Biomaterial-Associated Infection

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BIOMATERIALS

Biomaterial-Associated Infection: Locating the Finish Line in the Race for the Surface

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Biomaterial-associated infections occur on both permanent implants and temporary devices for restoration or support of human functions. Despite increasing use of biomaterials in an aging society, comparatively few biomaterials have been designed that effectively reduce the incidence of biomaterial-associated infections. This review provides design guidelines for infection-reducing strategies based on the concept that the fate of biomaterial implants or devices is a competition between host tissue cell integration and bacterial colonization at their surfaces.

BACTERIA VERSUS HOST TISSUE

Currently, we face unusually long life expectancies, while at the same time, people expect a high quality of life to accompany aging. Yet, no matter how well human life-styles are adapted to protect against injury and to age in healthy ways, the body eventually reaches a state exceeding its capacity for effective natural repair. In some cases, severe trauma damages human tissues beyond repair. Also, tumor resections—a common procedure as we age—can create irreparable damage or structural defects that do not heal. More naturally, increasing wear within musculoskeletal joints becomes less amenable to repair by innate host processes.

Today, however, irreparable damage to the human body does not necessarily imply functional loss or reduced quality of life. Frequently, functional restoration is achieved surgically using permanently implanted biomaterials and devices like total joint arthroplasties and the artificial heart, respectively, or using instruments and temporary devices for transient intervention to help promote tissue regeneration, functional restoration, and healing, like a pulmonary assist device and urinary or intravenous catheters. Implant and device composition and application may differ widely—from the artificial heart to prosthetic joints to vascular prostheses to dental implants to contact lenses—but all attract microorganisms, thus representing niches for infection in vivo (Table 1). Continued microbial presence interferes with the intended function of an implant or device and adds risk to human use. Indeed, infections surrounding foreign body materials have been recognized since the 14th century when the French surgeon Guy de Chauliac, Pope Clement VI's personal physician, advised removal of all foreign bodies from infected wound sites in his surgical handbook, *Chirurgia Magna* (*9*). Implant infections have a substantial and largely unchanged clinical incidence, associated mortality and morbidity, and significant costs shared across all implant and device categories.

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Increasing use of biomaterial implants and devices accompanies our growing life expectancies and exacerbates these problems. In particular, permanent, totally internal biomaterial implants and devices face two challenges with respect to their extended use in vivo: biomaterial-associated infection and lack of native tissue integration (*10*). Anthony Gristina (*10*) coined the phrase the "race for the surface," suggesting that tissue cell integration and bacterial adhesion compete for real estate on the implant's surface. One hopes that the race is won by the host tissue, "defending" the implant surface from invading pathogens by implant integration and vigorous immune competence.

Nevertheless, bacteria can also "win" with dire patient consequences. Because implant microbial colonization is the prelude to biomaterial-associated infection (*11*, *12*), biomaterial surface properties have long been the focus for understanding microbial adhesion and infection mechanisms as well as for the design of preventive measures. Nonetheless, progress in improving biomaterial surfaces and the design of coatings to prevent microbial colonization has been limited. Central to this lack of progress is the failure of design technologies for both coatings and new biomaterials that reduce ubiquitous bacterial adhesion occurring on every material surface, as well as strategies that effectively block microbial phenotypic changes upon adhesion, including the production of extracellular polymeric substances (*13*) in which bacteria embed and protect themselves in their biofilm mode of growth. Organisms in a biofilm mode of growth may be metabolically less active, facilitating resistance to antimicrobial agents (*14*). Such metabolically reduced or senescent phenotypes allow organisms to survive in long-lasting dormant states despite antibiotic treatment. This highly protective biofilm phenotype enables microorganisms colonizing a biomaterial surface to evade antibiotics and host immune responses for up to several years before awakening in more virulent modes (*15*). The immune responses that should clear bacteria from the surrounding tissue are severely compromised by the trauma associated with surgical implantation as well as by the resulting presence of a foreign body in the tissue, thus frustrating phagocytic activity and deranging the host immune response. This ultimately allows bacterial survival both on and around the implant or device. More than 3 centuries ago, Antonie van Leeuwenhoek discovered that vinegar, an antimicrobial used at the time to kill oral biofilm, did not penetrate in a biofilm and killed only bacteria at the outside of the biofilm, therewith referring to the protective, biofilm mode of growth of microorganisms on his tooth surfaces. We now find these films on biomaterial implants and devices.

Table 1. Incidence of biomaterial-associated infection for different implants and devices. Incidence data are given over the entire implant or device lifetime, unless stated otherwise.

Tissue implant site	Implant or device	Infection incidence over lifetime (%)	References
Urinary tract	Catheter	33 (per week)	(*1*, *2*)
Percutaneous	Central venous catheter	2–10	(*1*)
	Temporary pacemaker	4	(*3*)
	Short indwelling catheter	0–3	(*2*)
	Peritoneal dialysis catheter	3–5	(*2*)
	Fixation pin or screw	5–10	(*1*)
	Sutures	1–5	(*4*)
	Voice prosthesis	25 (per month)	(*5*)
	Dental implant	5–10	(*1*)
Subcutaneous	Cardiac pacemaker	1–7	(*1*)
	Penile prosthesis	2–5	(*1*)
Soft tissue	Mammary prosthesis	1–7	(*2*)
	Abdominal wall patch	1–16	(*6*)
	Intraocular lens	0.1	(*2*)
Eye	Contact lens	0.1–0.5	(*7*)
Circulatory system	Prosthetic heart valve	1–3	(*1*)
	Vascular graft	1.5	(*1*)
Bone	Prosthetic hip	2–4	(*2*)
	Prosthetic knee	3–4	(*2*)
	Tibial nail	1–7	(*8*)

