Preclinical performance testing of medical devices with antimicrobial effects: shifting the focus from “bench” to “bedside”

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Abstract

- The rate of infection must be considered throughout the product development pathway, including the design and development of new technologies as well as any adverse events that arise postmarket.
- Microbial biofilm plays a key role in medical device-associated infections, resulting in strong motivation for development of medical devices with antimicrobial effects.
- In this poster, we show how preclinical testing can be improved by shifting the focus of *in vitro* performance testing from bench to bedside.
- Researchers should incorporate clinically meaningful challenges, use realistic simulated environments and conditions, and measure appropriate endpoints.
- Material characterization and pharmacologic modeling, including computational simulation, can bolster our understanding of the relationship between *in vitro* test parameters and *in vivo* outcomes.
- A systems approach is needed to understand the necessary relationships *in vivo* that need to be recapitulated *in vitro* for a given device, anatomy and usage scenario.
- We introduce a rubric to standardize the systems approach in a scalable and flexible manner.

The Product Development Pathway

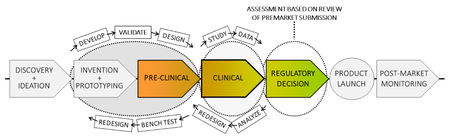


Figure 1. The medical device product development pathway is an iterative process of continual improvement. Pre-clinical testing is an essential part of a feedback loop that precedes clinical testing and regulatory assessment.

Current Preclinical Test Methods

In vitro methods should identify potential safety or efficacy issues before more costly *in vivo* testing. But *in vitro* success does not always correlate with clinical benefit. Improved preclinical testing methods would lead to reduced costs and more efficient time-to-market for novel medical devices.

	Test Method	Description	Format	Endpoint	Key Limitations
1a	Pharmacologic Test Methods				
	CLSI M02-A11	Antimicrobial disk susceptibility	Agar plate	Zone of inhibition	Static system, unrealistic substrate
	CLSI M07-A9	Antimicrobial susceptibility	Agar plate	Direct culture	Static system, unrealistic substrate
	USP S1	Preservative	Liquid medium	Direct culture	Static system, measures inhibition
1b	Material Test Methods				
	Static adhesion assay	Bacteria on material surface	Batch container	Harvest and culture	Static system, unrealistic environment
	Flow perfusion assay	Bacteria on material surface	Flow chamber	Harvest and culture	Unrealistic solution, environment and time points
	Biofilm based assay	Biofilm on material surface	Batch of flow container	Harvest and culture	Unrealistic environment and time points
	ISO22196, JIS Z 2801	Biofilm growth on material surface	Batch container	Harvest and culture	Static system, unrealistic environment and time points
1c	ASTM Biofilm Test Methods				
	ASTM E2196	Biofilm growth on coupon surface	Rotating disk reactor	Harvest and culture	No antimicrobial dilution, unrealistic time points
	ASTM E2647	Biofilm growth on coupon surface	Drip flow reactor	Harvest and culture	Unrealistic environment and time points
	ASTM E2562	Biofilm growth on coupon surface	CDC flow reactor	Harvest and culture	Unrealistic environment and time points
	ASTM E2799	Biofilm growth on plastic pegs	MBEC assay	Absorbance	Unrealistic environment and materials
	ASTM WG32449	Single tube assay		Direct culture	No antimicrobial dilution, unrealistic time points
1d	Highly Cited Test Methods in the Literature				
	Certika assay	Biofilm growth on coupon surface	Micro-comb model	Absorbance?	Unrealistic solution, environment and time points
	Microtiter dish assay	Biofilm growth on plastic walls	Microtiter dish	Crystal violet absorbance	Unrealistic environment and materials
	Calgary biofilm device assa	Biofilm growth on plastic pegs	Calgary Biofilm Device (CBD)	Harvest and culture	Unrealistic environment and materials

Moving from Bench to Bedside

The *in vitro* testing of many antimicrobial biomaterials often shows high performance in the literature. Large zones of inhibition, reductions in bacterial CFU, strong inhibitory effects read by optical density. Current *in vitro* methods do not predict clinical outcomes.

