difference between the untreated reference and the anti-microbial sample. The major limitations are the required sample size (ISO 22196 5 x 5 cm, ASTM 3 x 3 cm) and the flat and smooth surface geometry, which is often not a given for orthopaedic implants [4]. In addition, hydrophobic surfaces can be unsuitable for testing according to ISO 22196, and the applied agar film (ASTM E 2180) can be too thick for non-leaching surface bound anti-microbials, thus leading to false-negative results.

Proliferation assay-based methods, first described by Bechert et al. [3], measure the antimicrobial efficacy based on the reproduction and release of daughter cells, monitoring the growth activity of these offspring bacteria over time. The main advantage of the proliferation-based assays is a broad applicability to flexible sample geometries (e.g., 2D and 3D), surface properties (e.g., smooth, textured, porous) and test conditions (e.g., leaching and non-leaching) [3–5]. Moreover, this method allows a parallelized investigation of many different setups in one test run ensuring a direct comparability, which results in increased explanatory power and higher sensitivity as given in the ISO and ASTM test methods [3,4]. However, the interpretation of test results is somehow more sophisticated, since growth of the offspring bacteria is analyzed rather than the vital cells on the sample surface [3,4]. In case of more complex surface structures and 3D geometries, which is the case for orthopaedic implants, the most reliable test method is a proliferation-based assay [4]. An important additional aspect is the contact of the implant to body fluids (such as blood, serum or interstitial liquid), having typically a high concentration of proteins, covering the device surface by a protein layer, which can have an impact on the antimicrobial performance of the material. Moreover, the influence of sterilization, aging degradation and persistence of the anti-microbial effect should be examined and testing should always be performed at least against gram-positive and gram-negative bacteria strains [4]. However, a direct transferability of in vitro results to in vivo performance is not stringently given. Thus, animal data are required to substantiate the antimicrobial efficacy in vivo.

To demonstrate unimpaired osseointegration for implant materials and surfaces that are modified by new anti-infective technologies in hip and knee arthroplasty, an appropriate animal study should be performed using controls based on long-term, clinically-established implant surfaces for cementless fixation, and also the base material and surface structure without the anti-infective treatment. Eto et al. [6] described a rat model with intramedullary implantation of a titanium rod to evaluate the osteoconductivity and osteogenesis in the meta- and diaphyseal region of the distal femur for experimental silver-oxide-containing hydroxyapatide coatings. They examined the implant anchorage strength at 2, 4 and 12 weeks post-implantation in a pull-out test, and performed a histological examination using a contralateral femur implantation with the same surface [6]. Analyzing the surface coverage with bone, they used this procedure to quantify the active peri-implant osteogenesis and osteoconductivity in the meta- and diaphysis of the femur in a comparison of anti-microbial surface treatments to a clinically-established hydroxyapatite (HA) coating [6]. Combining biomechanical and histological examinations, the model by Eto et al. [6] is valuable during the development phase of new anti-microbial implant surfaces to detect favorable solutions. The limitations of size, not allowing for testing multiple implants simultaneously and also significant dissimilarities between rat and human bone make a rat model unsuitable for clinically relevant osseo-integration testing [7].

To evaluate new anti-microbial surface solutions for a clinical use in orthopaedic implants, their biocompatibility, peri-implant osteogenesis, osteoconductivity and ability of osseointegration should be tested in an animal model of a higher species, like sheep, goat, pig or dog [7,8]. Preferably a load-bearing model of the proximal tibia or distal femur in direct implantation site, or autologous left-right comparison should be performed, in reference to a clinically established surface (e.g., HA or porous coating) under a mid-term implantation duration of at least 26 weeks, to evaluate the osseointegration in a substantiated manner [7–10].

Animal models with osteomyelitis have been used previously to investigate potential treatment options using implants. After a review of the existing literature, it was found that a wide variety of osteomyelitis animal models exist [9]. However, no ideal single animal model exists to address implant associated osteomyelitis. Therefore, we propose that researchers and clinicians should ask indication and disease-specific questions and build on established appropriate animal models capable of answering their questions and enabling translations to the clinical situation [9]. Traditional methods to quantify bacterial load via colony forming unit (CFU) assays should be replaced with in vivo bio-luminescent imaging and radiological outcome quantification. New anti-microbial treatments should be evaluated in regard to the host immune response utilizing biomarkers, and shoud be based on new technologies like the detection of bacteria by fluorescent in-situ hybridization in bone infection [9,11].

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