Although lack of progress over 3 centuries to design biomaterial surfaces that prevent biofilm formation is frustrating scientifically, our inability to prevent biomaterial-associated infections is increasingly considered socially and medically unacceptable. Opinions differ on whether current infection incidences in various applications (Table 1) are high or low with respect to the benefits of a biomaterial implant or device intended to improve the patient's quality of life. Higher risks can be accepted, for instance, in case of potentially fatal disturbances of cardiac rhythm using pacing devices, more so than for implant surgeries solely aiming at cosmetic effects. However, there is increasing social intolerance toward implant-associated infection: Resulting infections are frequently accompanied by patient morbidity and discomfort and can lead to surgical replacement of the implant after lengthy, unsuccessful attempts to mitigate infections with antibiotic treatments. This tedious clinical routine incurs additional health care costs and patient morbidities. Revision surgery to replace a total hip arthroplasty triples the cost of the primary implant procedure and amounts to an average of $75,000 (*16*, *17*). Since 2008, the U.S. Centers for Medicare & Medicaid Services have stopped reimbursing hospitals for eight conditions with evidence-based prevention guidelines, including coronary artery bypass graft and catheter-associated infections. Hospitals in various countries worldwide are held directly accountable for biomaterial-associated infections (*18*).

This historical inability to produce clinically effective, infection-resistant biomaterial implants and devices can be attributed in part to the complexity of the problem. The various scientific disciplines addressing the issues may not approach the problem in a sufficiently interactive manner. Therefore, this Review aims to describe major hurdles that impede clinical progress after initial discoveries by van Leeuwenhoek more than 300 years ago and by Gristina more than 25 years ago, who provided a more modern, unique, comprehensive, and multidisciplinary insight into biomaterial-associated infections.

PROTECTING THE PRIMARY IMPLANT PATIENT

A technically successful operation is no guarantee against biomaterial-associated infections. In the absence of skin-penetrating trauma, it is likely that the organisms causing implant infection have either entered the wound site or attached to the implant during surgery (termed perioperative contamination) or during hospitalization, before wound closure (termed early postoperative contamination). No surgical suite is truly sterile (*19*), and sources of contaminating pathogens are present in most operating theaters (*20*); therefore, both routes of microbial contamination are common in all surgeries and postoperative hospitalizations but pose greater risks in biomaterial implant surgeries because organisms that adhere to implant surfaces and revert to their protective biofilm phenotype and/or enter senescent states can survive—often unnoticed by the host immune system. Under these conditions, pathogens can cause clinically significant infections, even many years after surgery (*21*).

Sterile implant surgery may therefore be considered a myth, and estimates are that in a standard operating theater during a surgical procedure of 1 hour, the total number of bacteria-laden particles falling into a wound amounts to about 270 bacteria/cm^2 (*22*). More recently, through the use of modern, better-ventilated operation theaters (20 changes of air per hour) and impermeable clothing, perioperative bacterial contamination may be less (*23*). Nevertheless, many surgical procedures in which implants are placed in the body last longer than 1 hour, sufficiently long for considerable bacterial contamination to occur. Contamination is generally higher during periods of personnel movement and surgical activity, and when more people are present in the operation theater. Hence, discipline among personnel in the operation theater is also involved in the prevention of perioperative contamination (*24*). Apart from these nonpatient sources of perioperative contamination, commensal bacteria residing in the deeper layers of patients' skin cannot be killed by regular disinfection and may be "released" to the wound site upon scalpel insertion and incision (*25*–*27*).

Figure 1 exemplifies adverse events possibly occurring after a technically successful implant surgery. Other than infection from peri- or early postoperative contamination that can be noted almost immediately (if not falsely taken for an occasional influenza), clinical signs of infection may not appear until many years later. A biomaterial implant or device remains at risk of infection by hematogenous spread of bacteria disseminated from infections elsewhere in the body or entering the bloodstream after routine dental treatments or minor skin-penetrating trauma. In such circumstances, effective protection is only offered by integration of the biomaterial into host tissues and establishment of a normal host immune response at the implant site.

Gristina (*10*) was acutely aware that biomaterial surfaces needed to be modified to improve compatibility and tissue integration to resist

microbial colonization. Notably, surface modification remains the most frequently adopted route to reduce the incidence of biomaterial-associated infection (*28*–*30*). Unfortunately, performance demands and expectations imposed on many coatings by clinicians and patients to address infection risks are ill-defined. These demands and expectations neglect the crucial distinction between a host tissue environment before primary implantation and the environment surrounding an infected implant or device that must be replaced (revision surgery). Primary implant surgery involves low numbers of contaminating bacteria, wherein tissue surrounding the implant site is initially uncompromised by bacteria. This is distinct from a failing, infected implant requiring replacement, where the tissue surrounding the implant can also be infected, compromised, inflamed, and possibly necrotic. In many cases, systemic antibiotics, local antibiotic release from implant coatings (*31*), or device fixation materials, such as antibiotic-loaded bone cements (*32*), suffice to mitigate microbial contamination developed during primary hospitalization. However, revision surgery often requires retraumatizing the already-infected implant site, tissue debridement with the risk of disseminating resident bacteria, further loss of tissue, and creation of poorly perfused tissue defects and voids.