Device	Antimicrobial Agent	Year	n (#)	Clinical Significance?	In Vitro Significance?
Bone Cement	Cefuroxime, Gentamicin, Tobramycin, Erythromycin, Ciprofloxacin, Vancomycin, Cefotaxime	2018	34664	No	Yes
Bone Cement	Cefuroxime, Tobramycin, Erythromycin & Colistin	2017	3903	No	Yes
	Gentamicin or Vancomycin		189	Yes [a]	
Bone Cement	Antibiotic-loaded bone cement	2021	671,246	No [c]	Yes
			371,977	Yes [c]	
				No [d]	
Bone Cement	Antibiotic-loaded bone cement	2014	123,788	Yes [e]	Yes
CVCs	Chlorhexidine & Silver Sulfadiazine, 5-Fluorouracil, Vancomycin, Benzalkonium Chloride, Teicoplanin, Miconazole & Rifampicin, Minocycline & Rifampin	2018	10464	Yes	Yes
	Silver			No	
CVCs	Minocycline & Rifampicin, Silver	2017	3079	Yes	Yes
	Heparin, Silver platinum-carbon, Chlorhexidine, Chlorhexidine & Silver sulfadiazine, Miconazole & Rifampicin, Benzalkonium, Cefazolin, Chlorhexidine, 5-Fluorouracil		9918	No	
Urinary Catheter	Silver Alloy, Nitrofural	2006	13392	Yes	Yes
Urinary Catheter	Silver Alloy, Nitrofural	2014	12422 27876 [f]	No	Yes
Wound dressings	Iodine, Zinc Oxide, Honey, Sulfamethoxazole & Trimethoprim, Aloe Vera, Hydrocolloid, Silver Sulfadiazine, Lysozyme, Framycetin Sulphate, Gentamicin, Chlorhexidine	2018	16093	No	Yes

[a]for primary TKA
[b]for revision TKA
[c]revision PJI
[d]all cause revision for primary THA
[e] primary THA-related SSI when both systemic antibiotics and antibiotic loaded bone cement are used together
[f]12,422 hospitalized adults in 25 parallel group trials, and 27,878 adults in one large cluster-randomized cross-over trial

- Antibiotic-loaded bone cement (ALBC) is a common approach to dealing with prosthetic joint infections (PJI) that arise in orthopedic patients receiving procedures such as total knee arthroplasty (TKA). A 2018 systematic review of 34,664 TKA patients revealed that ALBC did not reduce the prevalence of PJI and cost more than plain bone cement.
- A similar “bench side / bed side” mismatch exists with wound dressings, urinary catheters, and central venous catheters.
- Our literature review revealed that even when *in vitro* success is claimed, clinical success only follows about 36% of the time. This disconnect may be due to the microbial pathogenesis being more complex than a simple “race to the surface.” There are multiple simultaneous interactions: bacteria and host, bacteria and biomaterial, biomaterial and host, antimicrobial agent and host, and antimicrobial agent and bacteria.

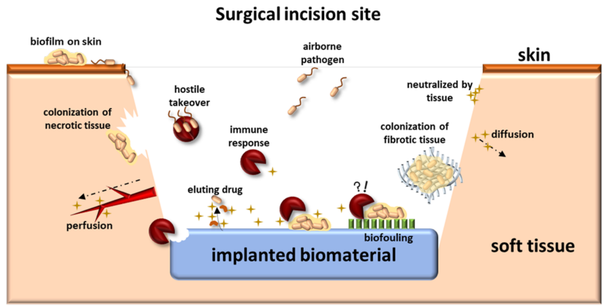


Figure 2. Top: surgical incision site; Bottom: biomaterial; Stars: eluting antimicrobial agent; Pacman: immune cells. Mixed species colonies normally present on the skin surface can invade deeper tissue at the incision site, where they colonize fibrotic tissue and/or the surface of implanted biomaterials, resulting in inflammation and tissue necrosis. The foreign material response and extracellular matrix of biofilm both increase the challenge for immune cells to clear pathogenic microbes. Biomaterials with antimicrobial surface effects can kill pathogens on contact but are subject to passivation by biofouling or dead cells. Drug eluting materials can kill bacteria in surrounding tissue, but are limited by dilution due to diffusion, perfusion and neutralization of the antimicrobial. Some antimicrobials may hinder the immune response or healing process and present toxicity to mammalian cells.