Costs and health consequences of implant-associated infections are substantial, and research efforts that more effectively address implant failure resulting from both peri- and early postoperative contamination during hospitalization are needed. This encompasses biomaterial surface modifications that ensure patients that all hospital-acquired organisms endangering the implant or device are killed and cleared rapidly upon or immediately after implantation. Societal pressure to achieve such design improvements is increasing because we all desire a "warranty," or guarantee, that the service or implant will have lasting, positive effects without a risk of infection. Antimicrobial implants and devices should not impair the local host immune competence. Depending on the application, implants are also expected to stimulate tissue integration while simultaneously preventing microbial adhesion, killing all organisms both active and dormant, present on and around the implant or device. These performance requirements put a restraint on the time period that these coatings should remain reliable and effective. In general, coatings that negate the effects of microbial contamination acquired during hospitalization or surgery should complete this task within 2 to 3 weeks after implantation. Longer implant protection is of course desirable to prevent infection of an implant site through the hematogenous route, but this long-term performance is currently technically not possible by lack of appropriate biomaterials and coatings.

PROTECTING THE REVISION IMPLANT PATIENT

Revision surgeries after implant infection are cumbersome because they suffer from higher infection incidences than primary implants. For orthopedic joint prostheses, the growing number of national and international registries, such as the Swedish Hip Arthroplasty Registry (*33*), comprising thousands of patients followed over many decades, provides clear, statistically supported evidence that secondary prostheses placed after infections are at greater risk for infection than primary prostheses. In general, however, reliable literature data to this point are scarce, because only a small fraction of all implant patients undergo revision surgery due to infection. Penile prostheses, for instance, have an infection incidence of 2 to 5%, but in revision patients with implant infections, this increases to 10% (*34*).

Secondary implants and devices placed after infections require different approaches to mitigation because bacteria residing in peri-implant tissue can proliferate if not adequately eliminated before revision surgery, causing resuscitation of infection. In one study, routine hospital cultures of tissue sampled from regions surrounding failed joint prostheses exhibited bacterial growth in 9 of 22 cases, increasing to 14 cases after extended culturing (*35*). In live bone tissue excised from a patient with recurrent, long-term osteomyelitis, bacteria were observed inside osteoblasts and osteoclasts (*36*). Moreover, the importance of peri-implant tissue reservoirs for reseeding biomaterial-associated infection has been extensively shown in murine implant models. *Staphylococcus epidermidis* colonized the peri-implant tissue surrounding silicone rubber subcutaneous implants in mice where bacteria survived in high numbers, even within resident macrophages (*21*). Bacteria in the implant-associated tissue actively replicate (*37*).

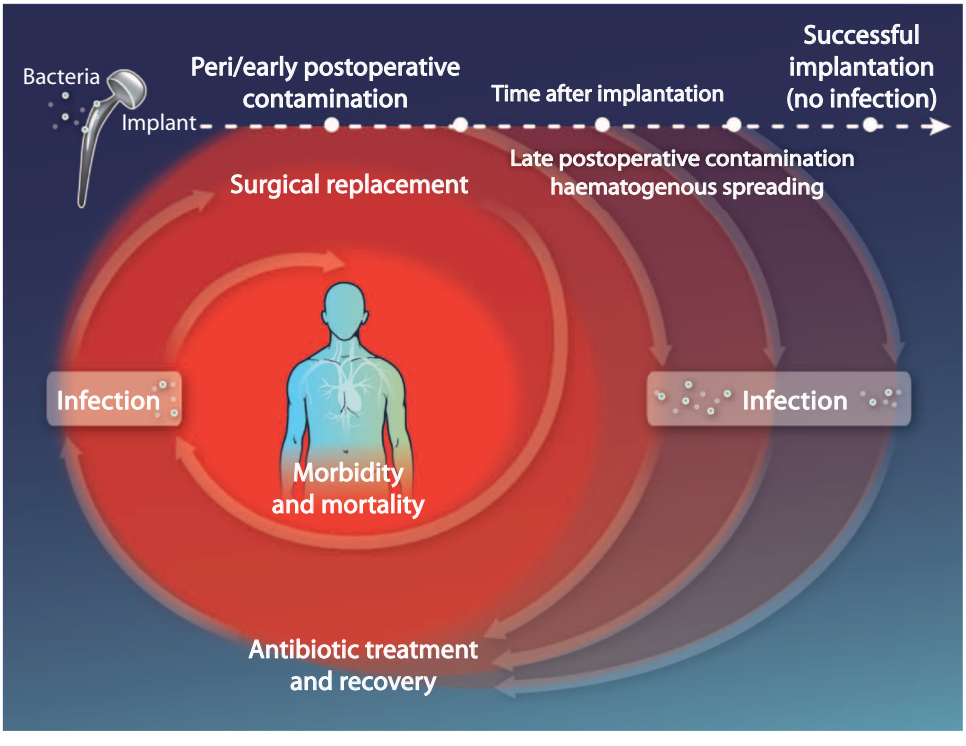

Fig. 1. Patient risk factors for developing a biomaterial-associated infection. Revision surgery patients are at greater risk than primary surgery implant patients, whereas the risk of an implant or device becoming infected hematogenously decreases with time after implant placement due to more extensive host tissue integration.

Similarly, pericatheter tissue samples of intensive care unit patients who had died from noninfectious causes were positive for bacteria both in culture and immunohistology, even in biopsies from tissues not bordering the catheter (*38*). This suggests that pericatheter tissues also provide a niche for infecting bacteria.

Clinically, antibiotics are chosen to be active against bacteria in biofilms, but the additional need to kill bacteria in niches, such as periimplant tissue or intracellular locations, is not always considered, or even possible. Antibiotics such as vancomycin and gentamicin have low—if any—activity against intracellular bacteria. Other antibiotics, such as rifampicin or fluoroquinolones, target intracellular bacteria, but pathogen resistance to these antibiotics develops rapidly, only requiring single point mutations. In the case of rifampicin-vancomycin combinations often used to treat biomaterial-associated infection, only rifampicin reaches intracellular bacteria; so, these bacteria are actually subjected to rifampicin monotherapy, yielding a high risk of resistance development. Combinations of rifampicin with other antibiotics may in part derive their efficacy for optimized clearance of intracellularly resident bacteria from results in animal infection models (*39*–*41*) and in patients (*42*, *43*).

In clinical orthopedics, elimination of bacteria from infected periimplant tissue in revision surgery is primarily achieved by two-stage revision surgery in which the infected prosthesis is removed, the host bed is rigorously cleaned, antibiotics are systemically and locally delivered for a prolonged period of time, and a new prosthesis is placed only when the infection has fully cleared and surrounding tissue is no longer compromised. Not all applications, however, allow a two-stage revision, and secondary implants sometimes have to be placed into contaminated tissue in single-stage surgical exchanges, like with an infected vascular graft. Aggressive and long-term antibiotic therapy is then essential to protect the revision implant or device in its compromised tissue environment to prevent recontamination over time by persister organisms that have survived initial antibiotic treatment. Initial antibiotic treatment not only comprises systemic antibiotics but may also include biomaterial coatings, such as surgical meshes and tibial nails with gentamicin incorporated in a biodegradable matrix or antibiotic-releasing bone cements for fixation of joint prostheses (*3*). Usually, however, the clearance of organisms from infected surrounding tissues requires high local concentrations of antibiotics at these tissue sites, extending the time periods over which current coatings release effective concentrations of antibiotics (usually 3 to 4 days, including a high initial burst release within 1 to 2 days).

DEVICE COATING AND DESIGN STRATEGIES

All biomaterial implants and devices can be adversely affected by microbial contamination and clinical infection. However, device coating strategies are often evaluated and applied without considering important details unique or specific to each application. This is flawed for many reasons. First, temporary implants, such as feeding tubes, urinary or vascular catheters, and contact lenses, do not require tissue integration. Therefore, nonadhesive (*44*, *45*), antibiotic-releasing (*46*), silver-impregnated (*47*), or coatings that kill bacteria immediately upon their adhesion to the coating (*48*) can prevent implant infection in these contexts. Regardless, infections arising from temporarily implanted medical devices should not be considered less severe: Even infection caused by devices that can be easily removed can result in a life-threatening situation where the physician has to balance the risks of infection versus the consequences of removing a potentially life-saving device.

Permanent, totally internal implants and devices designed to selectively favor host tissue integration over bacterial adhesion and biofilm growth are elusive: Biomaterial surfaces facilitating host cell adhesion, spreading, and growth are also adhesive to microorganisms because microorganisms use many of the same adhesive mechanisms as host tissue cells. An important example is the extracellular matrix (ECM) protein fibronectin (Fn) (*49*), a host protein that adsorbs to implants and devices and is characterized by an integrin receptor–binding motif, which is also recognized by Staphylococci having Fn-binding proteins (*50*). Alternatively, surfaces and coatings designed to prevent bacterial colonization do not effectively integrate with host cells and tissues. Functional duplicity between friend and foe has prompted increasing awareness of the futility of using monofunctional surfaces to both repel and kill bacteria while at the same time promoting tissue cell adhesion.

Surfaces with multiple functionalities (Fig. 2) that reliably select host cells over microbes must be created (*51*). Dual-function coatings use unique surface chemistries and topological patterns comprising both adhesive and nonadhesive sites in densities and configurations that selectively encourage tissue cell attachment yet impede adhesion of the much smaller microorganisms like bacteria (*28*, *52*–*54*). The number of functionalities added to a coating can be increased further by including moieties that kill bacteria upon their initial adhesion to a surface (*55*) or, in realization that biocompatible means more than anti-infection, moieties that demonstrate, for example, both antimicrobial and antithrombotic properties (*56*). Such multifunctional coatings give host cells a leg up in winning the race for the surface, as opposed to often-reported monofunctional surface chemistries and morphologies that either discourage microbial adhesion and biofilm growth or promote host tissue integration but cannot achieve both simultaneously. The realization that monofunctional coatings will not offer sufficient relief to the problem of implant and device infection is recent, and therefore, multifunctional coatings are still in their infancy. Moreover, appropriate methods for in vitro evaluation of multifunctional coatings are only slowly emerging.