Ways to Improve Test Methods

1. *Use more realistic endpoints*. Antimicrobials can achieve a 3-log reduction in bioburden (CFU) against lush, thick biofilm formed in a flow cell. But testing the same intervention against a buildup biofilm—a multi-day biofilm exposed to increasing concentrations of antimicrobials—may yield less removal. Explants often resemble cells seen in buildup biofilm, which are more resistant to antimicrobials. The duration of tests is also mismatched with clinical use time.
2. *Incorporating the tissue environment into models*. Bacteria have adhesins for tissue surfaces. Compromised tissue may be a source of biological molecules that signal and stimulate bacterial migration, growth, or virulence factors. Tissue can absorb antimicrobials through direct binding or sequestration, reducing their effectiveness. Where tissue is in contact with a medical device, it may provide an alternative route for migration or colonization.
3. *Implement microphysiological systems (MPSs)*. These live tissue models that recapitulate some basic aspects of biological systems would provide even more realism.

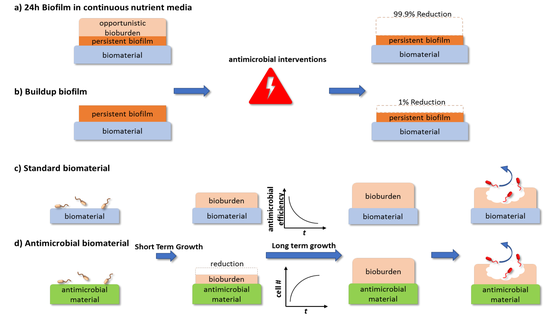
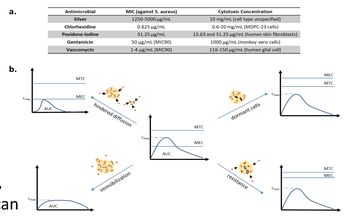


Figure 3. a,b) Testing of a thick biofilm may overestimate antimicrobial performance by not considering the importance of persister cells in infection risk; c,d). Short term testing may show reductions in bioburden, but a longer-term endpoint can reveal decreasing antimicrobial efficacy and a surface overrun by rapidly multiplying cells.

Figure 4. The combination of computational modeling and *in vitro* data can be used to generate more realistic starting estimates for the performance of eluting devices. For some antimicrobials, there is a very narrow therapeutic window, or none, where antimicrobial concentrations can be achieved that are sufficiently high to prevent infections, but sufficiently low to avoid toxicity (Figure 3a). This is further exacerbated by the presence of biofilms (Figure 3b). Biofilm can slow diffusion through steric hindrance and immobilize drugs through electrostatic and hydrophobic interactions. Dormant cells may not take up drugs and are difficult to kill, while changes in phenotype and propagation of resistance genes can raise the minimum effective concentration.

a.

Antimicrobial	MIC (against S. aureus)	Cytotoxic Concentration
Silver	1250-5000 µg/mL	50 mg/mL (cell type unspecified)
Chlorhexidine	0.625 µg/mL	0.6-20 mg/mL (MDPC-23 cells)
Povidone-Iodine	31.25 µg/mL	15.63 and 31.25 µg/mL (human skin fibroblasts)
Gentamicin	50 µg/mL (MICRO)	1000 µg/mL (monkey vero cells)
Vancomycin	1-4 µg/mL (MICRO)	110-150 µg/mL (human glial cell)



c.