EVALUATING ANTI-INFECTION POTENTIAL IN VITRO

Few in vitro studies reliably culture both bacteria and tissue cells under conditions relevant to in vivo infection niches. Microbial strains and tissue-derived cell lines used should be matched to the implant site's phenotypes. Urinary catheters, for instance, are predominantly colonized by *Escherichia coli* (*57*). Voice prostheses harvest a mixture of skin Staphylococci, oral Streptococci, and yeast (*58*). *Pseudomonas aeruginosa* is the primary causative species in contact lens–induced microbial keratitis (*59*). Joint prostheses and metal fixation implants usually fail owing to staphylococcal infections (*60*). The appropriate bacterial strains should be used in vitro when evaluating coatings for a specific application in vivo. In vitro evaluation methods are furthermore distinguished by the absence or presence of fluid flow, with fluid volumes varying with respect to biomaterial surface areas and microbial challenge doses applied.

Often, these parameters are unknown for the surrounding of an implant or device in the body, such as around the stem of a total hip prosthesis. These factors are particularly important for evaluation of

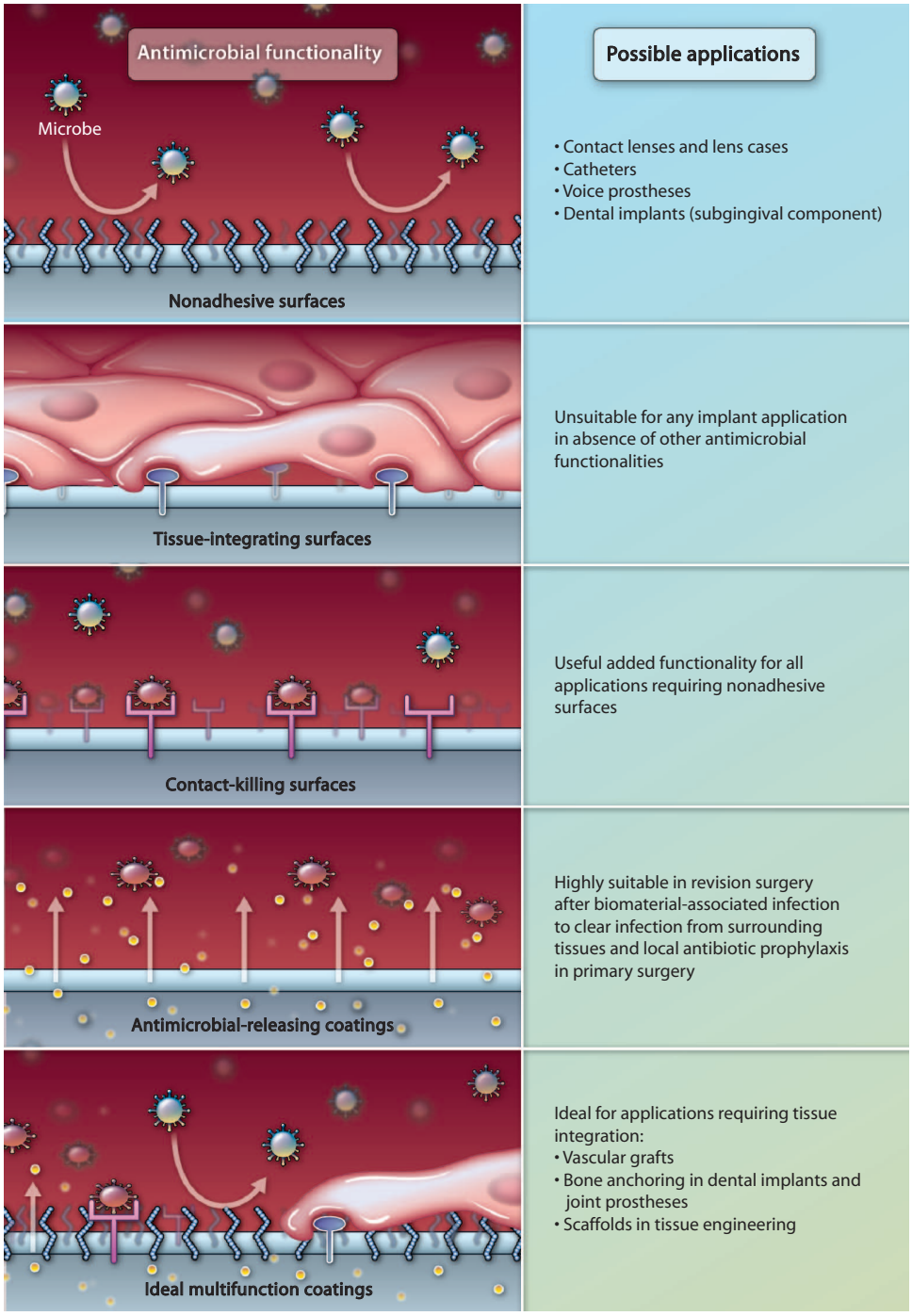

Fig. 2. Function requirements to biomaterials and coatings in different clinical applications. A schematic presentation of different antimicrobial functionalities that can be added to the surface of a biomaterial implant or device, together with their possible application.

coatings that release antimicrobials, and the design of the assay has a direct impact on the outcome of any evaluation. Under flow, for example, biofilms grow on antibiotic-releasing bone cements used for orthopedic prosthetic fixation, but in small, convection-free fluid volumes, bacteria are effectively killed, inhibiting biofilm formation (*61*). Another point often neglected in the design of in vitro studies is that in vivo biomaterials are rapidly covered with layers of adsorbed proteins from plasma, saliva, tear fluid, or other bodily fluids, depending on the implant site. Adsorbed protein films likely interfere with coating functionalities, which is why in vitro testing should include the effects of protein adsorption to a biomaterial or coating to yield relevant results for translation into people.

Different microbial species—and even strains within one species—can exhibit widely differing virulence and patterns of adhesion and growth on different biomaterial surfaces (*62*). Culture media for many in vitro microbiological assays have no physiological relevance and may yield different microbial properties than those observed from organisms grown, for instance, in serum or saliva. Within species, strain virulence and antibiotic susceptibility vary widely with source and strains used in in vitro assays; therefore, bacterial strains for in vitro testing must be carefully selected to prevent a bias toward favorable results, for example, by using nonvirulent or highly antibiotic-susceptible strains.

The shift in philosophy and strategy toward the design of multifunctional biomaterials and coatings and their downstream translation into clinical application necessitates the development of new in vitro and in vivo approaches to evaluate them. Many different in vitro methods assess either microbial adhesion and growth or cell adhesion, spreading, and growth on biomaterial surfaces. Flow perfusion devices are often used to this end, either macroscopic in size (*63*) or miniaturized (*64*–*66*). Microfluidic devices allow higher throughput and use smaller volumes and lower numbers of organisms, including clinical samples, without culturing, but offer less versatility with respect to the material to be investigated. Development of improved in vitro coculture models requires a suitable culture medium in which both mammalian cells and microorganisms grow naturally and in balance with one another (*67*). In a coculture model using modified media, it was observed that adhering *Staphylococcus aureus* and *P. aeruginosa* caused rapid death of adhering human U2OS osteoblasts within 24 hours, whereas U2OS cells survive for at least 48 hours in cocultures with adhering *S. epidermidis* (*68*). Because clinical infections arising from *S. aureus* and *P. aeruginosa* usually progress much more aggressively than those caused by *S. epidermidis*, this in vitro result has considerable

significance as a validation of the coculture model and the modified medium used.

Coculture models can also be applied to mimic perioperative or late hematogenous spreading of infectious bacteria, depending on whether bacteria are adhering to a surface before or after mammalian cell seeding (*67*, *69*). Evaluations in coculture models have shown that when cells are allowed to first reach a critical cell surface coverage before bacterial challenges, the chances for bacteria to colonize a surface are strongly reduced (*69*). This supports the notion that tissue integration is the best protection for a permanent, totally internal implant or device. This is also consistent with a clinical study on infection of total hip arthroplasties that demonstrated that infection opportunities from hematogenous sources are greatest in the first 6 postoperative weeks, during which time tissue integration is still incomplete and ongoing (*70*).

In vitro coculture studies are needed to provide different conclusions from the long-used and poorly predictive monoculture studies with either bacteria or tissue cells. On a bifunctional surface having specific tissue-reactive sites, coculture studies have demonstrated that favorable effects of such sites on mammalian cell interaction seen in monoculture studies may disappear in the presence of Staphylococci owing to the preferential adsorption of bacterial extracellular polymeric substances on the tissue-reactive groups, thereby blocking host tissue cell accessibility (*71*). Microfluidic devices have been used to investigate simultaneous interactions of osteoblast-like cells and *S. epidermidis* on a titanium surface (*64*) and were also extended to assay microbial biofilm formation on three-dimensional tissue cell growth (*65*) and collagen scaffolds (*66*), as in tissue-engineering scenarios. Unfortunately, despite their more predictive capabilities than monoculture counterparts, coculture studies are tedious and time-consuming, which does not make them popular for general use.

VIRTUES OF IN VITRO VERSUS IN VIVO ANIMAL EVALUATIONS

A comprehensive overview of the many in vitro and in vivo evaluation methods available and their pros and cons with respect to demonstrating antimicrobial efficacies of biomaterials and coatings in clinical applications is beyond the scope of this paper. Nevertheless, it is important to point out the long-standing challenge to establish reliable correlations between in vitro results, in vivo data obtained in animal experiments, and subsequent human in vivo performance. Table 2 summarizes frequent flaws in design of both in vitro and in vivo animal (preclinical) experiments that we consider central to this lack of correlation with human outcome.

Apart from the flaws listed in Table 2, most in vivo experiments are confined to small animals, like rats, mice, and rabbits, that may have different immune responses than each other as well as humans. Therefore, the bacterial challenges have to be carefully adjusted for the animal selected to prevent acute death due to infection. Infection doses for rabbits, for example, are usually much lower than for rats. Subcutaneous evaluations of the combined presence of a biomaterial with infecting bacteria are generally preferred, because in small animals, it is difficult to shape the biomaterial and surgically implant it at the application site aimed for.

Despite the drawbacks of current animal models, there are interesting new developments that may improve translation of results to the clinic and allow more rapid screening of biomaterials for their infection resistance and biocompatibility. To screen large numbers of novel biomaterials for immune responses and infection, a zebrafish embryo model is being developed (*72*). Zebrafish embryos have an innate immune system with high similarity to that of humans and are translucent (immune processes can be visualized in real time), and their transcriptomes can be easily analyzed (*72*). Biomaterials may then be selected for their capacity to induce specific desired expression of marker genes. In combination with fluorescently labeled biomaterials and bacterial pathogens, this model holds promise for speeding up development of infection-resistant biomaterials and coatings.