Device	Infection	Cost $/y (2009)	Resistance Threats	CDC Levels
Urinary Catheters	CAUTI	$2.0 billion	extended spectrum ß-lactamase producing Enterobacteriaceae	Serious
			carbapenem-resistant enterobacteria	Urgent
Central Catheters	CLABSI	$1.2 billion	drug-resistant Candida, vancomycin resistant Enterococcus, methicillin resistant Staphylococcus aureus	Serious
Ventilators	VAP	$1.3 billion	multidrug-resistant nontuberculous mycobacteria	Serious
Orthopedic Implants	PJI	$1.0 billion	methicillin resistant Staphylococcus aureus, drug resistant Enterobacteriaceae, drug resistant Pseudomonas aeruginosa	Serious

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A Systems Approach

An ideal preclinical testing strategy needs to be scalable to incorporate complexity of related testing (such as Biocompatibility), and flexible to incorporate technological gains over time. A systems approach is a problem-solving paradigm that uses hierarchical grouping to address complexity. Construction of the system is an iterative process using refinement and convergence, which is confirmed through validation in the actual operating environment. A systems approach could implement a rubric that would answer questions like:

1. What is the anatomic location of the device? Where in the body is it located? Diagram the device and local anatomy.
   a. What is the material of the device? For example, is it metallic, polymer based, or ceramic? Does it have materials with potential to degrade *in vivo*?
   b. If an antimicrobial is to be eluted, what is the antimicrobial’s water solubility and its solubility in organic content? What are the release characteristics of the antimicrobial, including its dissolution and diffusion properties?
   c. What is the nature and duration of contact between the device and the patient’s body?
2. Diagram any physiologic systems in contact with the device (mechanical stress, perfusion or fluid flow, tissue compartments, etc.)
   a. What are the mechanical forces that the device will experience?
   b. What are the chemical and mass transport forces that would impact any elution of antimicrobials?
3. Diagram sources of microbial contamination.
   a. Are these sources a one-time event during device implantation, or are they ongoing sources (such as protrusion through the skin, indwelling devices, etc.)?
   b. What are expected types of bioburden based on literature or experimental data?
4. How will the clinical risk/benefit be determined?
   a. What is the appropriate antimicrobial release profile?
   b. What is the antimicrobial safety and effectiveness at the specified range of concentrations?
   c. What is the safe and effective dose of the antimicrobial agent?
   d. What are the toxicity risks?
   e. What are the antimicrobial resistance risks?
5. What information is needed from in vitro tests? What *in vitro* tests and endpoints can be used to obtain this information? What are the most important features of the physiological environment that need to be present in testing?

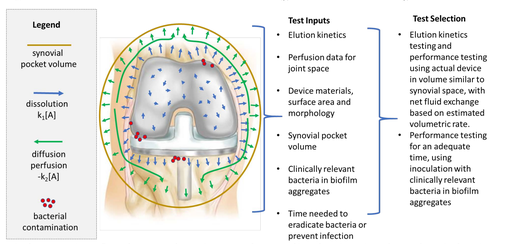

Figure 5. Schematic of a drug releasing orthopedic device in the physiologic space with key parameters diagrammed based on the rubric questions. a.)Diagram of key systems and their interactions; b.)Key inputs needed to assess performance and safety based on the systems diagram; c.)Test selection to obtain test inputs.
The background image (knee and spacer) have been used and annotated with permission from the American Academy of Orthopedic Surgeons (AAOS).

Conclusions

- Preclinical testing plays an important role in our effort to further reduce the risk of medical device associated infection.
- Current in vitro methods of testing antimicrobial performance are not predictive of clinical outcomes and can be improved through more realistic environments and endpoint measurements.
- Tissue models and Medical Devices on Chips are emerging as transformative in vitro simulation technologies.
- A scalable and flexible systems approach using a consensus-based rubric will enable development of rational preclinical testing approaches that can identify new technologies with significant patient benefit.