Regardless of any experimental design and animal choice, however, the best that in vitro and in vivo animal studies can provide is the following:

(i) How much microbial adhesion and biofilm formation is inhibited or delayed on one biomaterial (coating) relative to another;

(ii) How many more organisms are killed on one biomaterial (coating) versus another;

(iii) How tissue cells interact on different biomaterials (coatings); or, more specifically,

(iv) How the race for the surface between microbial colonization and tissue integration is influenced by surface design.

Although such information clearly is relevant to new biomaterial and coating designs, the assays provide relative assessments in simplified formats. These assays cannot answer the most important question often asked by funding and regulatory review panels, marketing

Table 2. Common flaws in the design of in vitro and in vivo experiments in animals aimed at demonstrating antimicrobial efficacy of an implant or device surface.

Evaluation should be in line with implant or device functionality
• Retains host immune system competence in clearance
• Facilitates host tissue integration
• Blocks microbial adhesion and growth
• Kills all organisms on and around the implant
• Evaluation for relevant duration of infection protection
Evaluation should be clearly aimed at primary or secondary implants or devices after biomaterial-associated infection
• Surgical competence and experience
• Hospital hygiene conditions and re-enforcement through appropriate protocols
• Presence of unaffected versus bacterially compromised tissue
• Duration of infection protection required
Evaluation method should be tailored to the physiological condition at the implant site (permanent, totally internal, temporary, and/or skin-penetrating)
• Ubiquitous presence of host protein conditioning films
• Use of relevant pathogenic species, causative to site-specific biomaterial-associated infection
• Use of relevant bacterial challenge doses

departments in industry, and physicians: By what percentage is this coating capable of reducing the incidence of biomaterial-associated infection in a given clinical application?

VALIDATION OF INFECTION-RESISTANT BIOMATERIAL COATINGS

Most of the hundreds of antimicrobial coating approaches reported in the literature fail in testing (*73*) or will not even reach the status of in vivo, animal experiments. Many in vitro evaluations of new antimicrobial coatings in the literature are rarely pursued by follow-up papers, presumably owing to negative results of further research or because finances for further research and necessary patents, including industrial interest, are lacking. Approaches that have been taken to clinical application very often encounter mixed enthusiasm, and it seems that no antimicrobial biomaterial or coating has been embraced in the clinic with the same enthusiasm as antibiotics when they were first discovered. An exception to this is the class of antibiotic-releasing biomaterials and coatings that have moved to clinical application (Table 3). Moreover, true validation of infection-resistant biomaterials and coatings required for effective downstream translation can only be obtained in human clinical trials, and unfortunately, this is often where development of an antimicrobial biomaterial or coating terminates.

Let's consider a hypothetical case: A design for an antimicrobial coating on total hip prostheses that prevents infection associated with perioperative microbial contamination has converged to a promising prototype coating through extensive in vitro and in vivo animal testing. Assuming an infection rate for total hip arthroplasties of 2%, a simple power calculation shows that studies aiming to demonstrate a 50% reduction in infection incidence, at a conservative statistical significance of $P < 0.05$, requires the inclusion of around 5000 patients that must be followed longitudinally over several years (and at a cost of tens of millions of dollars). To demonstrate a 25% reduction ($P < 0.05$), more than 22,000 patients have to be included (*95*). Clearly, these clinical trials are too large, costly, and lengthy to be realistic and encourage innovation, and hence, many such trials are never conducted. The result is that new technologies are not clinically validated or introduced.

We might consider a new reality in which the safety and efficacy of biomaterials that prevent implant infection will not be substantiated with clinical trials but instead will have to be derived from properly designed in vitro and in vivo animal experiments that include benchmarked, clinically widely accepted biomaterials for reference; this is similar to the process taken by many orphan drugs, where insufficient economic incentives exist to stimulate translation into clinical

Table 3. Exemplary materials and coatings undergoing clinical translation. The table only comprises a fraction of the hundreds of antimicrobial ideas and concepts reported in the literature but which either fail early in translation or cannot be translated for the reasons described in this article.

Functionality	Chemical basis	Current status	Examples of clinical application	References
Nonadhesive	Hydrophilic polymer coatings	Clinically applied	Contact lenses, hydrocephalic shunts, endotracheal tubes, urinary catheters	(*74–76*)
	Polymer brush coatings	In vitro and in vivo animal experiments	Unspecified	(*77*)
Tissue-integrating	Arginine–glycine–aspartic acid (RGD) peptide as a cell adhesion promoter	In vitro	Vascular graft	(*78*)
	Hydroxyapatite coatings	Clinically applied	Dental and orthopedic implants	(*79*)
	Titania thin-film coating	In vitro	Dental implants	(*80*)
Contact-killing	Immobilized quaternary ammonium compounds	In vitro	As yet unspecified	(*48*)
	Selenium coatings	In vitro	Contact lenses	(*81*)
	Silver coatings	Clinically applied	Urinary catheters	(*82*)
Antimicrobial-releasing	Antibiotic-releasing acrylates	Clinically applied	Orthopedic joint prostheses	(*31*, *32*)
	Silver carbonate–, chlorhexidine diacetate–releasing	Clinically applied	Surgical meshes	(*83*, *84*)
	Antibiotic-releasing	Clinically applied	Endovascular stent	(*85–87*)
	Silver, chlorhexidine, rifampicin, or minocycline coatings	Clinically applied	Vascular catheters	(*88*, *89*)
	Triclosan-releasing sutures	Clinically applied	Sutures	(*90*, *91*)
	Gentamicin/biodegradable polymer coating	Clinically applied	Tibia nail, surgical meshes	(*3*, *92*)
Multifunctional coating	Polymer brush/antibiotic co-grafted	Chemical drawing board, in vitro	Unspecified, possibly for titanium implants	(*56*, *93*, *94*)
	Polymer brush/cell-ligand co-grafted	Chemical drawing board, in vitro	Unspecified	(*52–54*)

use. Animal trials with relatively high bacterial challenge doses may show antimicrobial efficacy for biomaterial surfaces or coatings, but to demonstrate an actual reduction of infection incidences with lower, clinically relevant challenge doses, more comparable to the clinical situation with patients, the numbers of animals required would be as high as the numbers of patients, making the costs of such animal studies prohibitive as well. This poses a dilemma not only to biomaterial implant and device companies but also to funding and regulatory agencies, because it implies that the true clinical impact and economic value generation of new, anti-infection biomaterial designs cannot be accurately estimated.

The implant infection issue is too important to patients, however, to be deadlocked in this maze of uncertainties and impossibilities. Therefore, to facilitate translation of new coating approaches to clinical application, so-called combination devices—implants or devices supplemented with secondary on-board drug therapies—are offered a unique regulatory review process (*3*). In combination devices, clinically approved components are combined into a single platform, such as the gentamicin-releasing orthopedic tibia nail or paclitaxel-eluting coronary stent. Despite good intentions of regulatory bodies, such as the U.S. Food and Drug Administration, combination devices still undergo lengthy approval processes that include clinical trials to substantiate claims for antimicrobial efficacy of biomaterials and coatings for implants and devices. Academic institutions, industries, and funding and regulatory agencies will all have to find better ways to rely on indirect in vitro and in vivo animal experiments to move the field forward.

TISSUE-ENGINEERED DEVICES

Given the competition between tissue integration and bacterial colonization inherent in the race for the surface, a frequently asked question is whether new tissue-engineered constructs or devices will provide new opportunities to win the race. Tissue engineering may be the ultimate solution to restore function when the human body is compromised beyond natural repair. Tissue-engineered scaffold materials are implanted directly, or after being seeded and cultured with cells (*96*, *97*), ECM proteins, and growth factors most appropriate to the in vivo application site. However, such constructs and devices must also be surgically implanted to regenerate the desired tissue form and functionality and to integrate with host tissues. As biomaterial-based scaffolds, both acellular and cellularized implants are at equal risk of peri- and early postoperative microbial contamination.

Furthermore, host immune response may not develop sufficiently around implantation sites to clear microbial contamination. In a unique evaluation of 228 implanted polymeric, porous scaffolds in rabbit knee osteochondral defects, 6 scaffolds without cells (3.6%) and 4 identical scaffolds seeded with cartilage cells (6.3%) appeared to demonstrate clinical signs of infection within 3 to 9 weeks (*98*). These infection incidences are comparable with those currently seen for acellular biomaterial implants and devices in patients (Table 1). Apparently, even preseeded cells on a biomaterial cannot outcompete bacteria in their attempt to colonize a scaffold surface, indicating that infection control measures should be part of the development process of any tissue-engineered constructs and devices. Here, too, the concept of tissue integration over microbial colonization, as proposed by Gristina (*10*), provides an important guideline for which coculture assays may be indispensable tools.

A FINISH LINE IN THE RACE FOR THE SURFACE

The challenges in producing new infection-resistant, antimicrobial biomaterials and coatings for implants and devices have stubbornly persisted for many years. This Review identifies difficult barriers in understanding the backgrounds of this problem and therewith provides direction to accelerate progress as demanded by society, by outlining flaws in current research and development processes that impede downstream translation of new ideas to human use. Moreover, this Review enforces a proposed shift to multifunctional surface coatings, which are expected to perform better than current generations of monofunctional coatings. We also highlight an experimental need to simultaneously evaluate tissue integration, bacterial colonization, and immune responses before biomaterials can be tested in clinical trials.

Clinical trials, however, are cumbersome because the number of patients acquiring a biomaterial-associated infection is low and such trials necessitate inclusion of thousands of patients, which are often too large, costly, and lengthy to be pursued. Because downstream translation into clinical practice is the ultimate goal, these considerations yield the unavoidable, simple conclusion that claims for medical implant and device infection prevention should be accepted without clinical trials on the basis of in vitro and in vivo studies. Such studies are expected to extrapolate clinical efficacy as long as clinically biomaterials are used for reference, and the primary implant or device function is not adversely affected by adding antimicrobial coatings. To ensure patient safety, we need to scrutinize post-marketing surveillance results for unanticipated adverse effects.

Production of infection-resistant antimicrobial biomaterials and coatings will not only benefit the current generation of patients relying on biomaterial implants or devices. Past prototypes of the artificial heart, for instance, have bluntly ignored the risks of infection. Surprisingly, the upcoming field of tissue engineering largely also continues to neglect the risks of infection. With an eye on the future, prototypes of tissue-engineered constructs and devices are needed that include infection-resistant antimicrobial biomaterials and coatings. Otherwise, smooth translation to clinical applications will be cumbersome, positioning the finish line in the race for the surface farther away than needed.

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